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Poster

113. Nervous System Patterning and Developmental Cell Death

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 113.01/A1

Topic: A.01. Neurogenesis and Gliogenesis

Title: Spatially organized ciliary beating in ependymal cells compartmentalizes CSF flow in the brain and regulate ventricular development

Authors: *N. JURISCH-YAKSI, E. W. OLSTAD, C. RINGERS, A. WENS, J. N. HANSEN, C. BRANDT, E. YAKSI
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Abstract: Motile cilia, which are small extensions emanating from the surface of cells, generally beat in a concerted fashion to generate a directional fluid flow. In the brain ventricles, ependymal cells extend their cilia into the cerebrospinal fluid (CSF) and thereby contribute to CSF flow. Although cilia are not the only contributors to the overall flow, their functioning is crucial, as human patients having defective motile cilia develop clinical features including hydrocephalus. Traditionally, the functions of CSF flow were regarded as transport of nutrients and disposal of waste. However, recently, the complexity of this flow and its interplay with the nervous system are increasingly recognized. Nevertheless, these interactions, as well as the mere generation of the CSF flow, remain poorly understood. To address these shortcomings, we study the motile cilia and CSF flow in the ventricles of larval zebrafish. Our results show that ependymal cells, which harbor motile cilia are spatially organized in the brain ventricles and generate a directional CSF flow upon active ciliary beating. While the cilia-driven flow is prominent within an individual ventricular cavity, there is surprisingly little exchange of fluid between ventricles. Interestingly, these ventricular boundaries are abolished during bodily movement, thus highlighting the influence of external factors on the overall CSF flow. As our results imply that CSF flow is important for regionalizing molecules, we are currently investigating the developmental significance and regulation of such compartmentalization. Altogether, we expect our work to provide a better understanding of the interaction between the brain and the CSF, and to ultimately unravel the neural mechanisms regulating the flow of molecules in the brain.

Disclosures: N. Jurisch-Yaksi: None. **E.W. Olstad:** None. **C. Ringers:** None. **A. Wens:** None. **J.N. Hansen:** None. **C. Brandt:** None. **E. Yaksi:** None.

Poster

113. Nervous System Patterning and Developmental Cell Death

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 113.02/A2

Topic: A.01. Neurogenesis and Gliogenesis

Support: NSF CAREER AWARD 1253126 to SR

Title: Characterizing the development of GFP expressing GnRH3 neurons in the spinal cord in the developing zebrafish embryo

Authors: *N. R. DILLON[1], L. OLLERENSHAW[2], A. DEMARAIS[3], S. RAMAKRISHNAN[2]
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Abstract: The gonadotropin-releasing hormone (GnRH) pathway in the brain is the primary controller of the hypothalamic pituitary gonadal (HPG) axis and hence reproduction and allied behaviors in most vertebrates. While previous studies have focused on GnRH neurons in the HPG axis, the observation of GnRH neurons of the spinal cord have been limited. However there have been studies indicating the presence of some GnRH neurons in the spinal cord and effects of GnRH on spinal control of animal behavior. To date there has not been a comprehensive study of these neurons in the spinal cord of the developing embryo. In a stable transgenic line of zebrafish with GnRH3 neurons tagged with green fluorescent protein (GFP), we have observed GFP expressing neurons along the developing spinal cord starting 1 day post fertilization (dpf) persisting through hatch at 3dpf. This study characterized the development of these neurons in living embryos using immunohistochemistry, epifluorescence and confocal microscopy. Embryos were dechorionated on day 1 and embedded in 1% agarose made in embryo medium and examined under the microscope at 20x magnification and 488nm excitation/512 nm emission. Embryos were returned to the incubator post observation to be reexamined on both 2 and 3dpf. We observed a steady decrease in neuron numbers beginning 1dpf through 3dpf ($n = 12$, $p < 0.01$). While there was a decrease in neuron area between 1 and 2 dpf, no differences in neuron area was observed between 2 and 3 dpf embryos ($n = 12$, $p < 0.01$). Aside from discrete observations, we have also performed continuous confocal monitoring of these spinal cord neurons to observe and record dynamic changes in the spinal cord during this early period of development. Proximity to motor neurons were also assessed using whole embryo immunohistochemistry with mnx-1 antibody at various developmental stages. We are in the process of quantifying levels of apoptosis in the spinal cord between 1 to 3dpf using caspase immunohistochemistry to determine if neural numbers are decreasing because of cell death or migration. This is a novel study characterizing GFP expressing GnRH neurons in the early developing embryo.

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Poster

113. Nervous System Patterning and Developmental Cell Death

Location: SDCC Halls B-H

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Program #/Poster #: 113.03/A3

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH T32 (HL110852-05)
BCH Pilot Grant
Pediatric Hydrocephalus Foundation
National Science Foundation (GRFP)
William Randolph Hearst Fund
AFAR
NIH R01 (NS 088566)

Title: Translational control of the cerebrospinal fluid (CSF) stem cell niche

Authors: *R. M. FAME[1], K. F. CHAU[1,2], M. L. SHANNON[1], N. DANI[1], M. L. CALICCHIO[1], H. STEEN[1], S. ALEXANDRESCU[1], M. K. LEHTINEN[1,2]
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Abstract: The mammalian brain forms around cerebrospinal fluid (CSF)-filled ventricles. Secreted signals in the CSF instruct neural progenitor cell health, identity, and neurogenesis. While CSF components can reflect the overall health of the entire CNS, the choroid plexuses (ChP) are the tissues specialized to secrete CSF. Our goal is to elucidate mechanisms by which the ChP secretome and CSF proteome are regulated and how fluid system interacts with adjacent neural tissues, with direct diagnostic and/ or therapeutic applications to neurodevelopmental diseases.
To investigate the changing CSF composition during neural tube closure, we analyzed the developing amniotic fluid and CSF proteomes by mass spectrometry. We observed downregulation of protein biosynthetic machinery throughout early brain development. Parallel transcriptome analyses of the adjacent forebrain neuroepithelium uncovered a similar trend in progenitor cell transcriptomes. Specifically, transcriptome analysis suggests that c-MYC is normally downregulated in forebrain precursors after neural tube closure, as is ribosome biogenesis. Expression of c-Myc is required for neural tube closure. Using mouse models, we tested the molecular mechanisms regulating the protein biosynthetic machinery in forebrain precursors, because any missteps in their development can have devastating consequences on later brain function. We found that persistent c-MYC expression in neuroepithelial progenitor

cells results in a mouse with a large brain and fully penetrant choroid plexus carcinoma (CPC) and ciliary body medulloepithelioma (CBME).
These initial data suggest that selective protein translation provides an additional regulatory step for producing the CSF proteome. We therefore directly tested the hypothesis that translation is differentially regulated in the ChP. Using translating ribosome affinity purification (TRAP) in the embryonic and adult ChP after neural tube closure, we identify a changing landscape of specific age-dependent translation targets as the ChP matures. We also identify differential translation targets in the adult choroid plexus over the much shorter diurnal time scale. These data implicate translation as a key regulatory step governing ChP activity and the CSF proteome over time.

Disclosures: R.M. Fame: None. **K.F. Chau:** None. **M.L. Shannon:** None. **N. Dani:** None. **M.L. Calicchio:** None. **H. Steen:** None. **S. Alexandrescu:** None. **M.K. Lehtinen:** None.

Poster

113. Nervous System Patterning and Developmental Cell Death

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 113.04/A4

Topic: A.01. Neurogenesis and Gliogenesis

Support: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)
Instituto Nacional de Neurociência Translancional (INNT-INCT)
Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)
Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ)

Title: CB1/2 receptor activation induces death of chick embryonic retinal cells

Authors: *H. R. FREITAS[1], A. R. ISAAC[1], A. L. M. VENTURA[2], K. C. CALAZA[2], M. Z. P. GUIMARÃES[1], F. G. MELLO[1], R. A. DE MELO REIS[1], G. R. FRANÇA[3]
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Abstract: The vertebrate embryonic retina is composed by multipotent progenitors in a neuroblastic layer, which generate diverse cell types. Intrinsic and extrinsic factors modify the rate of proliferation, differentiation, maturation and cell death, accelerating or slowing the appearance of new retinal cells. As cannabinoid receptors are highly expressed at early stages of embryonic retina, we asked whether the mixed CB1/CB2 receptor agonist WIN 55,212-2 (WIN) could modulate death, proliferation, whole cell and mitochondrial stress and functional calcium responses to known transmitters on retinal progenitors. Retinal cells were obtained from chick (*Gallus gallus domesticus*) embryos at the seventh embryonic day and cultured for two days (E7C2). A fluorescence-based viability (Live/Dead) assay was performed exactly as suggested

by the manufacturer (Invitrogen, Carlsbad, CA). Quantification of live and dead cells was carried out using Image J (NIH, Bethesda, MD). In single cell calcium imaging experiments, free intracellular calcium levels ($[Ca^{2+}]i$) were measured in retinal cells in culture using the protocol adapted from (de Melo Reis, 2011) and (Freitas et al., 2016). To evaluate proliferation, retinal cultures at E7C1 were stimulated for 24 h with increasing concentrations of the selected drugs and incubated with 0.25 µCi [^{3}H]-thymidine for 60 min, at 37 °C. Samples were filtered, dried and the radioactivity determined by scintillation spectroscopy. Intracellular ROS accumulation was evaluated in live cells using a chloromethyl derivative of H2DCFDA, CM-H2DCFDA. Readings were carried out at 485/535 nm using a VICTOR X Multilabel Plate Reader (Perkin Elmer, Inc). Additionally, a fluorogenic assay (MitoSOX Red) targeted to mitochondrial superoxide in live cells, was performed exactly as suggested by the manufacturer (ThermoFisher). Quantification of fluorescence intensity, and subtraction of background values, were performed using Image J (NIH, Bethesda, MD). Treatment of retinal progenitors with a CB1/2 agonist, WIN, reduced cell viability (up to 4-fold decrease), increased mitochondrial stress (similar to H2O2-induced stress), abolished functional (KCl, glutamate and GABA) and upregulated ATP shifts in terms of $[Ca^{2+}]i$ responses. Finally, WIN and endocannabinoid-degrading enzymes regulated proliferation of these retinal progenitors (up to 5-fold reduction). Cannabinoid receptors play a role in the regulation of survival and proliferation in the chick embryonic retina. This effect may involve mitochondrial stress and increases in purinergic responses from retinal progenitor cells.

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Poster

113. Nervous System Patterning and Developmental Cell Death

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 113.05/A5

Topic: A.01. Neurogenesis and Gliogenesis

Support: NHMRC Grant APP1061291
Monash University FGRIS scholarship
Monash University MGS-Co funded scholarship

Title: Differential gene expression in the cortical plate during gyrification in fetal sheep

Authors: *S. QUEZADA[1,2], M. CASTILLO-MELENDEZ[3,2], N. HALE[3], M. TOLCOS[4], D. W. WALKER[4]
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Australia; [3]The Ritchie Centre, Hudson Inst. of Med. Res., Melbourne, Australia; [4]Sch. of Hlth. & Biomed. Sci., RMIT Univ., Melbourne, Australia

Abstract: The process of folding of the cerebral cortex (gyrification) has been intensely studied, and recent theories and investigations have largely focused on the role of the cortical plate. However, differences in cortical plate gene expression between gyri and sulci, and a possible role in the process of gyrification, remain unexplored. We used Laser Capture Microdissection to sample the cortical plate of the fetal sheep brain at mid-gyrification (90 dg, term ~147 dg) and used RNA Seq approaches to determine differentially expressed genes between gyri and sulci. While no differentially expressed genes were detected *in silico* using standard RNA Seq approaches, alternative approaches for selecting differentially expressed genes and further *in vitro* validation of selected genes display significant differential expression between gyri and sulci. Two-way ANOVA analyses found significant differences in gyri and sulci expression of BDNF ($p < 0.0001$), CDK5 ($p < 0.0001$), HDAC5 ($p < 0.01$) and MeCP2 ($p < 0.001$) at 90 dg, that are absent in the cortical plate at 70 dg; i.e., pre-gyrification. Also, there were no differences in expression rostro-caudally of these genes at either stage, showing that the differences between gyri and sulci were consistent throughout the cortex. A common feature of these genes is their participation in the neurite outgrowth process of maturing neurons, which coincides with the time of gyrification. This raises the possibility that the impact of neurite outgrowth on regional volume expansion may be a fundamental cause of gyrification (i.e. this process is different between gyri and sulci); a process currently under investigation.

Disclosures: S. Quezada: A. Employment/Salary (full or part-time):; Monash University. **M. Castillo-Melendez:** A. Employment/Salary (full or part-time):; Hudson Institute of Medical Research. **N. Hale:** A. Employment/Salary (full or part-time):; The Ritchie Center, Hudson Institute of Medical Research. **M. Tolcos:** A. Employment/Salary (full or part-time):; RMIT University. **D.W. Walker:** A. Employment/Salary (full or part-time):; RMIT University.

Poster

113. Nervous System Patterning and Developmental Cell Death

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 113.06/A6

Topic: A.01. Neurogenesis and Gliogenesis

Support: NSF GRFP DGE 16-44869
NIH- NINDS 5R03NS099920

Title: Non-apoptotic caspase function plays a role in retinal vascular development

Authors: *K. V. JOHNSON, M. AVRUTSKY, C. M. TROY
Pathology, Columbia Univ. Med. Ctr., New York, NY

Abstract: The retina being an easily accessible extension of the central nervous system (CNS) provides an opportunity to study many of the vital processes occurring in this elaborate system. The CNS, while comprised of some of the most metabolically active tissues of the body, is unable to store nutrients and is therefore completely reliant upon the vascular system to supply its needs. Vascular development in the CNS is tightly regulated by a variety of signaling pathways. Vascular growth in the mouse eye begins postnatally and progresses into the first 3 weeks of life making it an exceptional model to explore the mechanisms involved in angiogenesis. In this study, we investigate previously unexplored mechanisms and signaling pathways involved in the outgrowth and subsequent remodeling of the retinal vasculature. The caspase family of proteases is typically confined to roles in apoptosis and inflammation, however several members of this family have been found to have essential non-apoptotic functions. Preliminary data has revealed that activation of caspase-9, an initiator in the intrinsic apoptosis pathway, in endothelial cells does not lead to death of these cells suggesting an alternate role for this protease in the vasculature. To confirm the presence of caspases in the retina during angiogenesis, we performed immunofluorescence analysis on the retinas of wild-type C57BL/6 pups isolated during the first 2 weeks postnatal. We find that caspase-9 and caspase-8, as well as downstream effector caspases, are present at varying levels in endothelial cells and microglia during the 2-week time course with little indication of apoptosis occurring in those cells at the time points analyzed. Ongoing studies aim to explore the specific roles of these proteases in retinal angiogenesis. We employ cell-specific genetic knock out models of caspase-9 and caspase-8, as well as whole tissue caspase inhibition using protease-specific cell permeant inhibitors followed by immunofluorescence analysis. This study potentially reveals a novel pathway in the regulation of retinal angiogenesis and may provide insight into mechanisms behind pathologic vessel formation in the eye.

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Poster

113. Nervous System Patterning and Developmental Cell Death

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 113.07/A7

Topic: A.01. Neurogenesis and Gliogenesis

Title: An investigation into the long-term impact of prenatal zika virus infection on the developing brain using a rat model of maternal infection

Authors: *M. L. SHERER[1], P. KHANAL[2], M. PARCELLS[2], J. M. SCHWARZ[3]
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Abstract: Zika virus (ZIKV) is a mosquito-borne flavivirus that is of significant concern due to the association between ZIKV infection in pregnant mothers and an increased risk of

microcephaly and other neurological conditions in the fetus. While the widespread 2015-16 epidemic in North and South America may be behind us, there remains much to be learned about the virus's pathogenesis and mechanism of infectivity, as well as the potential long-term neural consequences and behavioral deficits in the infants born to infected mothers. By developing a rat model of maternal ZIKV infection, our lab is working to address some of these unanswered questions. In order to deepen the current understanding of ZIKV infection, we have measured the level of infectivity in the mother and the pups, the rate of viral clearance, the effect of ZIKV on the expression of inflammatory genes, cortical thickness, microglia morphology, apoptosis, and other important aspects of brain development in the affected neonatal rat pups. Unlike other Flaviviruses, ZIKV has an apparent selective tropism for developing neural stem cells, thus we are currently examining the long-term consequences of prenatal ZIKV infection on later-life neurogenesis in brain regions important for learning and memory. Overall, this model and our findings will help us to better understand the underlying mechanisms of ZIKV infection in the maternal-fetal interface and the immediate and long-term impact of the virus on the developing brain.

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Poster

113. Nervous System Patterning and Developmental Cell Death

Location: SDCC Halls B-H

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Program #/Poster #: 113.08/A8

Topic: A.01. Neurogenesis and Gliogenesis

Support: NSERC
NSERC PGS(D)
Killam Doctoral Award

Title: Adult neurogenesis and regulation of dentate gyrus neurons born in early postnatal development

Authors: *S. P. CAHILL[1], A. MARTINOVIC[2], T. CIRIC[2], J. COLE[2], D. R. SEIB[2], M. B. COOKE[2], J. S. SNYDER[2]
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Abstract: The dentate gyrus (DG) is a subregion of the hippocampus that is made up of two distinct populations: a formative population added in development and an additive population created in adulthood. We have previously shown that cells born in development show stable survival until young adulthood, where they undergo a delayed period of cell death, a pattern not seen in adult-born cells. It is possible that this cell death balances out the continued addition of

adult born neurons. Here, we looked at the relationship between these two populations in terms of activity patterns. We hypothesized that activity in older, developmentally-born neurons depends on the numbers of adult-born neurons that are present. To test this, we either inhibited or enhanced adult neurogenesis and quantified activity using immediate-early gene expression in developmentally-born cells after rats explored a novel environment. Rats were injected with the mitotic marker BrdU at the peak of DG development and were given one of two treatment types in adulthood (2 to 6 months of age). To inhibit adult neurogenesis, a transgenic rat model (GFAP-TK) was used. We found that inhibition of adult neurogenesis increased activity in the developmentally-born cells. To increase adult neurogenesis, rats received a combined treatment of running and memantine. Increasing adult neurogenesis led to compensatory decreases in activity of developmentally-born cells. These data suggest that adult-born neurons inhibit developmentally-born neurons, and there is a homeostatic regulation of activity levels in the DG. We are also examining whether rates of loss of developmentally-born neurons are proportional to adult neurogenesis rates across the lifespan. If there is a balance between these cell populations, we would predict that as adult neurogenesis is known to decline with age, we can see how this relationship changes as animal age progresses, as it is not known how the survival of the developmental population is effected by the age related changes in adult neurogenesis. To test this, we injected rats with the mitotic marker CldU at the peak of DG development and rats were given a second mitotic marker IdU in adulthood (@ either 1, 3, 5, 11, or 17 months) and cells were visualized 4 weeks later. Collectively, these data will clarify whether there are interactions between neurons born at different stages of development, which may shape how information is retained in the hippocampus.

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Poster

113. Nervous System Patterning and Developmental Cell Death

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Program #/Poster #: 113.09/A9

Topic: A.01. Neurogenesis and Gliogenesis

Support: 1R21NS101151-01

Title: Effects of Zika virus exposure in a model of murine prenatal development

Authors: *S. M. SHELTON[1], A. R. SOUCY[2], J. H. CONNOR[2], T. F. HAYDAR[3]
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Abstract: The rapid spread of Zika virus (ZIKV) and its association with abnormal brain development constitute a global health emergency. Congenital ZIKV infection produces a range of mild to severe pathologies, including microcephaly. These pathologies are specific to fetal brain tissue, where neural stem cells are targeted. To understand the pathophysiology of ZIKV infection, an *in vivo* mouse model of fetal brain development was used to recapitulate the human cytoarchitecture of early to mid-gestation. ZIKV PRVABC59, isolated from the blood of a patient in Puerto Rico in 2015, was used to characterize ZIKV infection of neural progenitors and resulting changes in mouse brain development. Multiple dose concentrations, inoculation periods, and time points in development have been investigated to characterize the development of congenital Zika syndrome. Gross brain size and cortical thickness have been measured to test what age and inoculation periods result in microcephaly. The targeted neural precursor types were identified at various stages of brain development using in utero electroporation and immunohistochemistry. Changes in neural precursor proliferation and cell death were quantified to determine how these cellular processes may contribute to ZIKV-induced microcephaly. Infection of embryos allowed for the study of developing mice and whether there was cross-transmission between infected fetuses and the maternal circulation. This model provides a platform for robust *in vivo* screens of potential pharmacotherapeutics designed to limit ZIKV transmission and to promote healthy development in infected fetuses. Together, ZIKV infections in mothers and in developing mice at varying doses, inoculations, and stage in development combined with cell type specific in utero electroporation techniques will further elucidate the etiology of congenital Zika syndrome and microcephaly in an *in vivo* model of mammalian development. Future work will include single cell RNA sequencing to identify changes in neural precursor populations over short and long ZIKV inoculation periods.

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Poster

113. Nervous System Patterning and Developmental Cell Death

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Topic: A.01. Neurogenesis and Gliogenesis

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National Natural Science Foundation of China 81720108018
National Natural Science Foundation of China 81741034

Title: Autism-related chromatin remodeler CHD8 controls apical to basal progenitor specification and survival during cortical neurogenesis

Authors: *C. DONG[1,2], Y. LIN[1], W. ZHOU[1], R. Q. LU[2]
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Abstract: The chromatin remodeler *CHD8* has been identified as an autism spectrum disorder (ASD) associated high risk gene, mutations in which will cause severe developmental delay or ASD. CHD8 is highly expressed in the developing brain. CHD8 is detected in the neural progenitor cells, neurons and oligodendrocyte lineage cells. However, its function in neurogenesis has not been fully defined. Here we demonstrate a critical role of CHD8 in controlling cortical progenitor cell development. Genetic ablation of CHD8 in cortical progenitors leads to a loss of basal progenitors, while the $PAX6^{+}$ apical progenitors was unaffected in the subventricular zone. This results in a decrease in cortical thickness and the subventricular zone in postnatal stage. In addition, the mutant mice exhibit cortical dyslamination, especially in upper layer neurons. Intriguingly, the apoptosis of basal progenitors in the early stage is correlated with p53 activation. Furthermore, knocking out p53 partially rescues the neurogenesis defect in the mutant mice. Together, our observations demonstrate CHD8 regulates the survival of basal neural progenitors and is a critical factor for apical progenitors to basal progenitor transition.

Disclosures: C. Dong: None. **Y. Lin:** None. **W. Zhou:** None. **R.Q. Lu:** None.

Poster

113. Nervous System Patterning and Developmental Cell Death

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Program #/Poster #: 113.11/DP01/A11

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant MH113257

Title: MacBrainResource: Archived macaque brains available for neuroanatomical and neurodevelopmental studies

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Abstract: Primate research is on the decline worldwide due to several factors, including the exorbitant cost of primate housing and care, mounting regulatory hurdles, and changing attitudes toward primate use for medical research. MacBrainResource is a newly established resource that will foster new primate brain research by making archived macaque brains housed in the Department of Neuroscience at Yale available to researchers across the globe for their own

research purposes. MacBrainResource is currently comprised of five collections of histologically prepared slides and EM blocks derived from macaque studies conducted in the laboratories of Dr. Pasko Rakic and the late Dr. Patricia Goldman-Rakic. These include Collection 1: tritiated thymidine injections, Collection 2: tract tracing injections, Collection 3: prenatal and postnatal lesions, Collection 4: prenatal irradiation, and Collection 5: EM blocks. Brain materials may be analyzed on-site by arranged visit or remotely via website access (http://macbrainresource.org). Here is an example of how to use MacBrainResource. Click on Collection 1 (the same principles apply to the other collections) on the MacBrainResource website home page to open a list of 112 tritiated thymidine injected cases, i.e., monkey brains. The cases, e.g. 101673A E30-P76, are designated by case identifier (101673A), followed by age of experimental treatment (embryonic day 30: E30) dash age of sacrifice (postnatal day 76: P76). Request desired slides for your study based on age of exposure to tritiated thymidine, age of sacrifice, brain region, etc. The requested slides will be scanned at 20x or 40x (if justified by special request) on the Aperio CS2 Scanner and uploaded to the MacBrainResource digital database. Remotely, slides will be available via eSlide Manager, a free application for high resolution viewing of scanned slides, yielding zoomable images that can be saved as jpeg files. MacBrainResource is designed to be a "living" resource that enables contemporary analysis of these archived primate brain materials. Studies utilizing this resource can further our understanding of neurodevelopment and neuroanatomy without the need to sacrifice a single additional valuable animal.

Disclosures: L.D. Selemon: None. **A. Duque:** None.

Poster

113. Nervous System Patterning and Developmental Cell Death

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 113.12/A12

Topic: A.01. Neurogenesis and Gliogenesis

Title: Rab5 GTPase activity and JNK is required for apoptotic signaling by the p75 receptor in response to BDNF

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Abstract: Introduction: During the development of the sympathetic nervous system, the p75 neurotrophin receptor (p75) triggers apoptosis upon binding to BDNF. Apoptotic signaling through p75 requires activation of the stress kinase, c-Jun N-terminal kinase (JNK), that increases the regulated receptor proteolysis in response to pro-apoptotic ligands. The cleavage facilitated ubiquitination of the DNA binding protein NRIF, a zinc finger protein that interacts

with the intracellular domain of p75, resulting in nuclear translocation of NRIF. Moreover, this leads a second wave of activation of JNK. All this is required for a long-term apoptotic signaling of p75.
Rab5 regulates the trafficking of receptors by regulating early endosomes dynamics.
Results of our lab have shown that p75 transiently interacts with Rab5 positive endosomes in sympathetic neurons and accumulates in other endocytic organelles.
Our aim is to clarify the role of Rab5 activity on apoptotic signaling of p75 and the relevance of JNK in retrograde apoptotic signaling of p75.
Material and Methods: Sympathetic neurons were dissected from the superior cervical ganglia (SCGs) of postnatal day one Sprague Dawley female and male rats. We over-expressed a constitutively active (Rab5CA) or dominant negative (Rab5DN) Rab5 mutants in 6 DIV SCGs and then we treated with BDNF. Them, we evaluated the interaction between p75 and Rab5, the internalization of p75, NRIF nuclear translocation and cleaved caspase-3. Moreover, we used compartmentalized culture of SCGs to evaluate JNK activation in axons. In addition, we used SP600125 (JNK inhibitor) to evaluate the role of JNK in the trafficking and apoptotic signaling of p75.
Results: Here, we report that the interaction between p75 and Rab5 is preferentially with the active form of Rab5. When a Rab5CA mutant was expressed, p75 was accumulated in Rab5 positive organelles and we observed an increased nuclear translocation of NRIF in response to BDNF. Nevertheless, inhibition of Rab5 activity reduced cleaved-caspase 3 as well as nuclear accumulation of NRIF. Moreover, we found that BDNF activated JNK in axons, and that JNK activity mediated internalization and retrograde axonal transport of p75. Inhibition of JNK activity by SP600125 decreases the internalization and retrograde axonal transport of p75.
Discussion: Our findings suggest that the apoptotic signaling of p75 requires the activity of Rab5 when the receptor is activated by BDNF. In addition, our results are evidence of a new role of JNK in the axonal trafficking of p75.

Disclosures: C.A. Cabeza: None. **C. Escudero:** None. **F. Bronfman:** None.

Poster

113. Nervous System Patterning and Developmental Cell Death

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 113.13/A13

Topic: A.01. Neurogenesis and Gliogenesis

Support: Nina Ireland endowment to the UCSF Department of Psychiatry
NIMH R37 MH049428
NIGMS R35 GM119831
Science without Borders Fellowship by CNPq (Brazil)

Title: Dlx transcription factors organize a core gene regulatory network required for basal ganglia development and interneuron specification

Authors: *R. CATTA-PRETA[1], S. LINDTNER[3], J. PRICE[3], A. VISEL[4], D. E. DICKEL[4], A. S. NORD[2], J. L. RUBENSTEIN[3]
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Abstract: Mammalian brain development is guided by a network of activating and repressive signaling pathways orchestrated by transcription factors (TFs). The systems that control early neurodevelopment are strongly conserved among vertebrates at the level of cis and trans signaling factors. Major advances in characterizing gene regulatory networks (GRNs) have been achieved via application of genomic assays to understand TF function. We have been working towards defining the mechanisms guiding lineage specification and patterning in the brain governed by the interaction between transcription factors (TFs), regulatory DNA elements (REs), and chromatin structure. Using a comprehensive approach, we characterized the regulatory wiring of early telencephalic GABAergic neuron development organized by Dlx family transcription factors, integrating in situ hybridization, transcriptomic, and epigenomic data from wild-type and Dlx1/2 knockout ganglionic eminences (embryonic mouse basal ganglia - BG). We show that binding of Dlx2, Dlx1, and Dlx5 at proximal and distal DNA regulatory elements is required for the establishment and/or maintenance of both ganglionic eminence-specific chromatin and transcriptional states, and regulation of gene expression patterns during stages in the specification of BG-derived projection neurons and interneurons (including cortical interneurons). Dlx factors bind extensively across the genome, however, a small subset of the Dlx binding sites show strong perturbations to their chromatin state and relevant gene expression in the Dlx1/2 knockout. These sensitive loci form the hub of a core GRN that is organized by Dlx and controls patterning and specification in the developing basal ganglia. Our analysis of Dlx TFs contributes to the growing understanding of how combinatorial binding of REs by master transcriptional regulators regulates specific region- and cell-type restricted expression patterns during early brain development. By integrating across genetic, genomic, and neuroanatomical approaches, we hope to decode the genomic wiring guiding cell type specification in the mammalian brain.

Disclosures: R. Catta-Preta: None. **S. Lindtner:** None. **J. Price:** None. **A. Visel:** None. **D.E. Dickel:** None. **A.S. Nord:** None. **J.L. Rubenstein:** None.

Poster

114. Transplantation and Regeneration (PNS) and Neuroglial Interactions

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 114.01/A14

Topic: A.04. Transplantation and Regeneration

Title: The immune adaptor sarm1 breaks down myelin sheaths after peripheral nerve injury

Authors: *D. GUO, C. PAN, S. ZHANG, Z. YING, X. WANG
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Abstract: Axon degeneration and subsequent demyelination are crucial events in the nerve regeneration after peripheral nerve injury. Upon peripheral nerve injury, the activated Schwann cells start to clear myelin debris along with the inhibitory factors for axon growth. Sterile alpha and HEAT/Armadillo motif (SARM) is a highly conserved Toll/interleukin-1 receptor (TIR)-containing adaptor protein that is believed to negatively regulate signaling of the pathogen recognition receptors TLR3 and TLR4. Previous study has provided evidences that initial axonal cytoskeletal breakdown of injured nerves are blocked in SARM mutants *Drosophila* and $Sarm^{-/-}$ mice. However, whether SARM functions in the process of myelin breakdown is unclear. In the recent work, we found that two dominant-negative forms of SARM proteins can slow myelin breakdown in *ex vivo* nerve culture, indicating SARM regulates myelin breakdown besides its axon function. Mice with myelin specific depletion of Sarm is under-generation to further confirm the myelin breakdown of SRAM in the future. Myelin breakdown is required for axon degeneration after nerve injury as well as the pathogenesis of demyelinating diseases. Our results will provide new approaches to the regulation of these biological processes.

Disclosures: D. Guo: None. **C. Pan:** None. **S. Zhang:** None. **Z. Ying:** None. **X. Wang:** None.

Poster

114. Transplantation and Regeneration (PNS) and Neuroglial Interactions

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 114.02/A15

Topic: A.04. Transplantation and Regeneration

Support: NS095017

Title: Molecular and cellular identification of the immune response in peripheral ganglia following nerve injury

Authors: J. P. NIEMI[1], J. A. LINDBORG[1], M. A. HOWARTH[2], K. W. LIU[1], D. MAHAJAN[1], C. Z. MOORE[1], *R. E. ZIGMOND[3]
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Abstract: Neuroinflammation accompanies neural trauma and most neurological diseases. Axotomy in the peripheral nervous system leads to dramatic changes in the injured neuron: the

cell body expresses a distinct set of genes known as regeneration-associated genes, the distal axonal segments degenerate and their debris is cleared, and the axons in the proximal segment form growth cones and extend neurites. These processes are orchestrated in part by immune and other non-neuronal cells. Macrophage accumulation in peripheral ganglia play an integral role in supporting regeneration. We have explored the molecular and cellular components of the injury-induced immune response within these ganglia. Adult male wild type (WT) and *Ccr2* -/- mice were subjected to a unilateral transection of the sciatic nerve and axotomy of the superior cervical ganglion (SCG). Antibody arrays were used to determine the expression of chemokines and cytokines in the dorsal root ganglia (DRG) and SCG. Flow cytometry and immunohistochemistry were utilized to identify the cellular composition of the injury-induced immune response within ganglia. Chemokine expression in the ganglia differed 48 h after nerve injury with a large increase in macrophage inflammatory protein-1γ in the SCG but not in the DRG, while CCL2 was highly expressed in both ganglia. Differences between WT and *Ccr2* -/- mice were also observed with increased CCL6/C10 expression in WT DRG compared to *Ccr2* -/- DRG and increased CXCL5 expression in *Ccr2*-/- SCG compared to WT. Diminished macrophage accumulation in the DRG and SCG of *Ccr2* -/- mice was found compared to WT ganglia 7 d after nerve injury. Interestingly, neutrophils were found in the SCG but not in the DRG. Cytokine expression, measured 7 d after injury, differed between ganglion type and genotype. Macrophage activation was assayed by colabeling ganglia with the anti-inflammatory marker CD206 and the macrophage marker CD68, and an almost complete colocalization of the two markers was found in both ganglia. Thus, both molecular and cellular differences in the nerve injury-induced immune response were found between DRG and SCG and between WT and *Ccr2* -/- mice. (Supported by NS095017).

Disclosures: J.P. Niemi: None. **J.A. Lindborg:** None. **M.A. Howarth:** None. **K.W. Liu:** None. **D. Mahajan:** None. **C.Z. Moore:** None. **R.E. Zigmond:** None.

Poster

114. Transplantation and Regeneration (PNS) and Neuroglial Interactions

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 114.03/A16

Topic: A.04. Transplantation and Regeneration

Support: NIH Grant K01NS105879-01
NIH Grant R01NS041596-11S1

Title: Can peripheral axotomy change the translational capacity of centrally projecting sensory axons?

Authors: *T. P. SMITH, J. L. TWISS
Biol. Sci., Univ. of South Carolina, Columbia, SC

Abstract: Axons of the PNS readily regenerate while those of the CNS do not because of differences in both intrinsic and extrinsic properties. Intra-axonal translation of mRNAs is a critical intrinsic property for regeneration in the PNS. Peripheral axotomy of DRG neurons induces retrograde signaling that increases regenerative capacity. Previous studies suggested that peripheral axotomy causes sprouting of centrally projecting dorsal root ganglia (DRG) axons (Woolf et al., 1992; Shortland and Woolf, 1993; Woolf et al., 1995). Here, we asked if peripheral axotomy changes localization and translation of mRNAs in the central axons of DRG neurons, which could contribute to sprouting in those axons. Since mRNAs localize in peripheral axons and intra-axonal translation of mRNAs is important for regeneration (e.g., GAP-43), we hypothesized that similar localization of growth associated mRNAs as well as a change in the translational capacity will occur in central DRG axons after peripheral axotomy. Using reverse transcriptase droplet digital PCR, we show that GAP-43, calcitonin gene related peptide (CGRP) and neuritin 1/candidate plasticity gene 15 (Nrn1/CPG15) mRNA levels are increased in the axoplasm of central axons of DRG neurons from adult rats 7 days after sciatic nerve crush compared to the naïve condition. Analysis of translational machinery showed alterations in levels in the centrally projecting axons 7 days following peripheral crush injury. Taken together, these data suggest that retrograde signaling responses following peripheral axotomy is propagated into the centrally projecting sensory axons, which could affect the growth capacity of these axons.

Disclosures: T.P. Smith: None. **J.L. Twiss:** None.

Poster

114. Transplantation and Regeneration (PNS) and Neuroglial Interactions

Location: SDCC Halls B-H

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Program #/Poster #: 114.04/A17

Topic: A.04. Transplantation and Regeneration

Support: National key research and development program Grant 2017YFA0104700
973 National Key Basic Research Program Grant 2014CB542203
National Natural Science Foundation of China Grant 31500927

Title: Comparative analysis of primary cultured fibroblasts between sensory and motor nerve

Authors: Q. HE, M. CONG, Y. SHEN, X. ZHOU, *F. DING
Nantong University, China, Jiangsu, China

Abstract: Recent study indicated that fibroblasts had pro-regenerative effects on Schwann cell behavior and neurite outgrowth. Whether fibroblasts express distinct sensory and motor phenotypes, which are associated with peripheral nerve development and regeneration, has not been fully addressed. In this study, we used whole rat genome microarray analysis and identified total of 121 genes differentially expressed between primary cultured motor and sensory

fibroblasts. The genes with higher level of expression in sensory fibroblasts were related to biological aspects including proliferation, migration, chemotaxis, motility activation, protein maturation, defense response, immune system, taxis, and regionalization, while the genes with higher level of expression in motor fibroblasts were related to biological aspects including neuron differentiation, segmentation, and pattern specification process. The significant difference in expression level of some key genes involved in proliferation and migration, including Abcc9, Cxcl10, Cxcl3, Syk, C5ar1, Pf4, and Ptgs1, was validated by quantitative real time PCR. The cell proliferation or migration analysis showed that the rate of cell proliferation or migration was higher in sensory fibroblasts than in motor fibroblasts. Besides, down-regulated chemokine (C-X-C motif) ligand 10 (CXCL10) and chemokine (C-X-C motif) ligand 3 (CXCL3) expression suppressed the proliferation rate of sensory fibroblasts, while enhanced the proliferation rate of motor fibroblasts. But the migration rate of sensory and motor fibroblasts were suppressed by down-regulated CXCL10 or CXCL3 expression. Our findings indicated that fibroblasts express distinct sensory and motor phenotypes that are associated with different gene expression and biological process. We believe this analysis may provide a basis for further study of the biological differences between sensory and motor fibroblasts, including the role in nerve regeneration.

Disclosures: Q. He: None. **M. Cong:** None. **Y. Shen:** None. **X. Zhou:** None. **F. Ding:** None.

Poster

114. Transplantation and Regeneration (PNS) and Neuroglial Interactions

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 114.05/A18

Topic: A.04. Transplantation and Regeneration

Support: The Health and Medical Research Fund (HMRF), Food and Health Bureau, Hong Kong Special Administrative Region Government (Ref. No.: 05160126)

Title: Basic helix-loop-helix protein is a crucial transcription regulator of axonal regeneration

Authors: *N. P. AU[1], G. KUMAR[1], S. K. CHIU[1], D. H. GESCHWIND[3], G. COPPOLA[3], C. H. E. MA[1,2]
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Abstract: Patients with proximal nerve injury such as brachial plexus nerve injury, motor functional recovery is largely limited even after surgical repair. One explanation for this phenomenon is that regrowth of injured peripheral axons is extremely slow (1-2mm/day) after peripheral nerve injury (PNI). Injured axons can regenerate over a long distance to reach their

target muscle; however, they fail to form functional synapses at motor end plate after prolonged denervation. Hence, it is necessary to develop new therapy to promote axonal regrowth, and the subsequent sensory and motor functional recovery in patients with PNI. Basic helix-loop-helix (bHLH) protein forms homodimers and binds to E-box motif CAGCTG of its target genes to regulate gene expression. bHLH protein is a key regulator of cell proliferation, migration and differentiation. In nervous system, bHLH protein is abundantly expressed in forebrain especially during developmental stages. Recent study demonstrated that an upstream regulator of bHLH protein could promote cell survival and axonal outgrowth of retinal ganglion cells after optic nerve injury. We thus hypothesized that bHLH could also promote axon regeneration resulting in early sensory and motor functional recovery after PNI. Here, we demonstrated that bHLH expression was essential for intrinsic growth of injured neurons after PNI. Marked up-regulation of bHLH was detected in 3 and 5 days after PNI. Knockdown of bHLH using target-specific short interfering RNA (siRNA) reduced neurite outgrowth from primary dorsal root ganglion (DRG) neurons, and abolished pre-conditioning effect of injured peripheral neurons. *In vivo* knockdown of bHLH resulted in delayed sensory and motor functional recovery following PNI. We then assessed the possible growth-promoting effects of bHLH overexpression using adeno-associated virus (AAV). Two weeks after injection, intrathecal injection of AAV8.2-bHLH successfully transduced over 60% of lumbar 4 and 5 DRGs that supply sciatic nerve directly. bHLH overexpression markedly enhanced the extent of axonal regrowth as assessed by nerve pinch test and regenerating axons quantification three days after sciatic nerve crush injury. Bioinformatics analysis to identify small molecules that activate signaling pathways associated with bHLH-induced growth-promoting effects will provide new insight for developing therapeutic approach to promote functional recovery in patients with PNI.

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Poster

114. Transplantation and Regeneration (PNS) and Neuroglial Interactions

Location: SDCC Halls B-H

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Program #/Poster #: 114.06/A19

Topic: A.04. Transplantation and Regeneration

Support: The General Research Fund grant from the Research Grant Council of the Hong Kong Special Administrative Region Government (Ref. No.: 11100417)

Title: Modulation of microtubule dynamics regulates axonal regeneration after peripheral nerve injury

Authors: *G. KUMAR, N. P. B. AU, X. WANG, C. H. E. MA
Dept. of Biomed. Sci., City Univ. of Hong Kong, Kowloon, Hong Kong

Abstract: Proximal peripheral nerve injury (PNI) requires injured neurons to regenerate its axons over a long-distance, and to re-establish connection with target sense organ and motor functional unit that could take months or even years. This results in limited functional recovery after proximal PNI due largely to the slow rate of axonal regrowth. Our previous study showed that injured axons must reach their target muscle within a 'critical period' of 35 days in adult mice for complete motor function recovery. Potential therapies aim to modulate and promote axonal regrowth can be made possible to improve functional recovery after PNI. Formin proteins which share highly conserved formin homology FH1 and FH2 domains, are abundantly expressed in both central and peripheral nervous system. Growing evidence suggests that FH1 and FH2 domains modulate microtubule stability and dynamic, which is one of the key events in regulating axonal regrowth. We first demonstrated that PNI induced downregulation of mRNA and protein expression level of formin significantly, while robust axon regeneration was observed after sciatic nerve crush injury. We therefore knock down formin protein expression in primary dorsal root ganglion (DRG) neurons by short interfering RNA (siRNA) silencing which promoted axonal regrowth and axon branching of primary DRG cultures. We further examined the *in vivo* promoting effect of formin protein knockdown on axonal regrowth and peripheral nerve regeneration. We knock down *formin* expression by *in vivo* siRNA silencing, and examined peripheral axonal regeneration by nerve pinch test. In line with our *in vitro* results, we showed that the distal extent of axonal regrowth was increased significantly 3 days after sciatic nerve crush. Neurobehavioral and electrophysiological studies on formin-siRNA-treated mice showed improved sensory and motor functional recovery. Ongoing experiments showed that similar results were obtained from formin completed knockout mice. An in-depth understanding of the molecular mechanisms underlying formin-mediated modulation of microtubule dynamics could lead to development of strategy to accelerate peripheral nerve regeneration.

Disclosures: G. Kumar: None. **N.P.B. Au:** None. **X. Wang:** None. **C.H.E. Ma:** None.

Poster

114. Transplantation and Regeneration (PNS) and Neuroglial Interactions

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 114.07/A20

Topic: A.04. Transplantation and Regeneration

Support: NIH Grant T32 GM007250
NIH Grant NS095017

Title: Investigating site-specific roles for macrophages after peripheral nerve injury using CCL2 conditional knockouts

Authors: *A. D. TALSMA[1], K. WANG[2,1], J. P. NIEMI[1], A. DEFRANCESCO[1], J. S. PACHTER[3], R. E. ZIGMOND[1]

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Abstract: Peripheral nerve injury stimulates a complex coordinated neuroimmune response that ultimately increases the regenerative capacity of injured neurons relative to uninjured counterparts; a phenomenon termed the conditioning lesion (CL) response. Recent work has shown that the innate immune system, and in particular macrophages, are necessary for the CL effect and can stimulate growth of CNS and PNS neurons. Production of the macrophage chemokine CCL2 is one of the early steps in the injury response, and CCL2 production by neurons in the dorsal root ganglia (DRG) and by Schwann cells distal to the injury serves to recruit macrophages to the DRG and distal nerve, respectively. However, the relative contribution of macrophages at each location to regeneration is still unknown. To address this question we used a floxed CCL2 allele with either Advillin-Cre, or P0-Cre to knock out CCL2 in sensory neurons or Schwann cells, respectively, and assessed degeneration and regeneration in a sciatic nerve injury paradigm. We show that the Advillin-Cre knock out of CCL2 is incomplete, implying that Advillin-Cre is not expressed in all DRG neurons or another cell type in the DRG expresses CCL2. Further, neither macrophage recruitment to the DRG nor *in vivo* regeneration is significantly impaired, suggesting that the CL response is intact. It may be that the remaining CCL2 is sufficient to preserve these effects, or another factor could compensate for the decrease in CCL2. We also show that loss of CCL2 in Schwann cells decreases macrophage recruitment in the distal nerve and decreases clearance of myelin after injury, but, importantly, does not impair regeneration *in vivo*, implying that the CL response in the cell bodies renders neurons insensitive to the inhibitory effects of myelin. Our results suggest distinct roles for macrophages in the DRG and the distal nerve, but a more complete knock down of CCL2 in DRGs is needed to demonstrate the role of macrophages recruited there. (Supported by grant NS095017)

Disclosures: A.D. Talsma: None. **K. Wang:** None. **J.P. Niemi:** None. **A. DeFrancesco:** None. **J.S. Pachter:** None. **R.E. Zigmond:** None.

Poster

114. Transplantation and Regeneration (PNS) and Neuroglial Interactions

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 114.08/A21

Topic: A.04. Transplantation and Regeneration

Support: VA Grant 1I01RX001471

Title: Bridging large peripheral nerve gaps with nerve lengthening

Authors: *H. M. HOWARTH[1], A. KADOOR[2], E. BLEVINS[2,3], R. SALEM[2], M. ESPARZA[2], S. SHAH[2,3,1]
[1]Bioengineering, [2]Orthopedic Surgery, UCSD, La Jolla, CA; [3]VA San Diego, La Jolla, CA

Abstract: INTRODUCTION: Functional recovery is often poor for individuals with peripheral nerve injuries, especially for more severe injuries or for delayed repairs. For small nerve gaps, an end-to-end repair is preferred, even under slight tension. Autologous or synthetic grafts/conduits are used to bridge larger gaps, to eliminate tension at the site of repair. However, because axons must grow into, through, and out of the graft to reach their targeted end organs, the efficiency of regeneration and functional recovery may be impaired. We propose the application of a novel device for gradual stretching of the proximal nerve stump towards the distal stump, to allow for an end-to-end repair. This strategy builds on data that tensile loading accelerates the growth of axons. METHODS: A 1cm gap was created in the sciatic nerve of Lewis rats. In the autograft (gold standard) group (n=8), the transected nerve segment was reversed and sutured to the proximal and distal stumps. Rats recovered for twelve weeks. In the experimental (device) group (n=8), the proximal stump was secured to the lengthening device, and the nerve was stretched 1 mm/day for two weeks using an extracorporeal actuator. The device was then removed, nerve ends trimmed, and an end-to-end repair was performed. Rats recovered for ten more weeks. Sciatic Functional Index (SFI) was used to assess functional recovery and immunolabeling to examine structure. RESULTS: In both groups, SFI improved over time after surgery, though not to pre-operative levels. While there was no significant difference in SFI between the device and autograft groups, there was significant improvement between two and eight weeks in the device group ($p < .0001$) but not the autograft group ($p < .2316$), suggesting improved recovery with nerve lengthening. Also, rats in the autograft group were more likely to display a contracture (Autograft: 7/8 vs. Device: 3/8 with contracture), consistent with significantly shorter paw length in autograft group vs. device. Axon counts were significantly higher ($p<0.05$) and more evenly distributed across the nerve cross-section in device vs. autograft. DISCUSSION: We conclude that nerve lengthening is a viable method for repairing large gaps in peripheral nerves. Nerve lengthening resulted in equal or better functional improvement compared to autografts, with reduced likelihood of contracture. This suggests more and/or more evenly distributed axons into tibial and peroneal branches of the sciatic nerve. This method has potential to be used to bridge large nerve gaps for which a graft is not viable.

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Poster

114. Transplantation and Regeneration (PNS) and Neuroglial Interactions

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 114.09/A22

Topic: A.04. Transplantation and Regeneration

Support: CIHR
Diabetis Canada
U of A DoM
UHF

Title: Inactivation of dual specificity phosphatases(DUSPs) attenuates axonal plasticity in adult sensory neurons

Authors: *A. CHANDRASEKHAR, D. W. ZOCHODNE
NMHI, Div. of Neurology, Fac. of Med., Univ. of Alberta, Edmonton, AB, Canada

Abstract: Diabetic polyneuropathy (DPN) is a neurodegenerative disorder that targets sensory neurons. In some neuropathy models, it has been reported that MAP kinases including DLK promote progression of axonal degeneration whereas Dual Specific Phosphatases (DUSPs) inactivate MAP kinases and may antagonize their actions. Microarray data showed that both DUSP1 and DUSP4, two DUSP variants, were upregulated in the dorsal root ganglia of mice with chronic (5 month) type 1 diabetes mellitus and neuropathy. Expression of both DUSP variants were confirmed in rat DRG neurons with or without axotomy. Interestingly, sciatic nerve axotomy was associated with a trend toward the reduction of both DUSP1 and DUSP4 mRNA and protein in rat DRGs. Confocal imaging suggested a decrease in both nuclear and cytoplasmic expression of DUSP after injury. In vitro knockdown of either DUSP1 or DUSP4 using siRNAs, in comparison to scrambled sequence siRNAs, decreased neurite outgrowth of adult sensory neurons, indicating an ongoing role for these proteins in baseline outgrowth and plasticity. Next, we analysed neurite outgrowth in primary sensory neuron cultures exposed to capsaicin, normally toxic to adult sensory neurons in higher doses, and studied whether DUSP1 knockdown influenced their ability to withstand capsaicin axonopathy. Knockdown of DUSP1 was associated with an apparent greater attenuation of neurite outgrowth at intermediate doses of capsaicin. DUSPs are intrinsic sensory neuron proteins that offer a constitutive role in supporting normal neuron plasticity and may protect neurons from axonopathy. DUSPs may be relevant to axon protection therapeutic strategies.

Disclosures: D.W. Zochodne: None.

Poster

114. Transplantation and Regeneration (PNS) and Neuroglial Interactions

Location: SDCC Halls B-H

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Topic: A.04. Transplantation and Regeneration

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NMSS-RG1507

Title: Histone deacetylase 3-dependent epigenetic regulation delimits myelin growth and functional regeneration

Authors: *X. LIU, L. ZHANG, X. HE, Q. LU
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Abstract: Reciprocal axo-glial communication is critical for establishing proper myelin thickness for saltatory nerve conduction, but how myelinating cells such as Schwann cells in the peripheral nervous system interpret a key axonal signal, neuregulin, to control myelin thickness has been unclear. Upon functional screening for small-molecule epigenetic modifiers, we identify a histone-modifying enzyme, Hdac3, as a potent inhibitor of Schwann cell maturation and an antagonist of neuregulin-PI3K/AKT signaling activity through a negative feedback inhibition loop. Pharmacological attenuation of Hdac3-mediated deacetylase activity markedly enhances myelin sheath growth and regeneration and improves functional recovery after peripheral nerve injury. Integrative genome-occupancy and transcriptome-profiling analyses revealed that Hdac3 not only represses the pro-myelinating program but also recruits p300 histone acetyltransferase to activate a myelination-inhibitory network. We identified a Hdac3/p300 target gene, encoding the HIPPO signaling effector Tead4, as a potent inhibitor of peripheral myelin sheath growth. Schwann cell-specific deletion of either Hdac3 or Tead4 results in a profound increase of myelin thickness in sciatic nerves, and remyelination is enhanced in Hdac3-deficient nerves after injury.
Moreover, in the central nerve system, ablation of *Hdac3* promotes remyelination in lysolecithin-induced demyelination lesion. HDAC3 inhibitor treatment not only promotes remyelination in cuprizone-feeding induced demyelinating mice, but also restores the motor function in the experimental autoimmune encephalomyelitis (EAE) demyelinating animal models. Thus, our findings identify an HDAC3-dependent pathway as a cell-intrinsic
inhibitory machinery that counters myelinogenic signals and maintains myelin homeostasis, highlighting a therapeutic potential of transient HDAC3 inhibition for improving myelin repair in both central and peripheral nervous systems.

Disclosures: X. Liu: None. **L. Zhang:** None. **X. He:** None. **Q. Lu:** None.

Poster

114. Transplantation and Regeneration (PNS) and Neuroglial Interactions

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 114.11/A24

Topic: A.04. Transplantation and Regeneration

Support: CNRS

Title: Effect of intensive motor training on accelerating axonal regeneration following peripheral nerve injury in rats

Authors: A. W. MADI[1], E. D. AL-CHAER[2], *N. LAWAND[3]
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Abstract: Peripheral nerve injury (PNI) results in a broad range of sensory and motor symptoms that depend on the severity and types of nerves involved. Many attempts to repair PNI have yielded poor outcomes and failed to attenuate the sensory and motor deficits. Since physical training has shown to promote the synthesis of nerve growth factors needed to facilitate axonal regeneration, we aim in the present study to examine whether intensive motor training improves the sensory and motor functions in rats with sciatic nerve compression. Adult male Sprague-Dawley rats that had their right sciatic nerve crushed were randomly divided into 4 groups and subjected, for a month, to different types of motor exercises (5 days/week). Rats in groups 1 and 2 were trained for 1 hour (two 30 min sessions separated by 10 min resting period). For group 1, rats were placed on a horizontal treadmill daily (8m/min), and those in group 2 were placed on the Rotarod (35 rpm; 8m/min). Rats in group 3 were trained using treadmill (30 min) followed by Rotarod (30 min). The training sessions were separated by a 10 min resting period. Group 4 served as control, in which rats were housed in standard cages for an equivalent period of time. To assess nerve regeneration, behavioral, histological and electrophysiological tests were performed. All rats were evaluated for sensory recovery, and hypersensitivity to thermal and mechanical stimuli at 1, 5, 12, 19 and 26 days post injury. Fine motor skills were also assessed using the staircase test. Counting the number of toes grips and the time taken to climb up and down the stairs were done before and at different time points post surgery. Whole mount immuno-florescence staining protocol was also used to examine the extent of regenerating axons using antibodies against neurofilament and myelin basic protein. Images of the stained nerves were then visualized using a laser scanning confocal microscope. Nerve conduction velocity (NCV) and the compound motor action potential (CMAP) were recorded twenty-six days after physical training to assess functional connections and recovery of functions in the compressed sciatic nerve. Our data have revealed that rats subjected to a combination of treadmill and Rotarod training showed significant recovery of sensory and motor functions

compared to other groups. The observed functional recovery highlights the role of intensive motor training in promoting axonal regeneration following injury.

Disclosures: A.W. Madi: None. **E.D. Al-Chaer:** None. **N. Lawand:** None.

Poster

114. Transplantation and Regeneration (PNS) and Neuroglial Interactions

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 114.12/A25

Topic: A.04. Transplantation and Regeneration

Support: Basic Science Research Programs through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2016R1D1A1B01014190).

Title: STAT3 phosphorylation by Cdk5 promotes axonal regeneration following sciatic nerve injury

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Abstract: Although Cyclin-dependent kinase 5 (Cdk5) is known to be involved in diverse neural functions, its role in nerve repair is not known well. Here, we explore functional involvement of Cdk5 activity in axonal regeneration after sciatic nerve injury in rats. Cdc5 protein levels were induced in the regenerating axons following nerve injury. Levels of p35, an activator of Cdk5, were decreased, but its proteolytically cleaved form p25 was upregulated in the injured nerve. We further find that the production of signal transducer and activator of transcription 3 (STAT3) was elevated in the regenerating nerve area, and its phosphorylated form at Ser727 (p727-STAT3) was increased in the injured nerve as well. Immunoprecipitation analysis revealed binding interaction between Cdk5 and STAT3. In vivo administration of Cdk5 inhibitor roscovitin abolished the production of p727-STAT3 in the injured nerve. However, treatment of MEK1 inhibitor U0126 did not alter Cdk5 phosphorylation, suggesting that STAT3 is phosphorylated by Cdk5 activity in the injured nerve. In animals administered with roscovitin, retarded axonal elongation was observed after nerve injury, and attenuated neurite outgrowth was seen in cultured DRG neurons. While cotransfection of the plasmid constructs overexpressing Cdk5 and p35 in DRG neurons increased the phosphorylation of STAT3 and neurite outgrowth, transfection of dominant negative forms of Cdk5 or non-phosphorylatable mutant STAT3 at Ser727 (S727A) also resulted in decreased neurite outgrowth, suggesting that Cdk5 phosphorylation of STAT3 may have growth-promoting effects on neurite elongation. Fractionation analysis of protein extracts and confocal image analysis for injured nerves revealed that both STAT3 and p727-STAT3 were heavily localized in the mitochondria of growth cone-

like structures in regenerating axons. We also identified that STAT3 in the injured nerve has a binding property with stathmin 1. Collectively, our data present new evidence that STAT3 and its phosphorylation by Cdk5 may be dynamically regulated in the injured nerve and play a role in promoting axonal regeneration.

Disclosures: J. Hwang: None. **U. Namgung:** None.

Poster

114. Transplantation and Regeneration (PNS) and Neuroglial Interactions

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 114.13/A26

Topic: A.04. Transplantation and Regeneration

Support: RO1NS085272
UL1TR000090
R01NS042617

Title: Neurotrophic skin stroma reprograming using tissue nanotransfection (TNT) rescues diabetic peripheral neuropathy

Authors: *S. KHANNA, A. CLARK, N. HIGUITA-CASTRO, D. GALLEGO-PEREZ, C. K. SEN
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Abstract: TNT is a non-viral nanotechnology-based platform that may deliver ABM (Ascl1, Brn2, and Myt1l) and induce neurogenic reprograming of murine skin in vivo (*Nat Nano* 2018). Induced neural cells (iN) develop in the skin and acquire functional electrophysiological properties. In this work we report that TNT-ABM, in addition to reprograming skin cells to iN, reprograms the skin stroma such that the resulting neurotrophic environment support development of iN in the skin. Neurotrophic stromal environment of the skin following TNT-ABM may be utilized to rescue pre-existing cutaneous nerve fibers in a setting of diabetes. The objective of this work was to apply TNT towards rescuing cutaneous nerve fibers from diabetic peripheral neuropathy (DPN). Although the pathogenic mechanism underlying DPN are not fully understood, it is known that bolstering the neurotrophins at the site of the target tissue, i.e. the skin is able to protect adult peripheral nerve fibers from the grasp of progressive DPN. In DPN, neurotrophin signaling mechanisms become dysfunctional, particularly in regard to nerve growth factor (NGF). Increasing the level of NGF within the skin has the potential to create a more neurotrophic environment that sustains nerve fiber density throughout the progression of DPN. Nanoelectroporation mediated transfection of mouse embryonic fibroblasts (MEF) with ABM caused reprogramming into induced neurons and increases NGF expression *in vitro*. TNT- ABM

converted skin cells in C57Bl/6 mice into induced neurons. Along with this cell conversion, we observed significant increase in NGF expression in ABM transfected skin in both C57Bl6 and genetically diabetic (db/db) mice. Following, TNT-ABM of the hindlimb of db/db mice, intraepidermal never fiber density (PGP9.5 positive nerve fibers), was significantly higher indicating a possible rescue from diabetes-dependent loss of fiber density. In these mice, TMT-ABM induced keratinocyte NGF. In summary, the current state of evidence points towards the possibility that topical TNT-ABM, a 0.1 sec procedure, of skin may induce a neurotrophic response that can be leveraged to rescue peripheral diabetic neuropathy.

Disclosures: S. Khanna: None. **A. Clark:** None. **N. Higuita-Castro:** None. **D. Gallego-Perez:** None. **C.K. Sen:** None.

Poster

114. Transplantation and Regeneration (PNS) and Neuroglial Interactions

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 114.14/A27

Topic: A.04. Transplantation and Regeneration

Support: Lenfest Foundation
Rubenstein Foundation

Title: Sensory axons inhibit motor axon regeneration *in vitro*

Authors: *T. M. BRUSHART, F. NAEF, R. SKOLASKY, Z. LI, R. WOLINSKY
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Abstract: The relationships between regenerating axons and the glia and substrates they contact have been examined in detail. The interaction of different classes of regenerating post-natal axons with one another, in contrast, are less well known. We now present an organotypic model of post-natal nerve regeneration in which sensory and motor axons are color-coded with fluorescent proteins so their interactions can be observed in real time. Spinal cord slices (P5) in which motoneurons express YFP (thy1-YFP-16) and DRG explants from mice (2-4 weeks old) expressing RFP (ROSA mT/mG) were used to populate cultured mouse femoral nerves (P5-P7) with either green motor axons alone (M) or both green motor and red sensory axons (SM). Sensory axons were fed retrograde into the femoral sensory branch, and motor axons into the femoral muscle branch. One week later the femoral nerve trunk, where sensory and motor axons intermingle, was transected and axons were grown out on unstructured collagen/laminin mats. Additionally, the regeneration environment was modified by crushing the femoral sensory branch to mechanically delay sensory outgrowth, or by treating the cultures with antibodies to NCAM or L1 to reduce axonal adhesion. After 5 days, cultures were imaged in *vitro* and axons were counted at each 0.25 mm increment from the cut nerve end. Motor axons regenerated

significantly farther and in greater numbers when they were not accompanied by sensory axons. Mean motor axon counts were: 0.25mm, SM=27, M=77, p=0.0; at 0.5mm, SM=10, M=70, p=0.0; at 0.75mm, SM=4, M=51, p=0.0002; at 1mm, SM=1, M=29, p=0.0016. Additionally, in the SM group a mean of 81% of total motor axon length was in direct contact with sensory axons. Motor axon growth was restored to normal in SM cultures by delaying sensory axon regeneration to eliminate contact between motor growth cones and sensory axons. Antibodies to L1 and NCAM equalized the length of sensory and motor axons by reducing sensory axon outgrowth. These experiments quantify the interaction of two distinct populations of regenerating post-natal axons. They reveal that sensory axons extend more rapidly than motor axons, and thus precede them during the early stages of regeneration. Furthermore, motor axons adhere to sensory axons throughout most of their length. As a result of this previously unappreciated interaction, sensory axons inhibit motor axon growth. Delay of sensory regeneration results in normal motor axon growth, while blocking function of the adhesion molecules NCAM and L1 to reduce axonal interactions retards sensory axon outgrowth but does not enhance motor regeneration.

Disclosures: T.M. Brushart: None. **F. Naef:** None. **R. Skolasky:** None. **Z. Li:** None. **R. Wolinsky:** None.

Poster

114. Transplantation and Regeneration (PNS) and Neuroglial Interactions

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 114.15/A28

Topic: A.04. Transplantation and Regeneration

Support: Volkswagen Experiment! Grant Az 91 437
Onassis Foundation

Title: Reawakening the growth potential of adult neurons by axonal mechanostimulation

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Abstract: During development extensive axonal growth continues for neurons following target innervation as the body continues to grow in length. In addition to this mechano-stimulated growth, mechanical properties of the surrounding environment play an integral role in developmental neuronal growth guidance. However, it remains unknown if mechanical stimulation plays a role in the axonal growth potential of adult injured neurons. Adult neurons of the peripheral nervous system (PNS) are able to regenerate following injury rather adequately

restoring function however their adult central nervous system (CNS) counterparts lack this ability. A prime model of this juxtaposition is the dorsal root ganglion (DRG) whose regenerative PNS branch differs in growth capacity from its non-regenerative CNS branch after axonal injury. The repetitive movement of the musculoskeletal system during exercise, such as extending and bending the knee in walking or running, applies cyclic mechanical tension upon peripheral nerves. Often attributed exclusively to neurotrophin release the biomechanics of such an event in the regenerative process has been primarily overlooked. Although the spinal column does flex, it remains far more rigid and lacking of repetitive stretch on enclosed neural structures. It has been discovered that cultured adult DRG neurons can surprisingly extend their neurites up to several millimeters in length in response to cyclic mechanostimulation.
Here, we aim to understand if cyclic mechanostimulation of either the PNS branch or the CNS branch of the DRG in an explant model leads to increased axonal growth potential *in vitro*. Moreover, we ask if the extent of stretch of the nerve (5-20%) leads towards growth potential differences in various DRG neuronal populations. For this we designed and built a bioreactor suitable for oscillatory stretch of explants from lumbar rat DRG (L4-L5) attached to either their central or peripheral (sciatic) nerve branches. We observed that cyclic mechanostimulation of the PNS and CNS DRG branches leads to enhanced neurite outgrowth (PNS: control 257.9±104.5um, 10% stretch 445.5±186.4um; CNS: control 244.4±94.0um, 10% stretch 466.2±101.08, unpaired t-test $P<0.05$). In addition, the extent of stretch appears to have an influence in the response of given neuronal populations ($TrkB^+$, $TrkC^+$ and $CGRP^+$). We show here that mechanostimulation may play a role in CNS axonal growth and we believe that deciphering the molecular mechanisms underlying this will allow us to pharmacologically mimic the relevant events *in vivo* and therapeutically reawaken the CNS regenerative potential to overcome traumatic axonal injury.

Disclosures: V. Kampanis: None. **L. Zhou:** None. **W. Roth:** None. **R. Puttagunta:** None.

Poster

114. Transplantation and Regeneration (PNS) and Neuroglial Interactions

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 114.16/A29

Topic: A.04. Transplantation and Regeneration

Support: Graduate Research and Creative Activity award, University of Nebraska at Omaha

Title: Developmental differences in immune response to gustatory nerve loss

Authors: *J. D. OMELIAN, S. I. SOLLARS
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Abstract: Transection of the chorda tympani nerve (CTX) results in well-characterized changes to the taste structures of the anterior tongue, and these outcomes vary across development. Following chorda tympani loss in adulthood, fungiform taste buds degenerate within 2 days and subsequently return following re-innervation by the chorda within several weeks. Conversely, CTX early in life (e.g., neonatal or juvenile ages) results in more extreme losses which are accompanied by permanent nerve degeneration. One proposed explanation for the developmental difference in chorda nerve regeneration is age-related differences in immune response to injury. Neutrophils have been shown to be upregulated following CTX in aged animals compared to adults, a group which also exhibits comparatively poor nerve regeneration and taste bud recovery. To examine the innate immune system across early development, we quantified the neutrophil response in female, Sprague-Dawely rats after CTX at neonatal (P10), juvenile (P25) or adult (P65) ages and counted myeloperoxidase positive leukocytes in the anterior tongue at 12, 24 or 48 hours after surgery. At no point did the average number of neutrophils significantly differ between the cut and intact sides of the tongue following unilateral CTX, regardless of surgical age. Comparisons were also made between the denervated side of the tongue in CTX animals and an equivalent area in age-matched, non-surgical animals. CTX at P10 or P65 led to neutrophil counts that were consistently elevated above controls at each post-surgical time point. Juvenile animals had a significantly increased immune response at 24 hours post-surgery. In total, these results suggest that the innate immune system responds to gustatory denervation across all ages of development. As an additional comparison, we evaluated the number of neutrophils on the CTX side of the tongue at each age, across the various post-surgical times. When compared within the denervated condition, a developmentally-mediated pattern of effects emerged such that the immune response increased more quickly after injury, the earlier the surgical age. By the end of the experimental window, the response in the younger animals had peaked and diminished but the adult immune response was comparatively elevated. This time course of suggests that younger animals undergo a greater immune response earlier, but that the adult response may be prolonged by comparison.

Disclosures: J.D. Omelian: None. **S.I. Sollars:** None.

Poster

114. Transplantation and Regeneration (PNS) and Neuroglial Interactions

Location: SDCC Halls B-H

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Program #/Poster #: 114.17/A30

Topic: A.04. Transplantation and Regeneration

Support: NIH Grant DE022000
NIH Grant NS099603

Title: mTORC1 activation enhances regenerative growth of nociceptive sensory neurons by altering their pro-regenerative gene expression landscape

Authors: *D. CARLIN[1], A. HALEVI[2], E. E. EWAN[1], A. MOORE[2], V. CAVALLI[1]
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Abstract: Axons in the peripheral nervous system regenerate relatively well after a traumatic injury, but poorly in the central nervous system. Mechanistic Target of Rapamycin Complex 1 (mTORC1) activation, through deletion of negative regulators Tuberous Sclerosis Complex (Tsc) or Phosphatase and tensin homolog (Pten), enhances regenerative axon growth in both peripheral and central neurons. However, the mechanism by which mTORC1 enhances axon growth in peripheral neurons is poorly understood, as are the types of sensory neurons that respond to increased mTORC1 activation. We have previously shown that Tsc2 deletion activates mTORC1 in all dorsal root ganglia (DRG) neurons, enhancing regeneration following sciatic nerve crush. We now extend those findings to show that Tsc2 deletion facilitates enhanced regenerative growth of nociceptors both in vivo and in vitro after sciatic nerve crush. Using fluorescence-associated cell sorting (FACS) of Nav1.8-positive DRG neurons followed by RNA-seq analysis, we identified several canonical regeneration-associated genes (RAGs) upregulated in uninjured neurons lacking Tsc2, suggesting that these neurons are in a primed state for axon growth. Interestingly, RAG expression levels were similar in injured control and Tsc2-deleted neurons. Consistent with this finding, axon growth rate after a conditioning lesion was similar in control and Tsc2-deleted neurons. Together these data suggest that Tsc2 deletion and mTORC1 activation in DRG neurons enhances initiation of regenerative axon growth. Current experiments are aimed at analyzing the rate of functional recovery of several sensory modalities to determine if constitutive mTORC1 activation confers a long-term growth advantage to regenerating axons after a peripheral nerve injury. We are also characterizing the effect of mTORC1 activation on regenerative axon growth following dorsal root crush, an injury that does not result in profound activation of RAGs such as observed after sciatic nerve injury. These studies show that Tsc2 deletion primes sensory neurons for axon growth in a manner similar to the case of a prior injury or conditioning lesion. Together these data expand our understanding of mTORC1 role in enhancing axon regeneration of peripheral sensory neurons.

Disclosures: D. Carlin: None. **A. Halevi:** None. **E.E. Ewan:** None. **A. Moore:** None. **V. Cavalli:** None.

Poster

114. Transplantation and Regeneration (PNS) and Neuroglial Interactions

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 114.18/A31

Topic: A.04. Transplantation and Regeneration

Support: R01DC014217-04

Title: Sculpting neural stem cell identity with the ubiquitin-proteasome system

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Abstract: The sensory neurons (OSNs) of the olfactory epithelium (OE) are vulnerable to injury because of their direct contact with the external environment. To maintain olfactory function, stem cells in the OE give birth to new OSNs throughout life, making the OE a powerful tool to study the mechanisms underlying neural regeneration. Two stem cell populations drive neurogenesis via a kind of dichotomous homeostasis; while the globose basal cells (GBCs) maintain the tissue when replacement of only neurons is needed, the otherwise dormant horizontal basal cells (HBCs) activate to multipotency and participate in epithelial regeneration after severe injury. Nonetheless, olfactory dysfunction is rife among the aged, with consequences for diminished quality of life, compromised nutrition, and endangered safety. The transcription factor (TF) ΔNp63 is the molecular switch that mediates the transition of HBCs from dormancy to active multipotency. A decrease in ΔNp63 protein level following injury is both necessary and sufficient to accomplish HBC activation. Consequently, we tested the hypothesis that the ubiquitin-proteasome system (UPS) mediates the transition from dormancy to activation. Treatment of *ex vivo* and primary *in vitro* HBCs with bortezomib (a proteosomal inhibitor) led to the enrichment of ΔNp63 protein via FACS and immunoblot analysis, respectively. When primary *in vitro* HBCs are treated with cycloheximide (a protein synthesis inhibitor) and bortezomib (a proteasomal inhibitor), ΔNp63 levels remain unchanged.

Disclosures: C. Barrios Camacho: None. **J.N. Peterson:** None. **J.E. Schwob:** None.

Poster

114. Transplantation and Regeneration (PNS) and Neuroglial Interactions

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 114.19/A32

Topic: B.11. Glial Mechanisms

Support: R01 NS097590

Title: LRP1 expression is decreased in a model of Schwann cell differentiation *in vitro* and in Schwann cell myelination during development

Authors: *K. FICHTER[1], C. BRIFAULT[2], S. L. GONIAS[3], W. M. CAMPANA[4]
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Abstract: The process of myelination in rats begins at birth and continues through the first 1-2 weeks of development. Myelinating Schwann cells (SCs) insulate large-diameter axons of the peripheral nervous system to promote salutatory conduction and to maintain the integrity of the axon. Following nerve injury in adults, myelinating SCs undergo de-differentiation to support axonal regeneration. Transcription of genes that produce myelin are down-regulated, and in turn, the Schwann Cell Repair Program is activated.
We have demonstrated that in response to peripheral nerve injury, SCs express greatly increased levels of the endocytic and cell-signaling receptor LDL receptor related protein-1 (LRP1). In conjunction with its co-receptor, the *N*-methyl-d-aspartic acid receptor (NMDA-R), which also is expressed by SCs, LRP1 mediates many of the changes in SCs that are necessary in the response to PNS injury by inducing robust c-Jun and ERK1/2 phosphorylation.
To study this transition in LRP1 expression in greater detail, we applied a model of SC differentiation *in vitro*. Rat SCs were induced to differentiate by culturing in the presence of ascorbic acid, NRG-1, forskolin, and L-glutamine for 48 hours. Differentiation was confirmed by increased expression of the mRNA for myelin basic protein, by increased P0 protein expression, and by decreased expression of p75.
Importantly, LRP1 expression in differentiated SCs was significantly down-regulated, consistent with our observations *in vivo*. As expected by changes in LRP1 expression, treatment with LRP1 ligands did not activate cell signaling, as illustrated by the absence of ERK1/2 phosphorylation.
During rat development, LRP1 expression appears to be inversely associated with development of the myelinating phenotype. We collected sciatic nerves from uninjured, developing rats on postnatal days 0, 7 and 29. LRP1 was maximally expressed at D0, and significantly decreased by day 7 (**P<0.01), a time when nerves are undergoing myelination. Further, LRP1 levels remained low at postnatal day 29 (*P<0.05). Collectively, these results suggest a model in which LRP1 is mainly expressed by SCs that are de-differentiated and specifically when LRP1 is necessary as a component of the Schwann Cell Repair Program.

Disclosures: K. Fichter: None. **C. Brifault:** None. **S.L. Gonias:** None. **W.M. Campana:** None.

Poster

114. Transplantation and Regeneration (PNS) and Neuroglial Interactions

Location: SDCC Halls B-H

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Program #/Poster #: 114.20/A33

Topic: B.11. Glial Mechanisms

Support: AUFF Grant AUFF-E-015-FLS-8-4

NNF Grant 14OC0011633
DM Grant 24858

Title: Schwann cell p75 neurotrophic receptor and type 2 diabetic neuropathy

Authors: *N. P. GONÇALVES[1,2,3], M. RICHNER[1,2], S. S. MURRAY[4], T. S. JENSEN[3], C. B. VAEGTER[1,2,3]
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Abstract: The most common cause of peripheral neuropathy in the United States and Europe is type 2 diabetes, affecting 30-50% of diabetic patients. The vast majority of clinical and basic research in diabetic neuropathy (DN) has focused on the neuronal component of the nerves, presumably from the perspective that the neurons are the functional signal transmitting cells. However, substantial amount of data categorically define Schwann cells (SCs) as indispensable components when it comes to maintaining neuronal structure and function. With this project, it is our main goal to expand the concept of Schwannopathy as an integral factor in the pathogenesis of type 2 DN, and evaluate how disruption of the interactions between SCs and axons contribute to the disease progression. By using a cell viability assay, our data indicate that high levels of glucose in the media induce primary wild-type (WT) SC death, possibly in a mechanism dependent on p75NTR activation since SCs lacking this receptor seemed to be significantly resistant to apoptosis when cultured in hyperglycemic conditions. Neuron-SC communication in conditions resembling diabetes *in vitro* was evaluated with the myelination assay. Briefly, SCs were seeded on top of purified and differentiated sensory neurons and allowed to expand for a week. After ascorbic acid stimulation, both under euglycemic and hyperglycemic conditions, myelination was assessed by confocal microscopy. Results highlight a compromised ability of WT SCs to myelinate axons when exposed to a hyperglycemic environment, which was even intensified in co-cultures with SCs lacking p75NTR. Due to the results *in vitro*, the role of SC p75NTR on type 2 DN was analyzed *in vivo* by crossing an MPZ Cre with a p75NTR fl/fl. About 30% reduction in both p75NTR protein and mRNA was observed when analyzing the whole sciatic nerve, indicating p75NTR expression by other cell types present in the nerve. Type 2 diabetes was induced in these mice, together with WT littermates, with a high fat diet (HFD). 24 weeks after, WT mice fasting blood glucose levels were increased in animals feed with the HFD. Surprisingly, in SC-p75NTR KO mice, fasting blood glucose levels were equally high in animals feed with either HFD or control diet. This suggests that peri-islets SCs must be targeted in this model and that p75NTR expressed in these cells may have a role for glucose metabolism. DN was confirmed by mechanical allodynia and decreased sensory and motor conduction velocities equally in WT or SC-p75NTR KO. We now will investigate pre-clinical signaling alterations in the peripheral nerve by RNA sequencing to disclose genetic regulation depending on p75NTR signaling and its modulatory role in DN.

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Poster

114. Transplantation and Regeneration (PNS) and Neuroglial Interactions

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 114.21/A34

Topic: B.11. Glial Mechanisms

Support: NIG Grant GM103554

Title: The expression and function of Kir4.1 in Schwann cells

Authors: *D. HEREDIA[1], K. NENNECKER[2], K. MOROSE[3], A. AGARWAL[3], T. W. GOULD[3]
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Abstract: Terminal/Perisynaptic Schwann cells (TPSCs) are a perisynaptic glial cell at the neuromuscular junction (NMJ) that regulate synaptic function, maintenance and regeneration after peripheral nerve injury. In a transcriptomic screen, the *KCNJ10* gene encoding the inwardly-rectifying potassium (K^+) channel Kir4.1 was upregulated in embryonic muscle with vs. without Schwann cells. During embryonic and early postnatal development, the expression of Kir4.1 was observed in both axonal Schwann cells (ASCs) along peripheral nerves as well as in TPSCs at the NMJ. At older ages, Kir4.1 expression was lost by ASCs but maintained by TPSCs. The expression of Kir4.1 in TPSCs was lost in *Wnt1-Cre*, conditional *KCNJ10* mutants which target neural crest derivatives including Schwann cells. *Wnt1-Cre*, conditional *KCNJ10* mutants survived but developed a tremor and exhibited muscle weakness. Ongoing genetic studies are determining whether this phenotype is caused by the selective absence of Kir4.1 in TPSCs, as well as whether the formation, function and maintenance of neuromuscular synapses are affected in *Wnt1-Cre*, conditional *KCNJ10* mutants. These results suggest that the decline of muscle strength observed in patients with seizures, sensorineural deafness, ataxia, mental retardation, and electrolyte imbalance (SeSAME/EAST syndrome), caused by congenital loss-of-function mutations in *KCNJ10*, may be caused by peripheral effects. Moreover, these studies highlight the importance of TPSCs in regulating neuromuscular function.

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Poster

114. Transplantation and Regeneration (PNS) and Neuroglial Interactions

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Program #/Poster #: 114.22/B1

Topic: B.11. Glial Mechanisms

Support: Neurosurgery Department at Brown University
Recruitment package Brown University and Rhode Island Hospital

Title: ErbB3 receptor tyrosine kinase is a mechanosensor in Schwann cells and modulates YAP-dependent transcription

Authors: *M. MARTINEZ MORENO, N. TAPINOS
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Abstract: Schwann cells are the glial cell of the peripheral nervous system. They wrap around axons and produce the myelin sheath, a specialized layer of lipids and proteins that allows the propagation of axon potentials through salutatory conduction. Recently, the activation of YAP by mechanical stimuli in Schwann cells and its role in the regulation of myelin length have been described. However, the molecular mechanisms that control YAP transcription and the mechanosensor responsible for the activation of YAP in Schwann cells are not known. Here we show that mechanical stretching of primary Schwann cells cultured in the absence of growth factors, ECM or serum induces tyrosine phosphorylation of ErbB3 receptor independent of heterodimerization with ErbB2. Phospho-proteomic analysis following mechanical stretching of Schwann cells reveals an ErbB3-dependent intracellular signaling cascade that leads to activation and nuclear translocation of YAP. PLA using phospho-ErbB3 and YAP antibodies revealed protein-protein interaction in the nucleus of Schwann cells. Finally, YAP ChIP-sequencing of mechanically stretched Schwann cells with and without ErbB3 knockdown, identified direct transcriptional targets of YAP that depend on mechanical activation of ErbB3 receptor. In conclusion, we identified an unknown role of ErbB3 receptor tyrosine kinase as mechanosensor that modulates YAP-dependent transcription in Schwann cells.

Disclosures: M. Martinez Moreno: None. **N. Tapinos:** None.

Poster

114. Transplantation and Regeneration (PNS) and Neuroglial Interactions

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 114.23/B2

Topic: B.11. Glial Mechanisms

Support: MEXT-Supported Program for the Strategic Research Foundation at Private Universities, 2015-2019

Title: Translational readthrough isoform, L-MPZ, exhibits unique localization pattern and functional aspects in the PNS myelin which differ from canonical myelin protein zero (P0/MPZ)

Authors: *Y. YAMAGUCHI, Y. OTANI,, J. CUI, H. BABA
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Abstract: Translational stop codon readthrough expands the potential of gene function to produce an isoform extended extra-functional domain at the C-terminus, which had been observed particularly in lower organisms. Recently, several groups have reported readthrough isoforms in physiological status of mammals including humans. Our identified large myelin protein zero (L-MPZ) is the first reported molecule among common mammalian proteins produced by stop codon readthrough of myelin protein zero (P0/MPZ) in the PNS. VEGF-Ax and AQP4ex are other readthrough isoforms of vascular endothelial growth factor A (VEGF-A) and aquaporin-4 (AQP4) gene, respectively. Thus, the translational readthrough is now gaining popularity in higher animals. Although VEGF-Ax and AQP4ex are demonstrated to modulate canonical gene function, the detailed distribution and the physiological role of L-MPZ in the PNS myelin is still unknown. In this study, to clarify these issues, we analyzed the localization of L-MPZ in developmental and adult mouse sciatic nerves (ScNs), and generated a mouse line (L-MPZ mouse) synthesized only L-MPZ by using CRISPR-Cas9 system. In the immunohistological analysis of ScNs prepared from postnatal day (P) 0-21 and adult ICR mice, L-MPZ-positive signals were colocalized with myelin basic protein (MBP) in compact myelin. These signals were dramatically increased during P0-10. In addition to the detection with MBP in compact myelin where P0/MPZ is mainly distributed, L-MPZ signals were enriched in Schmidt-Lanterman incisures (SLI) and paranodal regions at adult age. In the analyses of motor functions, homozygote of adult L-MPZ mouse exhibits motor impairment in rotarod and tail suspension tests, weaker grip strength, and abnormal conduction velocity. In the immunohistological analyses, L-MPZ mouse demonstrates aberrant myelin structure and shorter internode. While the primary structure of L-MPZ is identical with P0/MPZ except C-terminal L-MPZ-specific domain, P0/MPZ can not be replaced by L-MPZ. These results suggest that the role of L-MPZ is different from P0/MPZ in the formation and maintenance of the PNS myelin.

Disclosures: Y. Yamaguchi: None. **Y. Otani,:** None. **J. Cui:** None. **H. Baba:** None.

Poster

114. Transplantation and Regeneration (PNS) and Neuroglial Interactions

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 114.24/B3

Topic: B.11. Glial Mechanisms

Support: National Research Foundation funded by the Ministry of Education, Science and Technology (NRF-2016R1D1A1B01015042)

Title: Characterization of store-operated calcium entry in autonomic neuron-satellite glia unit

Authors: *S. KIM, S. KANG, S.-W. JEONG
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Abstract: Satellite glial cells (SGCs) ensheath the cell bodies of neurons within the autonomic ganglia. The unique anatomical arrangement suggests some signal exchanges between the autonomic neurons and SGCs. To date, however, little is known about the functional roles of the autonomic neuron-SGC units. In this study, we investigated the molecular mechanisms underlying calcium homeostasis in SGCs in responding to the various extracellular stimuli. In this regard, we isolated sympathetic superior cervical ganglion (SCG) neurons which are attached with SGCs by a partial enzymatic digestion, and then performed calcium imaging with Fura-2/AM. Both neurons and SGCs exhibited a store-operated Ca^{2+} entry (SOCE) when the internal storage of Ca^{2+} was depleted by cyclopiazonic acid (CPA) along with the removal of extracellular calcium. This depletion was further recovered rapidly on restoration of extracellular calcium via SOCE. The magnitude of SOCE was much larger in the SGCs than in the neurons. Unlike the neurons, interestingly, the SOCE in the SGCs was accompanied with a large Ca^{2+} oscillation. Using quantitative RT-PCR, we detected the expression of Orai 1/2/3 channels, stromal interaction molecules 1/2 (STIMs - endoplasmic reticulum calcium sensors), and the transient receptor potential cation channels 1/3/6 (TRPCs) which are responsible for SOCE. The blockade of TRPCs with lanthanides significantly decreased SOCE and severely attenuated the calcium signals of both neurons and SGCs, indicating the engagement of TRPC channels in SOCE. Taken together, the SOCE is mediated by both Orai and TRPC channels and may contribute to the calcium signaling pathways in the autonomic neuron-SGC units.

Disclosures: S. Kim: None. **S. Kang:** None. **S. Jeong:** None.

Poster

114. Transplantation and Regeneration (PNS) and Neuroglial Interactions

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 114.25/B4

Topic: B.11. Glial Mechanisms

Support: R01 NS28840
R37 NS083580 to NR
T32 NS007453 17

Title: Assessing the impact of P2RY14 on neurofibromatosis type 1

Authors: *J. PATRITTI CRAM, N. RATNER, J. WU, R. COOVER, S. KUNINAKA
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Abstract: Neurofibromatosis type 1 (NF1) is a common autosomal dominant disorder that affects 1:3,000 individuals worldwide. Currently, there is no effective pharmacological treatment for this disease. A predominant characteristic of NF1 is the growth of benign tumors called neurofibromas in peripheral nerves. All nerve cell types, including fibroblasts/perineurial cells, macrophages, and Schwann cells (SCs) are present in neurofibromas, but only SC contain inactivating mutations in both alleles of the *NF1* gene. Perineurial cells, on the other hand, form the blood-nerve-barrier, and it has been suggested that a leaky perineurium may enable nerve tumorigenesis. We found that the perineurial barrier is disrupted in our well characterized *DhhCre;Nf1* $^{flox/flox}$ neurofibroma mouse model. To find genes, other than *NF1*, that contribute to neurofibroma formation and/or affect the perineurium, we performed microarray analysis. We identified the purinergic receptor P2RY14 (GPR105) as overexpressed in SC precursor-like tumor initiating cells as compared to SCs, and P2RY14 enhanced self-renewal of these cells *in vitro*. We next examined P2RY14 expression in *Dhh-Cre;Nf1*$^{flox/flox}$ neurofibroma, in mice bred to *P2RY14* mutants with a β-galactosidase cassette inserted into the *P2RY14* locus. β-galactosidase immunostaining detected P2RY14 expression in Sox10+ SCs and in Glut1+ perineurial cells. An anti-P2RY14 antibody confirmed P2RY14 expression in human neurofibroma, in SC and perineurial cells. Importantly, perineurium disruption was reduced in *P2RY14*$^{-/-}$*Dhh-Cre;Nf1*$^{flox/flox}$ mice. Although double mutant mice ultimately succumbed to neurofibroma, preliminary data indicates that *P2RY14*$^{-/-}$ *Dhh-Cre;Nf1*$^{flox/flox}$ mice have a significant survival advantage compared to *Dhh-Cre;Nf1*$^{flox/flox}$ mice. We conclude that P2RY14 is expressed in Schwann cell precursor like-tumor initiating cells, in Schwann cells, and in perineurial cells, and that this receptor contributes to the aberrant perineurium in neurofibroma. We expect that understanding the mechanisms by which P2RY14 receptor contributes to tumorigenesis and perineurial integrity will allow us to identify novel points of therapeutic intervention to treat NF1.

Disclosures: N. Ratner: None. **J. Wu:** None. **R. Coover:** None. **S. Kuninaka:** None.

Poster

114. Transplantation and Regeneration (PNS) and Neuroglial Interactions

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 114.26/B5

Topic: B.11. Glial Mechanisms

Support: DFG SCHL21021

Title: A distinct and reversible regional pattern of myeloid cell activation induced by chronic peripheral inflammation

Authors: *J. SCHLACHETZKI[1], P. SÜß[1], A. HOFFMANN[1], T. ROTHE[1], G. SCHETT[1], G. KRÖNKE[1], C. K. GLASS[2], J. WINKLER[1]
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Abstract: Mounting experimental and clinical evidence shows that sustained chronic activation of the innate peripheral immune system in autoimmune diseases like rheumatoid arthritis is associated with altered pain perception, fatigue and mood disorders like anxiety and depression. However, a precise mechanistic understanding of how the peripheral immune system communicates with the central nervous system is currently lacking. We previously demonstrated in a human TNF-α transgenic mouse model of rheumatoid arthritis that chronic activation of the peripheral immune system induces severe erosive arthritis with increased joint and serum cytokine levels paralleled by an impaired locomotion (Suess et al, Brain Behavior Immunity 2017). Here, we interrogated the role of peripheral overexpression of human TNF-α on CNS myeloid cell function in the human TNF-α transgenic mouse model. We detected infiltration and activation of myeloid cells by flow cytometry and pinpointed $CD45^+$ leukocytes and $CD169^+$myeloid cells selectively in the cortex, striatum, and thalamus of TNFtg mice. The observed myeloid cell phenotype, in contrast, was absent in the hippocampus and cerebellum. Accordingly, we observed inflammatory gene expression by bulk RNA-seq and qPCR predominantly in the cortex, striatum, and thalamus, but not the hippocampus and cerebellum of TNFtg mice. Gene enrichment analysis showed overrepresentation of genes associated with leukocyte activation, response to cytokine stimulus and interferon-gamma. Myeloid cell activation and infiltration was reversible upon late-onset intervention with the anti-TNF-α antibody infliximab. Given these findings, chronic innate peripheral inflammation induces differential CNS myeloid cell activation and monocyte infiltration in distinct brain regions in rheumatoid arthritis and that neuroinflammation in this model is reversible upon treatment targeting TNF-α.

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Poster

115. Transplantation and Regeneration in the CNS

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 115.01/B6

Topic: A.04. Transplantation and Regeneration

Title: Impaired endothelial progenitor cell differentiation based on IL-10 secretory insufficiency from blood cells in Moyamoya disease

Authors: *E. NAGATA[1], H. MASUDA[2], T. NAKAYAMA[6], S. NETSU[3], H. YUZAWA[3], N. FUJII[3], S. KOHARA[3], T. SORIMACHI[4], T. OSADA[4], R. IMAZEKI[3], T. ABE[3], M. MATSUMAE[4], T. ASAHARA[5], S. TAKIZAWA[3]
[1]Tokai Univ. Sch. Med., Isehara, Japan; [2]Physiol., [3]Neurol., [4]Neurosurg., [5]Basic Clin. Science, Div. of Regenerative Med., Tokai Univ. Sch. of Med., Isehara, Japan; [6]Tokai Univ., Isehara, Japan

Abstract: Recently, it has been reported that a polymorphism of RNF213 gene or the numerical reduction of circulating endothelial progenitor cells (EPCs) might induce its abnormal vascular phenotype as the pathogenesis of MMD. However, the etiology and pathogenesis of Moyamoya disease (MMD) are still unknown. In this study, we investigated the biological activities of EPCs contributing to vascular formation in MMD patients using the newly developed vasculogenic culture of peripheral blood mononuclear cells (PBMNCs). In EPC colony forming assay, the cultured PBMNCs exhibited the reduction of definitive EPC colony forming potential in MMD patients, although the increase in healthy controls. In secretory cytokines of the cultured PBMNCs measured by cytometric bead array, the level of IL-10 was significantly lower in MMD patients than in healthy controls. The cultured PBMNCs of MMD patients with addition of human recombinant IL-10 restored the definitive EPC colony forming potential up to the same level in healthy controls. Furthermore, in flow cytometry following phorbol 12-myristate 13 acetate (PMA) stimulation of the cultured PBMNCs, intracellular IL-10 storage recognized in the main cell populations constituting the cultured PBMNCs, i.e., CD3+ T cells and CD11b+CD206+ M2 macrophages, was augmented in MMD patients, compared to healthy controls. In conclusion, these *in vitro* findings indicate that the abnormal vascular phenotype as the critical pathogenesis of MMD might derive from the impaired EPC differentiation based on the insufficiency of IL-10 secretion from blood cells under vasculogenic environment.

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Poster

115. Transplantation and Regeneration in the CNS

Location: SDCC Halls B-H

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Program #/Poster #: 115.02/B7

Topic: A.04. Transplantation and Regeneration

Support: National Multiple Sclerosis Society RG 5088-A- 1
Seventh Framework Programme of the European Union Num. 291730

Title: Blood vessels guide Schwann cell migration in the adult demyelinated CNS through Eph/Ephrin signaling

Authors: *B. GARCIA-DIAZ[1,2], C. BACHELIN[1], F. COULPIER[3], G. GERSCHENFELD[3], C. DEBOUX[1], V. ZUJOVIC[1], P. CHARNAY[3], P. TOPILKO[3], A. BARON-EVERCOOREN[1]
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Abstract: Myelination is supported by two different glial cell types, oligodendrocytes in the central nervous system (CNS), and Schwann cells (SC) in the peripheral nervous system (PNS). Despite the developmental PNS/CNS segregation, SC can invade and repair the CNS under pathophysiological conditions. To note, SC remyelination of CNS axons has been frequently observed close to blood vessels (BV). However, how SC invade the CNS to remyelinate central axons remains undetermined, and whether BV have a role in their CNS invasion has not been explored. To gain insights into the modalities of SC invasion of the CNS, we studied their migratory behavior *ex vivo* and *in vivo* after exogenous transplantation in the demyelinated spinal cord. Our data highlight for the first time that SC migrate preferentially along blood vessel perivascular space, avoiding CNS myelin. Among the myelin components involved in cell segregation and guidance, EphrinB3 has been reported as a ligand for several SC receptors and appeared as a good candidate for SC-CNS myelin inhibition. We demonstrate that SC migration within the CNS occurs by virtue of a dual mode of action of Ephrin/Eph receptor. We show *in vitro* and *in vivo* that EphrinB3, present in myelin, interacts with SC Eph receptors, to drive SC away from CNS myelin, and trigger their preferential adhesion to ECM components, such as fibronectin via integrinβ1 interactions. This complex interplay enhances SC migration along the BV-ECM network to reach the lesion. In addition, vascular remodeling occurring in the lesion site facilitates SC spreading into this lesion. These novel findings elucidate the mechanism by which SC can invade spinal cord lesions and contribute to their repair.

Disclosures: B. Garcia-Diaz: None. **C. Bachelin:** None. **F. Coulpier:** None. **G. Gerschenfeld:** None. **C. Deboux:** None. **V. Zujovic:** None. **P. Charnay:** None. **P. Topilko:** None. **A. Baron-Evercooren:** None.

Poster

115. Transplantation and Regeneration in the CNS

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Program #/Poster #: 115.03/B8

Topic: A.04. Transplantation and Regeneration

Support: Shriners Hospital for Children

Title: Non-canonical hedgehog signaling regulates spinal cord and muscle regeneration in xenopus laevis tadpoles

Authors: *A. M. HAMILTON[1,2], L. N. BORODINSKY[1,2]
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Abstract: Hedgehog signaling regulates a variety of essential biological processes, including cell proliferation, migration and differentiation. Recent studies have shown that stimulation of the Hedgehog signaling pathway also improves functional recovery following neural injury. However, abnormally elevated Hedgehog signaling is also associated with a number of forms of cancer. Therefore, if we are to manipulate Hedgehog signaling therapeutically, we must understand exactly how this pathway regulates the various processes underlying tissue regeneration. Using a tail regeneration model in *Xenopus laevis* tadpoles, we demonstrate that Hedgehog signaling through Smoothened is necessary immediately following amputation for normal regeneration of both spinal cord and muscle. Treatment with the Smoothened activator SAG blocks spinal cord regeneration, while inhibiting Smoothened with cyclopamine permits spinal cord regeneration, but blocks muscle regeneration. We also show that treating with GANT61 to directly inhibit Gli1/2, the downstream transcriptional activators of canonical Hedgehog signaling, has comparatively modest effects on regeneration, strongly suggesting that non-canonical Hedgehog signaling is a primary regulator of regeneration. Strikingly, we find that the activity of Gli1/2 is repressed following tail amputation, and that Gli1 actually acts as a brake on spinal cord and muscle regeneration. These results indicate that Hedgehog signaling acts through multiple pathways downstream from Smoothened, opening up opportunities for targeted manipulation of canonical and non-canonical hedgehog pathways in treating injury to the spinal cord and muscle.

Disclosures: A.M. Hamilton: None. **L.N. Borodinsky:** None.

Poster

115. Transplantation and Regeneration in the CNS

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 115.04/B9

Topic: A.04. Transplantation and Regeneration

Support: SC-CTSI
NANOS Pilot Grant
Research to Prevent Blindness

Title: Directed axon growth using applied electrical fields

Authors: *K. K. GOKOFFSKI[1], X. JIA[2], M. ZHAO[3]
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Abstract: Restoration of vision in patients blinded by optic neuropathies requires regenerating the optic nerve. Intraocular transplantation of retinal ganglion cells (RGCs) alone is insufficient to regenerate the optic nerve due to failure to sustain axon growth and/or insufficient directional cues. There is much interest in the potential role of electrical fields (EFs) in promoting long distance axon growth. The body has naturally occurring electrical currents and it is established that motor neuron and dorsal root ganglion cell axons can grow directionally when exposed to an EF. Whether EFs can direct RGC axon growth is unknown, as are the mechanisms through which EFs mediate directional growth. Members of the Rho Kinase signaling pathway have been shown to meditate EF-induced directional growth of spinal nerves. We hypothesize that EFs direct and sustain RGC neurite growth by signaling through the Rho kinase pathway. To test this hypothesis, retina was isolated from post-natal mice and cultured in an electrotaxis apparatus. Retina was then exposed to varying EF strengths with/without ToxinB, a non-specific inhibitor of Rho Kinase signaling. Time-lapsed microscopy was performed and videos used to quantify the direction and rate of neurite growth. In the absence of an EF, RGC neurites demonstrated indiscriminate directional growth from the tissue edge. Retinal cultures that were exposed to an EF of 200mV/mm, however, showed marked asymmetric growth: 81.2% were directed at the cathode, while 4.8% and 14.1% were directed towards the anode or perpendicular to the field, respectively ($p<0.001$). EF did not affect the rate of RGC axon growth. Interestingly, RGC axons retain their ability to respond to acute changes in EF polarity by changing their direction of growth. Finally, ToxinB neutralized EF-induced (200mV/mm) directional growth of retinal neurites. However, when the field strength was increased to 270mV/mm and 350mV/mm, directional growth was restored. Here, we demonstrate that RGC neurites exhibit directional growth when exposed to an EF. The acuity with which RGC neurites respond to changes in EF polarity suggests that the effect of EFs on RGCs is direct. Additionally, our data suggest that Rho

Kinase signaling is necessary to translate EFs into directional cues in RGC neurites. The significance of this work lies in its potential to advance the field of optic nerve regeneration. Application of electrical currents may be necessary to direct the growth of newly transplanted RGCs.

Disclosures: K.K. Gokoffski: None. **X. Jia:** None. **M. Zhao:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent Holder.

Poster

115. Transplantation and Regeneration in the CNS

Location: SDCC Halls B-H

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Program #/Poster #: 115.05/B10

Topic: A.04. Transplantation and Regeneration

Support: MRC Grant R004463
MRC Grant G1000864
Gates Cambridge Studentship
Gates Cambridge Academic Development Award
International Foundation for Research in Paraplegia P172

Title: Overexpression of protrudin in primary cortical neurons enhances regeneration after laser axotomy through multiple mechanisms

Authors: *V. PETROVA[1], R. EVA[1], J. W. FAWCETT[1,2]
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Abstract: Numerous extracellular and intracellular processes contribute to the failure of long-range regeneration in the adult central nervous system (CNS) after injury. One reason why adult CNS axons have poor regenerative capabilities is that a developmental change occurs where essential growth molecules such as integrins and growth factor receptors become excluded from axons. These growth-promoting molecules are normally transported along axons in Rab11-positive recycling endosomes by motor proteins/adaptor complexes. However, this transport declines with maturation leading to a decline in regenerative capacity. Protrudin, a member of the ZFYVE family of zinc-binding proteins is a membrane-associated protein involved in neurite outgrowth and directional membrane trafficking in HeLa, PC12 and primary hippocampal cells (Shirane *et al.*, 2006). Phosphorylated protrudin preferentially binds to Rab11-GDP, an association which is required for neurite outgrowth and for anterograde movement of this complex.
We hypothesised that increasing the phospho-protrudin/Rab11 interaction would result in

anterograde transport of growth-promoting molecules to the tip of injured axons enabling regeneration of primary cortical neurons after laser axotomy. To test this, two phosphomimetic forms of protrudin were created at phosphorylation sites known to play an important role for its association with Rab11. We found that both constitutively phosphorylated protrudin forms (64%, 70%) and also wild-type protrudin (60%) increased the proportion of regenerating axons compared to control (27%). Live-cell imaging experiments are currently being performed to investigate whether the increase in regenerative capacity is indeed due to its association with Rab11 and increased anterograde transport of integrins. Protrudin is a complex molecule with numerous cellular functions such as ER shaping, vesicular transport, interactions with spastin – a microtubule-severing protein and many more. In order to unpick the mechanisms of action of protrudin on regeneration, five mutants were created – each targeting a specific region of the protein important for its involvement in various molecular pathways (ΔFYVE, ΔRab11-binding-domain, ΔKIF5, ΔFFAT, ΔSpastin). These mutants were overexpressed in primary cortical neurons and their effects on regeneration were tested. Protrudin was found to promote regeneration through multiple mechanisms, some of which, such as ER function have not previously been associated with the process of axon regeneration. Protrudin's ability to promote regeneration is currently being tested *in vivo* in models of acute and chronic injury.

Disclosures: V. Petrova: None. **R. Eva:** None. **J.W. Fawcett:** None.

Poster

115. Transplantation and Regeneration in the CNS

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 115.06/B11

Topic: A.04. Transplantation and Regeneration

Support: International Spinal Research Trust (NRB110)
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Medical Research Council (G1000864 018556)
Cambridge Eye Trust (RG80564)
Fight for Sight (RG74504)

Title: Elevated phosphoinositide 3-kinase activity promotes axon regeneration of central nervous system neurons

Authors: *B. NIEUWENHUIS[1,2], R. EVANS[1], C. S. PEARSON[3], A. C. BARBER[1], J. CAVE[1], P. D. SMITH[4], J. FUCHS[5], B. J. EICKHOLT[5], H. M. GELLER[3], K. R. MARTIN[1], R. EVA[1], J. W. FAWCETT[1,6]
[1]Univ. of Cambridge, Cambridge, United Kingdom; [2]Netherlands Inst. for Neurosci.,

Amsterdam, Netherlands; [3]NIH, Bethesda, MD; [4]Carleton Univ., Ottawa, ON, Canada; [5]Charite Univ. Med., Berlin, Germany; [6]Inst. of Exptl. Med., Prague, Czech Republic

Abstract: Injury to the central nervous system (CNS) has severe consequences because adult CNS axons do not regenerate. PtdIns-3,4,5-P3 (PIP_3) signaling is essential for axon growth during development of the nervous system. Silencing PIP_3 phosphatase PTEN leads to increased regeneration in the corticospinal tract and optic nerve after injury, showing that PIP_3 is crucial for axonal regeneration. However as neurons mature, phosphoinositide 3-kinases (PI3Ks) activating receptors are excluded from axons. We hypothesize that a developmental decline in axonal PIP_3 signaling contributes to restricted axonal regeneration in the CNS. The objective of this study is to explore whether overexpression of PI3Ks, which generates PIP_3, could promote axonal regeneration of CNS neurons.

We first confirmed that there is a decline of PIP_3 levels in cultured cortical neurons in line with maturation by using immunocytochemistry. Overexpression of activated PI3K increased axonal growth by 50% in developing neurons (4 days *in vitro,* DIV). Expression of activated PI3K in matured cortical neurons (14 DIV) resulted in an enlarged the soma size (by 100%) and more complex dendritic morphology *in vitro*. Expression of constitutively activated PI3K, but not wildtype PI3K, resulted in a six-fold increase of the PIP_3 signaling pathway in these neurons – visualized by staining for the phosphorylated ribosomal protein S6. To investigate whether PI3Ks can enhance the regeneration capacity of CNS neurons, we overexpressed these in cortical neurons and applied *in vitro* laser axotomy. We confirmed that 14 DIV neurons have a restricted axon regeneration capacity after *in vitro* laser axotomy (only 15% of the axotomised neurons regenerate). Importantly, expression of PI3Ks increases the success rate of axonal regeneration (approximately 60% of the axotomised neurons regenerate). We recently started to explore whether PI3K can promote optic nerve regeneration *in vivo*. Using a transgenic mouse line with cre-inducible expression of constitutively activated PI3K, we found that the AAV2-cre condition had significantly increased axon regeneration beyond the optic nerve crush site compared to controls.

In summary, cultured cortical neurons have a decline of PIP_3 signaling and this could contribute to their decreasing regeneration capacity during maturation. Overexpression of activated PI3Ks promotes developmental axon growth, and axonal regeneration of CNS neurons *in vitro* and *in vivo*. The mechanisms of PI3K-induced axon regeneration have not been investigated in great detail. We are currently investigating whether PI3K acts by stimulating axonal transport of regeneration-associated proteins.

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Poster

115. Transplantation and Regeneration in the CNS

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Topic: A.04. Transplantation and Regeneration

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SHC-85100
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SHC-86200-PHI-16
SHC-85112-PHI-18

Title: Lin28 protein regulates CNS axon regeneration in adult mammalians

Authors: *S. LI, F. NATHAN, Y. OHTAKE, H. GUO, A. SAMI
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Abstract: Severed CNS axons fail to regenerate in adult mammals and there are no effective regenerative strategies to treat patients with CNS injuries. Several genes, including PTEN and Krüppel-like factors, regulate intrinsic growth capacity of mature neurons. However, none of these approaches were translated to clinics yet and thus there is a persistent need to identify better targets and improved delivery methods. Lin28, an RNA-binding protein, enhances translation of multiple genes including metabolic enzymes for increasing glycolysis and oxidative phosphorylation, and is essential for cell development and pluripotency in worms and mammals. Lin28 is a gatekeeper molecule to control switch between pluripotency and committed cells and its reactivation stimulates repair of several tissue systems, including hair, cartilage, bone, and mesenchyme. In this study, we reprogram mature CNS neurons with Lin28 activation to increase their growth capacity after CNS injuries. Especially, we evaluated the role of Lin28a in regulating regenerative capacity of different types of CNS neurons in adult mammals. Using neuron-specific Thy1 promoter, we generated transgenic mice that overexpress Lin28a protein in multiple neuronal populations, including multiple descending projection tracts and retinal ganglion cells. We demonstrate that upregulation of Lin28a in adult transgenic mice induces significant long distance regeneration of descending motor axons after spinal cord injury and optic fibres following optic nerve axotomy. Importantly, overexpression of Lin28a by post-injury treatment with AAV2 vector also stimulates dramatic regeneration of corticospinal tracts after spinal cord injury and optic axons after optic injury. Upregulation of Lin28a also enhances survival of retinal ganglion cells after injury and activity of Akt/mTOR signalling pathway in various CNS neurons. Therefore, Lin28a is critical for regulating growth capacity of CNS

neurons and may become an important molecular target for treating for CNS injuries, including traumatic spinal cord and brain injury.

Disclosures: S. Li: None. **F. Nathan:** None. **Y. Ohtake:** None. **H. Guo:** None. **A. Sami:** None.

Poster

115. Transplantation and Regeneration in the CNS

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 115.08/B13

Topic: A.04. Transplantation and Regeneration

Support: MBL - Eugene Bell Center for Regenerative Biology & Tissue Engineering

Title: Functional recovery and regeneration in sea lampreys after spinal cord re-transection

Authors: *K. L. HANSLIK[1], S. R. ALLEN[2], T. L. HARKENRIDER[1], S. M. FOGERSON[2], E. GUADARRAMA[3], J. R. MORGAN[4]
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Abstract: Many invertebrate and vertebrate species can reliably regenerate their tissues, including the nervous system. Sea lampreys (*Petromyzon marinus*) provide a highly regenerative model in which to study regeneration of the central nervous system (CNS) following spinal cord injury (SCI). Lampreys fully recover their swimming behaviors within 10-12 weeks after a complete spinal cord transection. This robust functional recovery is accompanied by lesion repair, as well as axon and synapse regeneration beyond the lesion site. However, very little is known about whether the regenerative processes are similarly as robust after a second spinal cord transection. To test this, we performed spinal transections on lampreys and allowed them to recover for 11 weeks. We then re-transected the spinal cords in the same location as the first and allowed them to recover for another 11 weeks. Compared to lampreys that underwent single spinal transections, the swimming behaviors of re-transected lampreys recovered along the same time course, as shown by semi-quantitative movement scoring. Similarly, repair of the spinal lesion was comparable in both transected and re-transected lampreys. Bulk anterograde labeling revealed similar axon regeneration patterns. Immunostaining also revealed similar re-distribution of axons, synapses and several cytoskeletal elements in both transected and re-transected spinal cords. We also took advantage of the large, identified reticulospinal (RS) neurons in lamprey brains, which possess known regenerative potentials with some being especially “good” or “poor” regenerators. Histological staining revealed that the same giant RS neurons survived after spinal re-transection as occurred after a single transection. Retrograde fluorescence labeling revealed that the same surviving neurons regenerated their axons. After spinal transection, the

giant RS neurons displayed a tight positive correlation between neuronal survival and axon regeneration ($R^2 = 0.90$) that was similarly observed after spinal re-transection ($R^2 = 0.93$). Together, these findings indicate that lampreys can recover just as well after a spinal re-transection as after the first injury, further indicating the high regenerative potential in the lamprey CNS. Understanding the regenerative processes in this model could help elucidate the factors that are limiting in regeneration-incompetent species and has important implications for pro-regenerative mechanisms after CNS injuries.

Disclosures: K.L. Hanslik: None. **S.R. Allen:** None. **T.L. Harkenrider:** None. **S.M. Fogerson:** None. **E. Guadarrama:** None. **J.R. Morgan:** None.

Poster

115. Transplantation and Regeneration in the CNS

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 115.09/B14

Topic: A.04. Transplantation and Regeneration

Support: CNPQ
CAPES
FAPERJ

Title: Intravitreal injection of transgenic mesenchymal stem cells expressing hIGF-1 increases mouse retinal ganglion cells survival after optic nerve crush

Authors: *J. VASQUES[1], C. A. ABREU[1], B. S. F. SOUZA[2], M. B. P. SOARES[2], R. MENDEZ-OTERO[1]
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Abstract: Introduction and objectives: Retinal ganglion cells (RGC), like other central nervous system neurons, have reduced regenerative capacity and, once damaged, the axons of adult RGC do not regenerate at long distances through the optic nerve. In addition, RGCs are extremely sensitive to axonal damage and most of them die after damage to the optic nerve. There are, to date, no clinically applicable therapies capable of protecting RGC and increasing the regeneration of their axons in an efficient and prolonged way. Recently, bone marrow-derived cells have been used to increase regeneration and/or functional improvement in several animal models of neurological diseases. In visual system, mesenchymal stem cells (MSC) were able to increase survival of RGC in glaucoma models and after axonal transection or optic nerve crush, as demonstrated by previous studies from our group. However, even inducing a significant increase in survival and regeneration of RGC, MSC therapy was not able to promote functional recovery. In this work our main objective is to potentiate this regenerative response through the use of a mouse transgenic MSC line expressing the human trophic factor IGF-1 (mMSC-hIGF1),

which has neuroprotective and neuroregenerative action. Methodology: SvEv129 adult mice were submitted to optic nerve crush and intravitreal injection of vehicle or 90,000 or 200,000 mMSC-hIGF1/control-mMSC. 14 days after surgery retinas were dissected and immunohistochemistry for TUJ1, marker of RGC, was performed. Results and discussion: Intravitreal injection of 90,000 mMSC-IGF1 cells after optic nerve crush was enough to induce the same significant neuroprotection evoked by 200,000 control-mMSC, leading to an increase of about 45% in the number of RGC compared to animals treated with PBS. Injection of 200,000 mMSC-hIGF1 promoted an even more robust and significant neuroprotective response, with an increase of about 90% in the number of RGC detected in relation to the PBS group. These data suggest that IGF-1 expression is capable of significantly potentiating the neuroprotective action of MSCs. It remains to be determined whether this effect is sustained after long periods and if it also results in an increase in axonal regeneration through the optic nerve.

Disclosures: J. Vasques: None. **C.A. Abreu:** None. **B.S.F. Souza:** None. **M.B.P. Soares:** None. **R. Mendez-Otero:** None.

Poster

115. Transplantation and Regeneration in the CNS

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Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

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Topic: A.04. Transplantation and Regeneration

Support: The University of Connecticut School of Medicine, Start-Up Funds (to E.F.T.)
The BrightFocus Foundation (Grant # G2017204, to E.F.T)

Title: Subtypes of axotomized adult retinal ganglion cells survive and regenerate long axons in response to an extrinsic cue in a permissive environment

Authors: *J. KIM, B. A. RHEAUME, M. S. SAJID, E. F. TRAKHTENBERG
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Abstract: The failure of mammalian central nervous system (CNS) projection neurons to regenerate injured axons and to restore long-distance connections between neurons severely limits recovery from ischemic or traumatic injury in the CNS. For example, blindness caused by trauma or stroke to the optic nerve disrupts the retinal ganglion cells' (RGCs) axons, through which the information from the eye is passed into the brain, resulting in irreversible blindness. Manipulating a number of cell-autonomous and extrinsic factors identified to date stimulates only modest axon regeneration in the CNS, and no therapeutics are available that could help patients with axonal injuries. Therefore, the failure of RGC and other CNS axons to regenerate over long distances after injury remains a major unmet problem. Here, we used bioinformatic analysis of RNA-seq transcriptome profiles of RGCs to predict extracellular matrix (ECM)

molecules with which embryonic RGCs (that grow axons robustly) could interact, and then tested their effect in culture on adult RGCs (which do not grow long axons in culture) isolated by immunopanning for Thy1.1. We found that one of these ECM molecules (identity masked due to proprietary information) enabled a subset of axotomized adult rodent RGCs to survive for a long period, and also stimulated long-distance axon growth in a permissive culture environment. Next, we analyzed developing RGCs with single-cell RNA-seq and clustering algorithms, which enabled segregating RGC population into 40 subtypes based on the differences in their transcriptomes. We found that consistent with our observation, that only a subset of RGCs responded to the predicted ECM, only certain RGC subtypes expressed the ligand that could interact with this ECM. Thus, the identified ECM molecule may hold potential for promoting axon regeneration of certain RGC subtypes after injury to the optic nerve and perhaps other parts of the CNS, in which neurons express this ligand.

Disclosures: J. Kim: None. **B.A. Rheaume:** None. **M.S. Sajid:** None. **E.F. Trakhtenberg:** None.

Poster

115. Transplantation and Regeneration in the CNS

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Program #/Poster #: 115.11/B16

Topic: A.04. Transplantation and Regeneration

Support: MSSOC Grant

Title: Inhibition of miR-383 promotes axon regeneration following injury

Authors: *C. JUZWIK[1], B. MORQUETTE[1], Y. ZHANG[1], E. GOWING[2], C. BOUDREAU-PINSONNEAULT[3], V. VANGOOR[4], R. PASTERKAMP[4], C. MOORE[5], A. BAR-OR[6], A. E. FOURNIER[1]
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Abstract: Neuroinflammation can positively influence axon regeneration following injury in the central nervous system (CNS) but the molecular mechanisms underlying this effect are not fully understood. We have evaluated how microRNAs may be regulated in the context of inflammation because they target multiple mRNAs simultaneously and may help to identify signalling hubs that can be targeted to promote regeneration. We identified miR-383 as a miRNA that is down regulated in neurons in response to neurite outgrowth-promoting astrocyte conditioned media (ACM). We also found that miR-383 was down regulated in Retinal Ganglion

Cells (RGCs) exposed to zymosan, a yeast cell wall protein that stimulates inflammation in the eye and promotes axon regeneration following optic nerve crush. miR383 down regulation promotes axon growth *in vitro* and injection of a miR-383 inhibitor into the eye promotes axon regeneration following optic nerve crush. Previous studies have demonstrated that astrocyte-derived CNTF, signals to injured RGCs and promotes their regeneration in response to zymosan injection. We found that neurons treated with CNTF down regulated the expression of miR-383. Further, we have acquired evidence that inhibiting miR-383 de-represses the expression of mitochondrial antioxidant genes and microtubule-associated proteins that are important for the pro-regenerative effects of the miR383 inhibitor. Together, we have implicated miR-383 as a molecule that suppresses axon regeneration and that can be further explored to identify novel signalling hubs that may be targeted to promote repair.

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Poster

115. Transplantation and Regeneration in the CNS

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 115.12/B17

Topic: A.04. Transplantation and Regeneration

Title: Insight into lipid dynamics of embryonic and early postnatal mouse growth cones

Authors: *A. M. TRZECIECKA[1], S. K. BHATTACHARYA[2]
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Abstract: Rationale: Postnatally, central nervous system (CNS) neurons rapidly lose their ability to regenerate axons. The limited capacity for repair in the CNS remains a significant medical challenge. Requirements for successful neuron regeneration and functional recovery include long-distance axon growth and correct pathfinding to reestablish synapses with proper targets. Growth, motility and guidance functions are executed by a specialized structure at the tip of a growing axon - growth cone (GC). Protein components of the GC has been reported to be important players in a damaged axon regrowth. Yet, the question of whether GC membrane has unique lipid composition has not been comprehensively addressed. Objective: The aim of this study was to investigate spatial compartmentalization and temporal dynamics of lipids in the neuronal GC. Method: GC particles and non-GC membrane (nonGCM) fractions (n=6) were prepared from embryonic day 18 (E18) and postnatal days 1, 3, 6 and 9 (P1, P3, P6 and P9) forebrains of C57BL/6J mice by subcellular fractionation (Pfenninger et al., 1983). To test for enrichment efficiency, fractions were analyzed by Western blotting. Lipids were extracted using chloroform, methanol and water mixture to obtain phase separation. We then used untargeted

high-performance liquid-chromatography tandem mass spectrometry approach to define GC and nonGCM lipid composition in a robust and unbiased manner. Results: For an overall assessment of lipidomic similarities and differences between GC and nonGCM, we employed principal component analysis (PCA). Clear separation occur between GC and nonGCM samples (PC1 82.1%). GC fractions show lipidome transition profile from E18 to P9 (PC2 6.9%). Using ANOVA-simultaneous component analysis (ASCA), we identified major patterns of lipid classes associated with group: sulfoquinovosyldiacylglycerol (SQDG) enrichment in GC and dihexosyl ceramide (CerG2) in nonGCM fractions; and time factor - ganglioside GD1a upegulation in GCs from P3-P9 mice. Conclusion: GCs have distinct lipid composition. Lipids associated with GC may regulate conversion of a neuron to a growth-plausible phenotype. Further elucidation of their role in GC biology could open up new directions to successful axon regeneration.

Disclosures: A.M. Trzeciecka: None. **S.K. Bhattacharya:** None.

Poster

115. Transplantation and Regeneration in the CNS

Location: SDCC Halls B-H

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Program #/Poster #: 115.13/B18

Topic: A.04. Transplantation and Regeneration

Support: CAPES
CNPq
FAPERJ

Title: Cellular mechanisms involved in retinal neuroprotection and neuroregeneration after optic nerve crush and mesenchymal stem cell therapy in rats

Authors: *L. CHIMELI-ORMONDE, R. F. M. SOARES, J. F. VASQUES, A. J. DA-SILVA-JUNIOR, L. C. TEIXEIRA-PINHEIRO, L. A. MESENTIER-LOURO, M. F. SANTIAGO, R. MENDEZ-OTERO
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Abstract: Cell therapy has been widely used in different animal models of neurological pathologies. Our group has been using a model of optic nerve crush in rats to study neurodegenerative conditions of the central nervous system, as the optic nerve is easily accessed and manipulated. This is also an interesting model to study specific ocular diseases that affect the optic nerve, such as optic neuropathies that lead to gradual death of retinal ganglion cells (RGCs) and often result in irreversible blindness. Previous work of our group have shown that one intravitreal injection of 500.000 rat bone marrow mesenchymal stem cells (rMSCs) immediately after optic nerve crush has a sustained effect increasing RGCs survival and axonal regeneration for 28 days. Adult Lister Hooded rats of both sexes (3-5 months old) were subjected to unilateral

optic nerve crush, keeping the contralateral eye as a control. Here, we investigated the glial response to optic nerve crush followed by rMSCs or vehicle intravitreal injection. We performed a blinded analysis of two different glial subpopulations, microglia and Müller cells, by immunohistochemistry against ionized calcium-binding adapter molecule 1 (IBA1) and glial fibrillary acidic protein (GFAP), respectively, in 20µm-thick retinal sections. There was an increase in the number of IBA1-positive microglia in the group treated with rMSCs 3, 7 and 14 days after crush in relation to vehicle-injected group. In contrast, the number of GFAP-positive processes in Müller cells decreased in retinas treated with rMSCs when compared to vehicle group. Thus, understanding the mechanisms and the cellular populations involved in the neuroprotective and neuroregenerative effects of rMSCs therapy after optic nerve crush may open up new perspectives for the treatment of neurodegenerative diseases.

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Poster

115. Transplantation and Regeneration in the CNS

Location: SDCC Halls B-H

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Topic: A.04. Transplantation and Regeneration

Support: Funds of Leading Talents of Guangdong Province(87014002)

Title: Controlled-releasing of ISP & ILP from functional self-assembling peptide nanohydrogel to promote neural regeneration after spinal cord injury

Authors: *H. LIU, S. RAMAKRISHNA, L. HE
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Abstract: The chondroitin sulfate polysaccharide proteins (CSPGs) are the main components of the scaring matrix that hinder the regeneration of axons following spinal cord injury. They are mainly secreted by activated astrocytes. In previous research, enzymatic digestion of chondroitin sulfate promoted the axonal regeneration. However, other studies have found that CSPGs, acting as an extracellular matrix, have an indispensable function in repairing spinal cord injury. In this study, proposed to intervene CSPGs by active short peptide nanohydrogels loaded short molecular compound “ISP&ILP” which can bind to PTPσ and LAR, relieving CSPGs-mediated inhibition. The motor tract was detected by biotinylated dextran amine (BDA, 10KDa) anterograde tracing. AAV-retro/GFP that was injected into sciatic never conformed sensory-motor tract regeneration. Moreover, at the mercy of the hind limb muscle, the gastrocnemius muscle be infected with pseudorabies virus (PRV-GFP). The behavior recovery was studied by

BBB locomotion assessment. Two weeks after injury, axon regeneration was obviously observed with many newly born neurons. Two months later, motor and sensory tracts were significantly reestablished. Hind limb functions were significantly improved as compared with the rats without interventions. Therefore, our study documented a feasible strategy for neural regeneration after spinal cord injury.

Disclosures: H. Liu: None. **S. Ramakrishna:** None. **L. He:** None.

Poster

115. Transplantation and Regeneration in the CNS

Location: SDCC Halls B-H

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Topic: A.04. Transplantation and Regeneration

Support: NRF South Africa Incentive Grant IPRR UID114780

Title: Post-injury regulation of Doublecortin (DCX) in the visual pathway of the lizard, Gallotia galloti

Authors: *D. M. LANG[1,2], A. NASIR[2], A. SCHUNDNER[2], D. ENGEL[2], H. WOLF[2], M. MONZON-MAYOR[3]
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Abstract: Unlike in mammals, retinal ganglion cells (RGCs) are able to survive and robustly regenerate their axons following optic nerve (ON) injury in the Canary Island wall lizard, *Gallotia galloti*. We have previously shown that this regenerative process occurs in the presence of CNS myelin and oligodendrocytes and that the regenerating lizard RGC axons are not sensitive to the neurite growth inhibitory (NI) protein, Nogo-A. Thus, we hypothesized that neuron-intrinsic properties might hold the key to survival and successful axon regeneration of RGCs in *G. galloti*. Doublecortin (DCX) family proteins are widely expressed in differentiating CNS neurons and are downregulated after RGC axotomy in mice, while DCX overexpression has recently been implicated in promoting neuron survival, growth cone formation and axon regeneration in the injured mammalian visual pathway. These observations suggest a central role of DCX family proteins in axon growth and regeneration - however, no information is available on the expression of DCX in the visual system of non-mammalian vertebrates during spontaneous RGC axon regeneration. We therefore studied the regulation of DCX expression after short and long-term post-injury intervals in the optic pathway of the lizard. Using a panel of antibodies to DCX and markers identifying neurons and glial cells, we observed significant upregulation of DCX immunoreactivity in RGCs following ON cut or crush injury. DCX upregulation in RGCs gradually increased up to 3 months post lesion and persisted at high levels

throughout the observation period of up to one year. Strong DCX upregulation was also observed in reactive astrocytes of the ON at all post injury intervals. Our results are consistent with an important role of DCX family proteins in neuronal survival, initiation and maintenance of the regenerative response of RGCs in *G. galloti*.

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Poster

115. Transplantation and Regeneration in the CNS

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Title: Epigenetic regulation during neurodevelopment and regenerative response

Authors: *X. WANG[1], C. QIAN[2], Q. LI[3], F.-Q. ZHOU[4]
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Abstract: Neurons in the adult mammalian CNS lose their capacity of regenerating axons after injury during maturation. On the contrary, neurons in the adult PNS can reclaim their intrinsic axonal growth competence following a spontaneous regenerative response toward injury. Emerging evidence has suggested that the switch from non-regenerative state to regenerative state of injured PNS neurons largely involves changes in the epigenetic landscape and chromatin accessibility. For example, a previous study demonstrated that the injury-triggered HDAC5 nucleus export increases histone acetylation to activate a regeneration-supportive gene expression program. A study in our lab showed that the level of H3K27 trimethylation is elevated in the PNS neurons after injury and such elevation is necessary for the neurons to regain axon regeneration potential and is rendered through orchestrated Ezh2 upregulation and Utx/Jmjd3 downregulation. Although these studies have characterized the functional roles of some epigenetic modifiers in axon regeneration, the functions of many other chromatin regulators are yet to be determined. Using a series of sequencing and array approaches, we

analyze how chromatin structures and related regulators change during neurodevelopment and after injury. The results provide a systematical view of epigenetic changes and related regulation of gene expression governing axon growth and regeneration.

Disclosures: X. Wang: None. **C. Qian:** None. **Q. Li:** None. **F. Zhou:** None.

Poster

115. Transplantation and Regeneration in the CNS

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Topic: A.04. Transplantation and Regeneration

Support: NEI EY026766
NEI U01
Research to Prevent Blindness

Title: The axon transportome in retinal ganglion cells isolates mediators of neuronal degeneration

Authors: *S. H. SHAH[1,2], L. M. SCHIAPPARELLI[3], Y. MA[3], M. ATKINS[2], J. YATES[3], H. CLINE[3], J. GOLDBERG[2]
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Abstract: After acute injury and in degenerative diseases like glaucoma, retinal ganglion cells (RGCs) lose their synaptic connections and eventually die. Decreased axoplasmic transport in the presence of increased intra-ocular pressure (IOP) remains a long-standing hypothesis for the pathology of glaucoma. Which specific proteins, and by what mechanisms, show altered transport after injury or glaucomatous insult? To study this question, we have developed new mass spectrometry strategies (Schiapparelli et al 2014) for analyzing proteome translation in the retina and its axonal transport into the optic nerve (ON). We call this population of axon-transported proteins in the ON “the transportome.” We are using these methodologies to address two main questions: (a) How are specific proteins transported from the cell body of RGCs affected in response to optic nerve injury? (b) Does disrupted protein transport affect local signaling dynamics in the cell body or in the axon?
To examine the transportome after injury, we labelled all retinal proteins with NHS-biotin, which binds covalently to all proteins present at time of injection, and isolated and analyzed biotin-modified peptides from the optic nerve. We identified proteins whose transport down the optic nerve decreased in the 24 hours after optic nerve crush (ONC), including key motor protein Kif5a, and others whose transport increased. To examine the retinal response to injury, we labeled newly synthesized retinal proteins with azidohomoalanine (AHA) following ONC in

adult rats and identified ~50 proteins whose synthesis and subsequent transport to ON significantly changed 24 hours after ONC, including RNA transport and translation machinery. We then analyzed if changes in transport of key proteins found in our proteomic results could be associated with ON injury-mediated RGC degeneration. We found that Kif5a loss alone leads to progressive RGC neurodegeneration resembling that observed after ONC. Kif5a knockout in adult RGCs led to progressive and total RGC death within 4 months. We are currently extending our methodology to identify and quantify differentially translated and transported proteins in RGCs in the context of glaucomatous injury. We hypothesize that identifying specific proteins that are synthesized and transported to an injury site will lead to new understanding of pathophysiology of injury and new approaches to promoting regeneration.

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Poster

115. Transplantation and Regeneration in the CNS

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BrightFocus Foundation (DJZ)

Title: SDF1 is highly expressed in macrophages and contributes to inflammation-induced optic nerve regeneration

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Abstract: Inflammation plays a key role in peripheral nerve regeneration and can also be recruited to promote regeneration in the CNS. Like other mammalian CNS neurons, retinal ganglion cells (RGCs), the projection neurons of the eye, normally fail to regenerate injured axons, but this failure can be partially reversed by inducing an inflammatory reaction in the eye. One key mediator of this phenomenon is Oncomodulin (Ocm), a small Ca^{2+}-binding protein that is expressed by neutrophils and macrophages and that binds to a high-affinity receptor on RGCs in a cAMP-dependent manner. Here, we report that stromal cell- derived factor 1 (SDF1, chemokine CXCL12) also contributes to the effects of intraocular inflammation and, when introduced exogenously, increases the level of regeneration induced by Ocm/cAMP. One day after intraocular injection of zymosan, invasive macrophages (but not neutrophils) express high levels of SDF1 mRNA and protein. Deletion of SDF1 in macrophages (using $cxcl12^{flx/flx}LysM^{Cre/+}$ mice) or deletion of its receptor (CXCR4) in RGCs ($cxcr4^{flx/flx}$ mice injected intraocularly with AAV2-Cre virus), resulted in ~ 30% reduction in optic nerve regeneration 2 weeks after nerve injury combined with intraocular zymosan. Thus, SDF-1 contributes to inflammation-induced optic nerve regeneration. In purified early postnatal RGC cultures, SDF1 showed direct effects on cell survival and neurite outgrowth, and in adult mixed retinal cultures, SDF1, uniquely among many growth factors tested, strongly enhanced the effects of Ocm/cAMP. The mechanisms of SDF1's activity involve increasing cAMP level and enhancing PI3K signaling and S6 phosphorylation. *In vivo*, exogenous SDF1 augmented levels of regeneration induced by Ocm or intraocular inflammation, and when combined with Ocm/cAMP and *pten* deletion, SDF1 increased the number of axons that crossed the chiasm and entered the brain. Thus, SDF1 is a constituent of intraocular inflammation and is potentially useful as an agent to augment regeneration after optic nerve injury.

Disclosures: Y. Yin: None. **L. Xie:** None. **H. Gilbert:** None. **C. Berlinicke:** None. **L. Cen:** None. **Y. Li:** None. **Q. Cui:** None. **D.J. Zack:** None. **L.I. Benowitz:** None.

Poster

115. Transplantation and Regeneration in the CNS

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 115.19/B24

Topic: A.04. Transplantation and Regeneration

Support: NIH Grant R01NS064288
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the Craig H. Neilson Foundation
the BrightFocus Foundation

Title: The histone demethylase inhibitor gsk-j4 promotes axon regeneration in optic nerve

Authors: *Q. LI[1], C. QIAN[2], X. WANG[3], C. ZHANG[4], A. OCHUBA[3], F.-Q. ZHOU[5]
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Abstract: Previous studies have shown that optic nerve axons seldomly regenerate after injury, leaving optic nerve injury no cure. One of the primary reasons for the CNS neurons failed to regenerate axons is the loss of intrinsic regenerative capability related to gene transcription. The process of axon regeneration after injury usually requires actions of coordinated genes, which suggests that a set of regeneration-associated genes may potentially be regulated by some epigenetic mechanisms. Indeed, our unpublished data shows that histone 3 trimethylation at lysine27 (H3K27me3) is elevated in mature mouse dorsal root ganglion after sciatic nerve axotomy. This increase of H3K27me3 was shown to be induced by an increase of methyltransferase EZH2 and a decrease of demethylase JMJD3/UTX in dorsal root ganglion after sciatic nerve axotomy. We hypothesized that a similar change in JMJD/UTX level could promote axon regeneration in the optic nerve after injury. GSK-J4 is a novel, selective inhibitor of the jumonji family of histone demethylases JMJD3/UTX. The specific aim of the current study is to examine if increasing the level of H3K27me3 by either genetical or pharmacological inhibition of JMJD3/UTX could promote optic nerve regeneration after axonal injury. First, intravitreally AAV2-Cre injected JMJD3$^{f/f}$/UTX$^{f/f}$ mice is utilized to investigate the effect of conditional double-knockout of JMJD3/UTX on optic nerve axon regeneration after injury. In addition, optic nerve axon regeneration is analyzed after the intravitreal injection of JMJD3/UTX inhibitor GSK-J4. Retinal ganglion cell survival rate after GSK-J4 treatment is analyzed to show the toxicity of the drug. Further, the levels of H3K27me3 and JMJD3/UTX are measured in fixed retina. Finally, GSK-J4-induced widespread changes in gene transcriptional programs are assessed with high throughput approaches, which provides insights into detailed mechanisms of CNS axon regeneration after JMJD3/UTX inhibition.

Disclosures: Q. Li: A. Employment/Salary (full or part-time):; Johns Hopkins University, School of Medicine. **C. Qian:** A. Employment/Salary (full or part-time):; Johns Hopkins University, School of Medicine. **X. Wang:** A. Employment/Salary (full or part-time):; Johns Hopkins University, School of Medicine. **C. Zhang:** None. **A. Ochuba:** None. **F. Zhou:** A. Employment/Salary (full or part-time):; Johns Hopkins University, School of Medicine.

Poster

115. Transplantation and Regeneration in the CNS

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 115.20/B25

Topic: A.04. Transplantation and Regeneration

Title: Resveratrol promotes neural regeneration and blocks ultraviolet stress induced attenuation of neurogenesis

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Abstract: Resveratrol is a polyphenolic compound which is found in various foods. The antioxidant and antiproliferative effects of resveratrol are well characterized and have resulted in the investigation of resveratrol as a potential treatment or adjuvant for treating diseases such as cancer, cardiovascular disease, autoimmune disorders and even attenuation of aging. Considering the widespread effects of resveratrol, we decided to investigate the effect of resveratrol on neural regeneration, using a planarian model system. Planaria were halved and left to regenerate in a 10 uM resveratrol solution, under normal conditions and under stress conditions (UV light) for seven days post amputation. UV light was applied for 12 h per day. With respect to locomotor activity after three days of regeneration, the anterior and posterior ends of the resveratrol exposed group showed a significant increase in mobility. Furthermore, resveratrol was able to rescue the sedentary phenotype that resulted from UV exposure. At 24 hours post halving, the anterior end of the resveratrol exposed groups evidenced a significantly larger population of neurons in the anterior blastema, with UV exposure attenuating neurogenesis. In these planaria, UV exposure increases reactive oxygen species formation and resveratrol works to reduce ROS formation. The sum of our data suggests that the antioxidant properties of resveratrol work to ameliorate environmentally mediated damage to the regenerating nervous system. Our work suggests that resveratrol may be an effective compound to promote neural regeneration.

Disclosures: N. Ferraro: None. **N. Longo:** None. **M. Bavaro:** None. **K. Otten:** None. **S.R. Guariglia:** None.

Poster

115. Transplantation and Regeneration in the CNS

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 115.21/B26

Topic: A.04. Transplantation and Regeneration

Support: Wellcome Trust Grant RG72598
Fight for Sight Grant RG74504
NERC Grant RG91322
Cambridge Eye Trust RG80564
MRC-Sackler PhD Scholarship RG70550

Title: Manipulation of the phosphoinositide 3-kinase pathway promotes axon regeneration

Authors: *R. S. EVANS[1], C. PEARSON[1,2], J. CAVE[1], S. S. DESHPANDE[1], R. CONCEIÇÃO[1], J. W. FAWCETT[1,3], R. EVA[1], K. R. MARTIN[1], A. C. BARBER[1]
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Abstract: We aimed to determine the effect of manipulating the phosphoinositide 3-kinase (PI3K) pathway and assess its effects on axon regeneration *in vivo*. The optic nerve, comprised of retinal ganglion cell (RGC) axons, carries visual signals from the retina to the brain. The optic nerve is easily accessible and provides a robust injury model when crushed behind the eye globe. The PI3K pathway converts phosphatidylinositol (3,4)-bis-phosphate (PIP2) lipids to phosphatidylinositol (3,4,5)-tris-phosphate (PIP3). Phosphatase and tensin homolog (PTEN) acts as a pathway regulator by converting PIP2 back into PIP3. PIP3 is a secondary messenger molecule for a number of pathways, including the mTOR pathway. Others have demonstrated that activation of the mTOR pathway promotes regeneration of both RGCs and cortical neurons. Transgenic mouse lines were generated that express different PI3K isoforms upon Cre recombination. In addition, two viral vectors where generated: AAV2.hyperexpressive-PI3K and AAV2.shPTEN.GFP. Validation studies were performed to confirm successful viral transduction and upregulation of the mTOR pathway. Adult transgenic mice were intravitreally injected with AAV2.Cre.GFP or AAV2.GFP as a control. In addition, adult wildtype C57bl/6 mice were intravitreally injected with either AAV2.hyperexpressive-PI3K, AAV2.GFP, AAV2.shPTEN.GFP or AAV2.shScram.GFP. For each virus, retinal tissues were collected 2 weeks post injection. GFP expression was analysed and mTOR pathway upregulation was assessed using immunohistochemistry (IHC) for pAkt and pS6. Regeneration studies were performed using adult mice who received intravitreal injections of the appropriate viral vector 2 weeks prior to optic nerve crush. The optic nerves and retinas were collected 4 weeks post-crush. Cholera toxin-B was used to visualise axons and the number of regenerating axons was assessed. GFP expression in retinal wholemounts demonstrated efficient transduction across the retina with all viral vectors. In addition, the percentage of GFP-labelled RGCs that co-localised with pS6 and pAkt was quantified. A significant upregulation of pAkt and pS6 was demonstrated in GFP-labelled RGCs in eyes treated with AAV2.Cre.GFP, AAV2.Hyperexpressive-PI3K and AAV2.shPTEN.GFP compared to eyes receiving injection of the control viral vectors including AAV2.GFP and AAV2.shScram.GFP. Together these data indicate successful activation of the mTOR pathway. Finally, a pro-regenerative effect was observed.

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Poster

115. Transplantation and Regeneration in the CNS

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 115.22/B27

Topic: A.04. Transplantation and Regeneration

Support: UWM Research Growth Initiative

Title: Identifying regulatory factors governing regeneration associated gene expression during CNS axon regeneration in zebrafish

Authors: S. P. DHARA[1], A. RAU[3], P. L. AUER[2], N. M. RECKA[1], M. J. FLISTER[4], *A. J. UDVADIA[1]
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Abstract: Axon degeneration accompanying CNS injury or disease typically results in permanent loss of function in human patients. This is largely due to an inability of mammals to reinitiate axon growth in adult CNS neurons. Mammalian optic nerve injury models have been successfully employed to discover signaling pathways that transform mature retinal ganglion cells into a growth competent state. Nevertheless, directed axon regrowth to appropriate brain targets continues to be a challenge for functional CNS regeneration in mammals. Zebrafish share common mechanisms with mammals for constructing the visual circuitry during development, and like mammals, they downregulate developmental growth and guidance signaling pathways during maturation of the visual system. However, unlike mammals, zebrafish respond to optic nerve injury by reinitiating programs of axon growth and guidance, leading to re-establishment of the visual circuitry. To identify pathways that contribute to functional CNS regeneration, we have conducted a temporal analysis of regulatory networks driving successful optic nerve regeneration in zebrafish. We are specifically interested in pathways governing distinct stages of regeneration, including initiation of axon growth, guidance across the midline, and re-innervation of the brain. Here, we examine changes in gene expression (RNA-seq) and chromatin accessibility (ATAC-seq) that accompany each stage of regeneration. Pathway analysis of the temporal changes in gene expression have identified distinct cellular processes characterizing each phase of the regenerative process. We are currently analyzing regeneration associated transcription factors induced in stage-specific patterns, and the accessibility of their putative binding sites. Together, this will enable us to distinguish key regulators of stage-specific transcriptional networks underlying the regeneration process. We anticipate that this approach will reveal key components of the gene regulatory networks that promote successful CNS axon

regeneration in zebrafish. Our long-term goal is to establish the efficacy of such pathways in promoting regeneration in mammalian CNS injury models.

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Poster

116. Axon and Dendrite Development: Axon Growth and Guidance: Adhesion and Cytoskeletal Dynamics

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 116.01/B28

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant F31NS100300
NIH Grant NS04496
NIH Grant NS069688

Title: Pleiotropic roles for Ankyrin-R in the Nervous System

Authors: *S. R. STEVENS[1], J. A. OSES-PRIETO[2], A. L. BURLINGAME[2], M. N. RASBAND[1]
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Abstract: Ankyrin (Ank) proteins, are found throughout the body and act as the primary link between the spectrin-based cytoskeleton and the cytoplasmic domain of many membrane-associated proteins. Although AnkG and AnkB are well recognized as important domain organizers within the nervous system, few studies have investigated AnkR's role. Our lab recently showed AnkR can compensate for a loss of AnkG and cluster Na^+ channels at nodes of Ranvier. Additionally, multiple studies have indicated various neurological disturbances have disruptions in AnkR, including cerebellar dysfunction. However, the role of AnkR in the nervous system remains poorly understood. Our expression analyses show, unlike the other ankyrin proteins found widely throughout the brain, AnkR is highly expressed in the somatodendritic domain of a subset of neurons, including cerebellar Purkinje cells and forebrain parvalbumin-positive (Pv^+) interneurons with 94 ± 1.2% and 93 ± 0.9% of Pv^+ cells expressing AnkR in the cortex and hippocampus, respectively. To elucidate the role of AnkR in these cells, we used the Cre/Lox system to create AnkR conditional knockout mice (AnkR cKOs). Exons 26 and 27 of *Ank1* were flanked by *loxP* sites, upstream of the spectrin binding domain. The Cre-mediated removal of these exons causes a frame-shift mutation resulting in a premature stop codon in exon 28. This strategy is similar to that successfully used to create the AnkB- and AnkG- cKO mice where *loxP* sites flank exons 23/24 and 22/23, respectively. We found AnkR cKO mice have

disrupted gait and cerebellar Purkinje cell degeneration marked by abnormal accumulation of beta-amyloid precursor protein (βAPP) and calbindin-D. In order to identify the molecular mechanisms underlying these changes, we have performed mass spectrometry screens and biochemical analysis yielding AnkR's interactors, which include multiple beta-spectrin binding partners in the brain. Taken together, these data suggest AnkR is important for tethering somatodendritic molecules to the spectrin cytoskeleton in specific neuronal populations.

Disclosures: S.R. Stevens: None. **J.A. Oses-Prieto:** None. **A.L. Burlingame:** None. **M.N. Rasband:** None.

Poster

116. Axon and Dendrite Development: Axon Growth and Guidance: Adhesion and Cytoskeletal Dynamics

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 116.02/B29

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant NS044916
NIH Grant NS069688

Title: β-spectrins maintain Na^+channels at axon initial segments and nodes of Ranvier

Authors: *C.-H. LIU[1], S. STEVENS[1], R. SEO[1], P. MOHLER[2], J. NOEBELS[1], M. STANKEWICH[3], M. RASBAND[1]
[1]Baylor Col. of Med., Houston, TX; [2]The Ohio State Univ., Columbus, OH; [3]Yale Univ., New Haven, CT

Abstract: Highly-concentrated ion channels at axon initial segments (AIS) and nodes of Ranvier are necessary to initiate and regenerate action potentials in axons. The cytoskeletal protein β4-spectrin is proposed to stabilize voltage-gated sodium (Nav) channels at nodes. Previous studies suggested that β1-spectrin can preserve nodal Nav channels after loss of β4-spectrin. However, the necessity of β-spectrins for Nav channel clustering at nodes has not been elucidated. Furthermore, the function of β1-spectrin in the nervous system is unknown. To determine if β1 and β4-spectrin are required at the nodes and AIS, we generated mice lacking these proteins in neurons. Mice lacking β1-spectrin retained Nav channel clustering at nodes, and loss of β4-spectrin also showed normal Nav clustering due to the compensatory effects from β1-spectrin. Mice lacking both β1- and β4-spectrin showed ataxia and reduced conduction velocity of compound action potentials. With increasing age, there was progressive loss of nodal Nav channels. In addition, loss of β1-spectrin in the central nervous system showed normal AIS integrity, whereas loss of β4-spectrin reduced Nav channel immunoreactivity. Unexpectedly, β1-spectrin was only detected at the AIS of parvalbumin-positive interneurons in β4-spectrin

deficient mice. Mice lacking both β1 and β4-spectrin showed severe seizures throughout development. These data suggest that β-spectrins are necessary to maintain Nav channels at axonal excitable domains. Furthermore, β4-spectrin is the primary stabilizer, while β1-spectrin performs secondary functions in a context-dependent manner.

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Poster

116. Axon and Dendrite Development: Axon Growth and Guidance: Adhesion and Cytoskeletal Dynamics

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 116.03/B30

Topic: A.05. Axon and Dendrite Development

Support: Research Grants Council Hong Kong
Health and Medical Research Fund (Hong Kong)
CUHK direct grant scheme
United College endowment fund
TUYF Charitable Trust

Title: Neuronal adaptor FE65 interacts with ELMO1 to stimulate neurite outgrowth

Authors: *K.-F. LAU[1], W. LI[2], W. CHAN[2]
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Abstract: FE65, also known as amyloid beta precursor protein binding family B member 1, is a brain-enriched adaptor that has been shown to stimulate Rac1-mediated neurite outgrowth. However, how FE65 promotes neurite elongation remains elusive. In a yeast-two hybrid screen, ELMO1, a subunit of ELMO1-DOCK180 bipartite Rac1 GEF, is found to be a novel FE65 N-terminal region interactor. In rat primary neurons, overexpression of FE65 and/or ELMO1 enhances whereas knockdown of FE65 or ELMO1 inhibits neurite outgrowth and Rac1 activation. The effect of FE65 alone or together with ELMO1 is attenuated by an FE65 double mutation that disrupts FE65-ELMO1 interaction. Intriguingly, FE65 is found to activate ELMO1 by diminishing ELMO1 intramolecular autoinhibitory interaction and to promote the targeting of ELMO1 to the plasma membrane where Rac1 is activated. Our study reveals a new mechanism that FE65 stimulates Rac1-mediated neurite outgrowth by the recruitment and activation of ELMO1.

Disclosures: K. Lau: None. **W. Li:** None. **W. Chan:** None.

Poster

116. Axon and Dendrite Development: Axon Growth and Guidance: Adhesion and Cytoskeletal Dynamics

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 116.04/B31

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant 5R01NS081674-05
NIH Grant 5R01NS083378-15

Title: Substrate specific regulation of CDK5 activity by intermediate filaments in developing cortical axons

Authors: *C. BOTT[1,2], C. YAP[2], B. WINCKLER[2]
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Abstract: The roles of intermediate filaments (IFs) are often thought to be primarily structural, including IFs in neurons, but IFs have also been implicated in regulating kinase signaling pathways in many cell types. Nestin is a unique IF protein required for Cyclin Dependent Kinase 5 (CDK5) activity and localization during development of neuromuscular junctions through a direct interaction between nestin and the CDK5 activator p35. In neurodevelopment, nestin is well established as a neural stem cell marker, but we have identified a population of embryonic cortical neurons (*in vitro* and *in vivo*) that transiently continue to express nestin in their axons early in the process of differentiation. The transient expression of nestin in neurons raised the question of what roles neuronal nestin might play, and we hypothesized that nestin may affect CDK5 dependent processes in neurons. Semaphorin 3A is an early developmental axon repulsive guidance cue and known to activate CDK5. We now show that nestin-expressing neurons are more sensitive to Semaphorin 3A filopodial retraction and growth cone collapse *in vitro*, compared to neurons not expressing nestin. We then evaluated a number of known CDK5 substrates for nestin binding and identified doublecortin (DCX), a microtubule binding protein important for growth cone guidance, as a novel nestin binding protein. Biochemical experiments suggest that nestin acts a scaffold for active CDK5 and DCX, and promotes phosphorylation of DCX. Phosphorylated DCX has a decreased affinity for microtubules, leading to a decrease in microtubule stability, and to greater sensitivity to Semaphorin3a. In agreement, we found that DCX null neurons were more sensitive to Semaphorin3a, but, unlike wild-type neurons, in a nestin independent manner. This suggests that nestin acts as a Semaphorin3a “gain control” modulator through its interactions with DCX, to allow for different levels of microtubule stability and responsiveness to Semaphorin3a. Evaluation of other neuronal IFs reveal that that Neurofilament Medium (NF-M), which is expressed later in neuronal maturation, suppresses CDK5-mediated DCX phosphorylation. Temporally expressed scaffolds (such as nestin and NF-

M) may allow for substrate specific regulation of CDK5 activity and set up different maturational states of neurons with distinct response profiles to extracellular cues. How this affects axon targeting *in vivo* is a concern for future investigations.

Disclosures: C. Bott: None. **C. Yap:** None. **B. Winckler:** None.

Poster

116. Axon and Dendrite Development: Axon Growth and Guidance: Adhesion and Cytoskeletal Dynamics

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 116.05/B32

Topic: A.05. Axon and Dendrite Development

Support: National Research Foundation of Korea (NRF), funded through the Ministry of Science, ICT South Korea (2018R1A2A2A05023615)

Title: Role of a small GTPase, Rheb, (Ras homologue enriched in brain) in axon formation in developing telencephalon

Authors: S. CHOI, J.-E. KANG, A. SADRA, *S.-O. HUH
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Abstract: Rheb (Ras homolog enriched in the brain) is a small GTP-binding protein and it plays an important role in cell signaling through mTORC1 (mammalian-target-of-rapamycin-complex-1), an essential regulator of the different biological process including protein synthesis and cytoskeleton formation. During development, Rheb's role in maintaing callosal axon projections in the brain is relatively unknown and promoting axonal elongation may have applications for a number of neurological disorders including autism. With a Rheb-dominant negative (DN) overexpression, it reduced mTORC1 activity, and promoted axonogenesis. However, treatment with rapamycin, a specific inhibitor of mTORC, reduced both mTORC1 and axonogenesis. With Rheb-DN overexpression, Rab35 was activated to promote axonal elongation with rapamycin-treatment not following a similar pathway in axonogenesis. Rab35-DN and Rheb silencing offset the positive effects of Rheb-DN on axonogenesis. The test model for Rheb-DN overexpression and its effect on callosal axon elongation was transfection of Rheb-DN via in utero electroporation (IUE) in the ventricles of mouse embryo by) at days E13.5 and E15.5 and observing callosal axon projections at E18.5 and P2. Rheb-DN transfected neurons had a longer axonal extension than the control DNA transfected neurons and we found that with the Rheb-DN mutant transfection the projections of callosal axon could be maintained with proper axon fiber conditions. As such, there could be therapeutic applications with the Rheb-DN construct for a number of neurological conditions due to defective axonal growth.
Significance statement

Contralateral axons projections from layer II/III neurons and in ASD callosal projections degenerate rapidly. One of possible cures for ASD could be restoration of the callosal projections. Rheb-DN therapy would be a solution for such a degenerative disorder. Moreover, this study provides an insight to the molecular pathways involving Rheb and Rab35 without involving mTORC1.
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Poster

116. Axon and Dendrite Development: Axon Growth and Guidance: Adhesion and Cytoskeletal Dynamics

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 116.06/C1

Topic: A.05. Axon and Dendrite Development

Support: BBSRC 6542

Title: The roles of Pax6 in regulating embryonic development of the prethalamus

Authors: *T. TIAN[1], C. GIASAFAKI[2], I. QUINTANA-URZAINQUI[2], T. PRATT[2], D. PRICE[2]
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Abstract: The transcription factor Pax6 is a pleiotropic player during neural development. In the central nervous system, Pax6 is mostly expressed by neural progenitors, where its functions have been most extensively studied. However, in the anterior diencephalon, the prethalamus, Pax6 is expressed in both neural progenitors and post-mitotic neurons. This distinctive expression pattern of Pax6 makes the prethalamus a unique place in which to further explore the functions of Pax6 and its mechanisms of action during development.
We have found that Pax6 removal from the prethalamus significantly deregulates the activity of various genes involved in canonical and non-canonical Wnt-signaling pathways. Our data indicate that in the prethalamic ventricular zone, where neural progenitors reside, Pax6 seems to suppress the expression of various canonical Wnt-signalling pathway effectors by promoting the expression of antagonists of Wnt-signaling pathways, such as Sfrp2 and Dkk3.
However, in post-mitotic prethalamic neurons, Pax6 seems to regulate the process of neuronal morphogenesis. Gene ontology analysis on our unpublished RNAseq data revealed that when Pax6 is removed from the prethalamus, genes involved in neuritogenesis, establishment of neuronal polarity, axon elongation and axon initial segment (AIS) were significantly differentially expressed.

To further explore this, we performed dissociated cell cultures of prethalamus at embryonic day 13.5. Various aspects of neuronal morphogenesis were analysed in these primary neurons cultured for 1-9 days in vitro (DIV). We found that Pax6-null prethalamic neurons constantly displayed fewer neurites and a disturbed rate of neurite elongation. Additionally, they showed expansion of AIS into their distal axons and dendritic branches, indicating a miss-specification of axonal-dendritic identities in these neurons when Pax6 is lost.
These results point to a cell-autonomous regulation of neuronal morphogenesis by Pax6 in the prethalamic post-mitotic neurons. Investigation on establishment of neuronal polarity and in vivo studies of neuronal morphogenesis of these prethalamic post-mitotic cells are currently underway.
These results reveal novel and distinct functions of Pax6 in prethalamic cells of different developmental stages, providing insight into the temporal and spatial regulation governed by master regulators such as Pax6 during brain development.

Disclosures: T. Tian: None. **C. Giasafaki:** None. **I. Quintana-Urzainqui:** None. **T. Pratt:** None. **D. Price:** None.

Poster

116. Axon and Dendrite Development: Axon Growth and Guidance: Adhesion and Cytoskeletal Dynamics

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 116.07/C2

Topic: A.05. Axon and Dendrite Development

Support: a National Research Foundation of Korea (NRF) (NRF-2017R1A2B4001846)
the Korean government (MSIP) (2016R1A5A2945889)

Title: Id2 regulates axon elongation, controlling α-tubulin acetylation

Authors: *T. YUN[1,2], H. KO[1,2], J.-Y. AHN[1,2]
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Abstract: The α-tubulin is a component of microtubules which are cytoskeletal organization of axons and growth cones and α-tubulin acetylation is associated with axon elongation. Sirt2, NAD-dependent deacetylase, plays a role in microtubules deacetylation in nervous system. In this study, we demonstrated that Inhibitor of DNA binding 2 (Id2) binds to α-tubulin and the interaction of Id2-α-tubulin disrupts Sirt2-α-tubulin complex formation. Moreover, we found that Id2 inhibits deacetylation of α-tubulin, inhibiting the access of Sirt2 to α-tubulin. Furthermore, Id2 localizes to the growing plus-ends of microtubules and interacts with plus-end tracking

protein (+TIP) EB3. Thus, our data suggests that Id2 promotes axon elongation acting as microtubules +TIPs, controlling α-tubulin acetylation.

Disclosures: T. Yun: None. **H. Ko:** None. **J. Ahn:** None.

Poster

116. Axon and Dendrite Development: Axon Growth and Guidance: Adhesion and Cytoskeletal Dynamics

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 116.08/C3

Topic: A.05. Axon and Dendrite Development

Title: Changes to neuronal cytoskeleton in response to small molecule modulators of GAG biosynthesis

Authors: *C. MENCIO, S. TILVE, C. AGBAEGBU, H. KATAGIRI, H. GELLER
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Abstract: Consisting of an aglycone attached to a xylose residue, xylosides are known to affect glycosaminoglycan (GAG) biosynthesis both *in vitro* and *in vivo.* Most commonly utilized in research at high concentrations (≥1mM), xylosides inhibit endogenous GAG chain production. At these high concentrations, xyloside treatment shows no obvious effect on neuronal morphology. However, we have found that acute treatment of isolated neurons with significantly lower concentrations of xyloside (≤500nM) leads to altered neuronal cytoskeleton *in vitro* evidenced by enlarged growth cones and lamellipodia. In enlarged growth cones, it is apparent that both microtubules (MTs) and actin are misregulated. MTs show extensive looping and altered F-actin staining. Examining two of the major players in actin turnover and MT stabilization, we found changes in phosphorylation in both cofilin and GSK3β signaling pathways. Additionally, increased expression levels of some cytoskeleton related proteins have been observed implicating possible abnormal regulation of gene expression. Chronic low concentration xyloside treatment of more dense neuron cultures leads to a significant increase in area of the well covered by neurites as compared to controls, a marked change from the high level of fasciculation that occurs with chronic high concentration xyloside treatment. This research indicates that exploring LCX treatment may provide new insights into the role of GAGs in regard to cytoskeleton stabilization and how xylosides may have additional actions to their being PG synthesis inhibitors.

Disclosures: C. Mencio: None. **S. Tilve:** None. **C. Agbaegbu:** None. **H. Katagiri:** None. **H. Geller:** None.

Poster

116. Axon and Dendrite Development: Axon Growth and Guidance: Adhesion and Cytoskeletal Dynamics

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 116.09/C4

Topic: A.05. Axon and Dendrite Development

Support: DBT-IISC partnership program

Title: Structure function analysis of the focal adhesion protein, vinculin in neocortical axon growth

Authors: *P. MANDAL, D. K. NAIR, V. BELAPURKAR, N. RAMANAN
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Abstract: Axon growth is promoted by several extracellular signals, which activate a complex network of intracellular effectors including signaling cascades and cytoskeletal proteins at the growth cone that convey information to critical transcription factors in the nucleus. This growth cone-to-nucleus signaling to coordinate gene expression is critical for sustained axon extension and connectivity both during development and for axon regeneration after injury. The stimulus-dependent transcription factor, serum response factor (SRF), is a major effector of gene expression, necessary for axon growth, downstream of actin-treadmilling in the growth cone. We had previously shown SRF is critical for axon growth during development *in vivo,* and *in vitro.* Following activation by GSK-3, SRF forms a complex with its cofactors, MRTF-A and -B and results in the expression of the actin-binding and focal adhesion protein, vinculin. Knockdown of vinculin resulted in attenuated axon growth in vitro. However, the molecular mechanisms by which vinculin regulates axon growth is poorly understood. In this study, we carried out structure-function analyses to understand how vinculin regulates axon growth. Vinculin has a head, neck and tail domain. It interacts with talin and integrin receptor through the head domain and helps to tether F-actin to focal adhesion site through the tail domain. We expressed several mutant forms of vinculin which were either truncated to express only the head or tail domains or were defective in binding with other proteins (talin, PIP2) in neocortical primary neurons *in* vitro. Interestingly we found expression of only the tail domain of vinculin (Vin-T) caused excessive branching in neurons with significantly shorter axons. Furthermore, when we mutated the actin binding region of vinculin from the tail part, this branching decreased. Through live imaging we found out that tail domain caused decrease in actin retrograde flow. Analysis at very early time point (12 hours) showed that Vin-T expressing neurons had more number of filopodia. In contrast, a constitutively active form of vinculin showed faster growth of axons and significantly longer axons both *in vitro* and *in vivo*. Interestingly, mutation of the talin binding domain did not have any appreciable effect on axon growth. Together, our findings suggest that

talin binding might be dispensable for vinculin function while constitutively active vinculin can promote faster axon growth

Disclosures: P. Mandal: A. Employment/Salary (full or part-time):; CSIR. **D.K. Nair:** None. **V. Belapurkar:** None. **N. Ramanan:** None.

Poster

116. Axon and Dendrite Development: Axon Growth and Guidance: Adhesion and Cytoskeletal Dynamics

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 116.10/C5

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant NS044916
NIH Grant NS069688
Adelson Medical Research Foundation

Title: A molecular dissection of the axon initial segment

Authors: *B. LIM[1], H. HAMDAN[1], M. KONNING[1], A. JOSHI[1], T. TORII[1], C. SMITH[1], J. OSES-PRIETO[2], A. BURLINGAME[2], M. RASBAND[1]
[1]Neurosci., Baylor Col. of Med., Houston, TX; [2]Mass Spectrometry Facility, Univ. of California, San Francisco, San Francisco, CA

Abstract: The axon initial segment (AIS) initiates action potentials and maintains neuronal polarity, and its disruption is prominently associated with nervous system diseases and injuries. It is located at the proximal axon and is defined by clustering of ankyrin-G (AnkG), neurofascin-186 (NF186), and βIV-spectrin, as well as voltage-gated ion channels. However, these known proteins likely represent only a subset of the 'toolkit' necessary for AIS function, assembly, and maintenance. For example, despite a few recent studies, the molecular determinants of neuronal polarity and vesicular trafficking at the AIS remain poorly understood. To identify the AIS interactome, we conducted an unbiased screen via proximity-dependent biotinylation in cultured hippocampal neurons. We introduced constructs containing known AIS proteins (NF186, NDEL1, and TRIM46) tagged with the biotin ligase BirA*, and identified neighboring biotinylated proteins after streptavidin pulldown and mass spectrometry. Importantly, the constructs target to different subdomains of the AIS and produced partially overlapping datasets that also contain other known AIS proteins. Here, we demonstrate the utility of this approach and identify a number of putative AIS components with roles in cytoskeletal organization and vesicle trafficking. Our results show that some of these candidates localize to the AIS and are capable of interacting with a variety of known AIS proteins. Furthermore, we investigated the role of these candidates through loss-of-function and gain-of function experiments and found significant

disruptions to AIS formation and maintenance. In summary, we have begun to map the AIS interactome and propose these studies provide a path for future studies of the AIS.

Disclosures: B. Lim: None. **H. Hamdan:** None. **M. Konning:** None. **A. Joshi:** None. **T. Torii:** None. **C. Smith:** None. **J. Oses-Prieto:** None. **A. Burlingame:** None. **M. Rasband:** None.

Poster

116. Axon and Dendrite Development: Axon Growth and Guidance: Adhesion and Cytoskeletal Dynamics

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 116.11/C6

Topic: A.05. Axon and Dendrite Development

Support: Neurological Foundation of New Zealand, Postdoctoral Fellowship 1641WF
Maurice and Phyllis Paykel Trust of New Zealand
Marsden Fund of New Zealand
Marine Biological Laboratory Post-course research grant

Title: A developmental role for the extracellular matrix sugar hyaluronan in regulating hippocampal neurite growth *in vitro*

Authors: *R. N. KARUNASINGHE[1,2], M. I. ABRAHAM[1], T. M. FOWKE[1], T. TANI[2], J. M. DEAN[1]
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Abstract: The targeted growth of neurons is a key event in development, and is fundamental for establishing neural circuitry. Hippocampal neurons develop in a morphologically stereotyped manner, involving the rapid outgrowth of processes (neurites) from their soma, and guided by actin-rich, sensory-motor structures called ‘growth cones’. However, the specific mechanisms that regulate neurite extension remain to be understood. Recent evidence implicates the brain extracellular matrix molecule hyaluronan in the control of mature synaptic functions. However, the role of hyaluronan in neuronal development is largely unknown. The aim of the present study was to examine the expression and function of neuronal hyaluronan in hippocampal neurite development. Dissociated hippocampal neuron cultures devoid of glia were generated from Sprague Dawley rat embryos (E18), and collected at 1-3 days *in vitro*. Cultures were fixed for immunocytochemical localisation studies. Live-cell images were obtained with a Nanolive 3D Cell explorer microscope, a label-free and non-invasive technique to resolve the leading edge of each neurite. Actin cytoskeleton dynamics were visualised with the fluorescent probe SiR Actin (40 nM, in phenol-red free neurobasal media), and live recordings were made using total internal reflection fluorescence (TIRF) microscopy. We found a neuron-specific expression of hyaluronan synthase (HAS 2-3) enzymes and the CD44 hyaluronan receptor on neuronal soma,

neurites, and growth cones (n=20 cells from 4 independent cultures). Pharmacological inhibition of hyaluronan synthesis with 4-methylumbelliferone (4-MU; 300μM) reduced the size and number of growth cones at DIV 1-3, and increased the length of neurites, compared with DMSO vehicle-control cultures (for each treatment, n=10 neurons from 2 cultures; images were processed by morphological tracings using Neurolucida software and blinded to the treatment). Further, in preliminary TIRF recordings, 4-MU increased the speed of cytoskeletal actin trafficking in neurites (quantified as an increased rate of SiR Actin signal recovery after photobleaching; n=11 cells) compared with DMSO controls (n=8 cells). These data suggest a role of hyaluronan, synthesised by neurons, in the early development of the neuronal cytoskeleton, including growth cones and neurites.

Disclosures: R.N. Karunasinghe: None. **M.I. Abraham:** None. **T.M. Fowke:** None. **T. Tani:** None. **J.M. Dean:** None.

Poster

116. Axon and Dendrite Development: Axon Growth and Guidance: Adhesion and Cytoskeletal Dynamics

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 116.12/C7

Topic: A.05. Axon and Dendrite Development

Support: Z01 NS003013

Title: A novel mechanism for axon growth revealed by live imaging of a pioneer axon in its native tissue

Authors: *H. FANG[1], A. CLARKE[1], R. KANNAN[2], P. MCQUEEN[1], S. WINCOVITCH[1], E. GINIGER[1]
[1]NIH, Bethesda, MD; [2]Natl. Inst. of Mental Hlth. and Neurosciences, Bangalore, India

Abstract: We show that live imaging of the TSM1 growth cone in the developing *Drosophila* wing reveals a novel mechanism of axon growth that is fundamentally different from the familiar adhesion-clutch model of growth cone motility. For a growth cone like that of TSM1 that is dominated by filopodia, rather than lamellipodia, we find that actin accumulates in a concentrated bolus in the distal portion of the extending axon, and that the axon advances by 'inchworming' of this actin mass, with cycles of de-condensation of the actin, followed by its re-condensation at a slightly more distal position. Additionally, we show that the actin mass locally creates a zone of active filopodial protrusion. Advance of the actin mass therefore causes advance of the protrusive zone, which in turn leads to directed extension of the axon. Specifically, we find that the peak of the actin distribution leads the peak of filopodial density by 3.2 ± 0.62μm on average, corresponding to a time delay of about 12-15 minutes between

advance of the actin and consequent advance of the active filopodial domain. We further show that the Abelson tyrosine kinase (Abl), which is an essential target of many guidance receptors, appears to facilitate axon growth primarily by regulating actin organization. Abl knockdown (KD) and overexpression (OE) cause the actin distribution to be grossly fragmented, and its orderly evolution over time to be disrupted, as revealed by quantitative metrics derived from information theory. Our analysis also reveals a systematic, dose-dependent modulation of the width of the actin bolus in these experimental conditions. Strikingly, Abl KD by RNAi causes an aberrant end-stage phenotype in 45% of TSM1 axons, which is enhanced to 84% by inactivation of a single copy of the Abl gene. Axonal defects also are observed in the Abl OE condition. These data strongly suggest that organized fluctuations in the structure of the actin mass is critical for TSM1 growth cone motility and axon extension. Taken together, our data suggest a simple, but unexpected, model for the regulation of axon growth, with receptors that activate Abl promoting extension of the actin mass, and thus the growth cone, toward the source of the signal, and receptors that reduce Abl activity promoting local consolidation of the growth cone. This work reveals a novel mechanism of growth cone motility, and one that can account for the growth and guidance of many axons, particularly pioneer axons in the developing nervous system.

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Poster

116. Axon and Dendrite Development: Axon Growth and Guidance: Adhesion and Cytoskeletal Dynamics

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 116.13/C8

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant NS044916
NIH Grant NS069688

Title: NuMA1 regulates the axon initial segment formation during early development

Authors: *T. TORII[1], T. S. HO[1], J. OSES-PRIETO[2], A. L. BURLINGAME[2], M. N. RASBAND[1]
[1]Neurosci., Baylor Col. of Med., Houston, TX; [2]Univ. of California San Francisco, San Francisco, CA

Abstract: The axon initial segment (AIS) initiates action potentials in neurons and is characterized by proteins including ankyrinG and Na^+ channels. However, the molecular mechanisms of AIS assembly and/or maintenance are still being discovered. To identify these

mechanisms, we determined down-regulated proteins in AIS-deficient mouse brain. Specifically, we performed a comprehensive proteome analysis using wild type mouse brain and Nestin-Cre; ankG$^{fl/fl}$ mouse brain homogenate. We identified nuclear mitotic apparatus protein 1 (NuMA1), which was significantly down-regulated in ankG KO mice compared to WT mice. NuMA1 is well known as a cell division regulator. However, we confirmed NuMA1 is also expressed in post-mitotic neurons and is enriched at the AIS. Also, the expression of NuMA1 increases through DIV7 and then decreases during later maturation. Thus, NuMA1 may play a role at the AIS as well as in cell division.
To determine if NuMA1 is associated with AIS function or not, we performed loss-of-function studies. Knockdown of NuMA1 by shRNA causes AIS disruption in early stage hippocampal neurons (DIV2-6), but not in middle-stage neurons (DIV9-12 and DIV9-16), suggesting that early stages of AIS formation require NuMA1, and NuMA1 does not contribute to AIS maintenance in neurons. Moreover, overexpression of NuMA1 and its truncated mutants disrupted AIS formation. Taken together, NuMA1 is transiently found at AIS and regulates AIS formation during development. We also confirmed the association between NuMA1 and the cytoskeletal protein 4.1B, which is regulated by cdk1/2-dependent phosphorylation. Notably, we found detergent insoluble 4.1B at the AIS. Our results suggest AIS structure is modulated in developing neurons by the interaction between NuMA1 and 4.1B. Since NuMA1 binds to Lis1 and dynein, which associates with Ndel1 and/or Nde1, the signal transduction may affect AIS function through Lis1/Ndel1/dynein-associated proteins.

Disclosures: T. Torii: None. **T.S. Ho:** None. **J. Oses-Prieto:** None. **A.L. Burlingame:** None. **M.N. Rasband:** None.

Poster

116. Axon and Dendrite Development: Axon Growth and Guidance: Adhesion and Cytoskeletal Dynamics

Location: SDCC Halls B-H

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Topic: A.05. Axon and Dendrite Development

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Title: Cyclin G2 is a cell cycle inhibitor well expressed in neurons that associates with β-catenin complexes in cerebellar and hippocampal tissues

Authors: A. HERGARDEN[1], A. S. A. DON[2], K. KIM[1], M. LE MAROIS[1], J. W. HELL[1,2], *M. C. HORNE[1,2]
[1]Pharmacol., Univ. of California Davis, Davis, CA; [2]Pharmacol., Univ. of Iowa, Iowa City, IA

Abstract: Cyclin G2 (CycG2) is an unconventional PP2A-associated cyclin homolog that antagonizes cellular proliferation. Here, we investigate the role of CycG2 in rodent hippocampal and cerebellar neurons. Previous findings showed that CycG2 gene (CCNG2) transcripts are enriched in adult mammalian brain tissue, particularly in the cerebellum. We determined that CCNG2 mRNA levels are substantially increased during cerebellar development, reaching peak levels as cerebellar granule cell (GC) neuron precursors exit the cell cycle and differentiate into neurons. This observation was subsequently substantiated by online cDNA microarray and in situ hybridization data confirming that CCNG2 transcripts are most abundant in human and rodent cerebellar and hippocampal tissues. We now show by immunoblot analysis and immunofluorescence microscopy that CycG2 protein expression is abundant in whole lysate and primary neuronal cultures made from rodent hippocampal and cerebellar tissues. Our biochemical analysis determined that CycG2 is abundant in rodent neuronal synaptosome fractions. Moreover, immunostaining of rat dissociated hippocampal cultures reveals that CycG2 localizes proximate to synaptic markers such as PSD-95 and Bassoon and super resolution STED microscopy has more precisely determined the proximity of CycG2 relative to these and other synaptic proteins. As our previous work demonstrated that CycG2 forms a catalytically active complex with PP2A/B' and C subunits in cerebellar tissues, we examined whether CycG2 interacts with other PP2A binding partners, including β-catenin. Immunoprecipitation and pulldown experiments provide compelling evidence that CycG2 directly associates with β-catenin, and have narrowed down the binding site to less than 50 residues distinct from the PP2A binding site in the carboxy terminus of CycG2. Interestingly, recent studies by others link CycG2 with β-catenin/Wnt signaling in mitotic cell types. Investigating the potential association of CycG2 with other β-catenin binding partners, we discovered that CycG2 co-immunoprecipitates with not only N-cadherin and α-N-catenin but also AKAP150, which acts as a scaffold for cAMP signaling proteins at postsynaptic sites. These interactions suggest that CycG2 may participate the structural stability of the synapse. We present our analysis of CycG2 subcellular distribution, associations and interactions with β-catenin/N-cadherin complexes and other potential binding partners. Our ongoing studies are investigating the consequences of CRISPR mediated CycG2 knockout on neuronal, synaptic, and dendritic morphology and β-catenin-associated complexes.

Disclosures: A. Hergarden: None. **A.S.A. Don:** None. **K. Kim:** None. **M. Le Marois:** None. **J.W. Hell:** None. **M.C. Horne:** None.

Poster

116. Axon and Dendrite Development: Axon Growth and Guidance: Adhesion and Cytoskeletal Dynamics

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 116.15/C10

Topic: A.05. Axon and Dendrite Development

Support: TWU Department of Biology
Dr. DiAnna Hynds

Title: Identifying signaling cascades involved in downregulation of Cofilin by functional, non-prenylatable RhoA and Rac1

Authors: *N. G. RAUT[1], J. M. REDDY[1], D. L. HYNDS[2]
[1]Biol., [2]Texas Woman's Univ., Denton, TX

Abstract: RhoA and Rac1 are small guanosine triphosphates (GTPases) known for their roles in regulating cytoskeletal rearrangements, cell motility, cell polarity, axon guidance, vesicle trafficking and the cell cycle. Rho GTPases also are critical regulators of signal transduction pathways in eukaryotic cells. Rho GTPases are active and able to interact with downstream effectors when they are bound to guanosine triphosphate (GTP) and inactive when bound to guanosine diphosphate (GDP). Rho GTPase signaling for cytoskeletal dynamics involves recruitment of a variety of downstream serine/threonine kinases including p21-activated kinase (PAK), mixed-lineage kinase (MLK), Rho-kinase (ROK), and protein kinase novel (PKN). Activated Rho GTPases, working through these kinases, can either stabilize actin filaments by inhibiting the activity of cofilin, or promote actin polymerization by activating actin nucleators. Rac1 activation of PAK or RhoA activation of ROK activates LIM kinases (LIMKs) that phosphorylate and inactivate the actin severing protein cofilin. Western blot and confocal microscopic analyses show a decrease in cofilin in the cytosol compared to that with associated with membranes after transfection with the wild-type RhoA. We have found transfection of these constructs in rat cortical neurons increase neurite outgrowth (for non-prenylatable RhoA) and neurite formation (for non-prenylatable Rac1). Both retained the ability to be made active independent of membrane targeting by prenylation. With emerging evidence of differential activation of these Rho GTPases based on their subcellular localization, elucidating the signaling cascades of the active GTPases may identify the distinct functions of these GTPases in the cytosol and can be used as novel targets to facilitate axon regeneration in neurodegenerative and neurological conditions.

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

Poster

116. Axon and Dendrite Development: Axon Growth and Guidance: Adhesion and Cytoskeletal Dynamics

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 116.16/C11

Topic: A.05. Axon and Dendrite Development

Title: Diaphanous Proteins in Filopodia

Authors: *A. HLEIHEL[1], D. L. HYNDS[2]
[1]Mol. Biol., Texas Wellmans Univ., Denton, TX; [2]Texas Woman's Univ., Denton, TX

Abstract: Proper human nervous system development requires neuronal differentiation and migration from birthplace into their final destination, where each type of neuron extends processes and establishesconnections with appropriate set of target cells. Many polarity proteins are involved in the molecular mechanisms that regulate the migration and differentiation, affecting the actin cytoskeleton to regulate growth cone motility, axon guidance, and development of dendritic spines. In newly born neurons, filamentous (F) actin rich structures, including lamellipodia (sheet-like) and filopodia (finger-like projections) are formed at the periphery of the immature neurons. These structures coalesce into minor neurites that eventually one of them will elongate as an axon and the rest as dendrites. At the tip of the elongating axon, a well-defined and organized growth cone. It is divided into peripheral, central, and transition zone domains. Filopodia in the peripheral domain contain bundled linear actin filament structures. Many actin binding proteins nucleate actin and promote polymerization and bundling. Formins are multi-domain proteins that nucleate and polymerize actin filaments, with the Diaphanous (Dia) subfamily regulating neurite initiation by promoting filopodia initiation and elongation. Rho guanosine triphosphatases (GTPases) regulate function of mammalial Diaproteins, but the molecular mechanisms directing these interactions are incompletely defined. In the current work, we hypothesize that Rho GTPase activate mDia 2, which interacts with additional actin nucleating proteins to promote growth cone filopodial extension. We have determined, using immunocytochemistry, real-time reverse transcription polymerase chain reaction and western blotting that mDia 2 is expressed in B35 neuroblastoma cells, while primary rat cortical neurons express mDia 1 and 3. Co-immunoprecipitation and knock-down studies are being performed to determine how different mDia proteins affect filopodial formation. We expect results from these studies to determine novel targets for treating neurodevelopmental or neurodegenerative conditions.

Disclosures: A. Hleihel: None. **D.L. Hynds:** None.

Poster

116. Axon and Dendrite Development: Axon Growth and Guidance: Adhesion and Cytoskeletal Dynamics

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 116.17/C12

Topic: A.05. Axon and Dendrite Development

Support: TWU Department of Biology

Title: Subcellular localization of CDC42: Implications for neurite extension

Authors: *K. P. BOON[1], D. HYNDS[2]
[2]Biol., [1]Texas Woman's Univ., Denton, TX

Abstract: Cdc42, a small GTPase belonging to the Rho sub-family, acts as a molecular on/off switch for signaling cascades that affect cell motility, morphology, and neural plasticity. Like other GTPases Cdc42 is active when bound to GTP, facilitated by guanine exchange factors (GEFs), and inactive when bound to GDP, catalyzed by GTPase activating proteins (GAPs). Active GTP-bound Cdc42 can interact with downstream effectors, but another form of regulation occurs by the binding of guanine dissociation inhibitors (GDIs) to inactive GDP-bound Cdc42, sequestering it to the cytosol. The dogma of Rho GTPase activation has been that it must undergo post-translational modification of prenylation to colocalize at the membrane with GEFs. Findings from our lab of activated non-prenylatable RhoA in the cytosol leads us to believe that prenylation is not needed for activation, but is a subset of signaling pathways for Cdc42. As well as canonical Cdc42, a splice variant found in the brain, bCdc42, differs in the c-terminus exon. Canonical Cdc42 carboxy-terminal ends with -CVLL and bCdc42 ends in -CCIF, the double cysteine at the c-terminal allows the splice variant bCdc42 to undergo both post-translational modifications of prenylation and palmitoylation.
To investigate the activation of Cdc42 in different subcellular locations, we have constructed a YFP-fused Cdc42 mutant by site-directed mutagenesis at the carboxy-terminal end to change the cysteine to an alanine therefor preventing prenylation by preventing the recognition for geranylgeranylation. To avoid any mistaken effector signaling of palmitoylated bCdc42 for our mutant Cdc42, we have acquired a mutant that prevents palmitoylation as well. Restriction digest mapping and sequencing indicate we have constructed the appropriate vectors. Current experiments are assessing how expressing Cdc42 that cannot translocate to the plasma membrane affect the development of axonal and dendritic processes. Furthermore, we hope to explore how this difference in subcellular localization affects activation and the signaling pathways of active non-prenylatable/non-palmitoylatable mutant Cdc42. It is likely that these experiments will elucidate novel sites for therapeutic interventions in neurodevelopmental, neurotraumatic and neurodegenerative conditions.

Disclosures: K.P. Boon: None. **D. Hynds:** None.

Poster

116. Axon and Dendrite Development: Axon Growth and Guidance: Adhesion and Cytoskeletal Dynamics

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 116.18/C13

Topic: A.05. Axon and Dendrite Development

Support: R01 MH062723

Title: Intrinsic regulatory factors governing formation of serotonergic ascending and descending axonal architectures

Authors: *L. J. DONOVAN, E. S. DENERIS
Neurosciences, Case Western Reserve Univ., Cleveland, OH

Abstract: Of the estimated 86 billion neurons in the human brain only about 400,000 make and use serotonin (5-HT) as a neurotransmitter. Yet, serotonin appears to modulate nearly all neural circuitry in the vertebrate CNS. Pervasive 5-HT signaling is made possible by the expansive axonal architecture issuing from this small group of neurons. 5-HT neurons generate two highly ramified topographically organized axonal subsystems, ascending and descending, that delivers the transmitter throughout the brain and spinal cord, respectively, to influence numerous behavioral and physiological processes. Intrinsic regulatory programs that govern the specification of these 5-HT neurons are known, however, little is known about the intrinsic regulators that enable the exuberant axonal growth of developing 5-HT neurons. The LIM-homeodomain (LIM HD) transcription factor (TF), Lmx1b, is a key intrinsic regulator of 5-HT neuron terminal differentiation through its activation of genes encoding 5-HT synthesis (Tph2), reuptake (Sert), and vesicular monoamine transport (VMAT2). Lmx1b deficient mice lack nearly all brain 5-HT. Notably, Lmx1b expression continues in all 5-HT neurons through fetal to early postnatal maturation stage during which 5-HT neurons build their axonal architectures. This led us to hypothesize that Lmx1b regulates other genes responsible for 5-HT axonogenesis. To investigate this idea, we generated conditionally targeted mice (*Lmx1b*^{CKO}) by crossing *Lmx1b*$^{flox/flox}$ mice with transgenic mice expressing Cre recombinase selectively in newborn 5-HT neurons and mice carrying the Ai9 reporter to enable marking of ascending and descending 5-HT axons with Td-Tomato. Strikingly, despite normal numbers of 5-HT cell bodies, the spinal cord of *Lmx1b*^{CKO} mice was nearly devoid of Td-Tomato^{+} axons from cervical to lumbar levels. Moreover, investigation of 5-HT terminal fields in the forebrain of *Lmx1b*^{CKO} mice revealed nearly complete absence of Td-Tomato^{+} axons in virtually all distal forebrain structures including the hippocampus, cortex, olfactory bulbs, and ventral hypothalamic nuclei. Further analyses suggest that 5-HT axons can initiate primary growth through the medial forebrain

bundle between E12-E15 and reach the thalamus but abruptly stop and fail to route to various forebrain structures. To define the Lmx1b regulated axonal transcriptome that governs serotonergic axonogenesis we are performing RNA-seq analyses with flow sorted *Lmx1b*^{CKO} vs. *Lmx1b*^{CON} ascending and descending 5-HT neurons. Our findings indicate that Lmx1b controls the major morphological features of 5-HT neurons that enable widespread serotonergic neuromodulation.

Disclosures: L.J. Donovan: None. **E.S. Deneris:** None.

Poster

116. Axon and Dendrite Development: Axon Growth and Guidance: Adhesion and Cytoskeletal Dynamics

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 116.19/C14

Topic: A.05. Axon and Dendrite Development

Support: 1F32MH115438-01
HHMI

Title: Visualizing development of the *C. elegans* pharyngeal nervous system

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Abstract: Despite the central importance of neural circuit development to brain function and behavior, we lack the genetic information required to assemble a complete circuit. We will take advantage of *C. elegans* biology -- its available ultrastructural connectome and facile genetics, to address questions of synaptic wiring in a complete circuit. We will use the pharyngeal nervous system, a small but largely functionally independent circuit, as a model to visualize neuronal neighborhood and connectivity establishment in live animals. While a wiring diagram for the adult pharynx exists, less information is known about connectivity and process placement in embryos. To provide a snapshot of pharyngeal development we first volumetrically reconstructed all cells in two embryonic (1.5 fold) samples. By extracting the adjacencies of all pharyngeal cells, we were able to determine the neighborhoods of all 20 neurons in the pharynx. Using these data as a platform, we are actively evaluating axon outgrowth and neighborhood formation of all neurons in embryos using a unique combination of membrane-bound fluorescent reporters. To visualize connectivity, we are using GFP Reconstitution Across Synaptic Partners (GRASP) labeling for individual and combinations of synapses. We are also developing inducible split-GFP drivers to reduce inter-individual variability in transgene expression and synaptic puncta.

Together our approaches and results will be useful in the broad context of understanding the genetic logic of circuit formation.

Disclosures: S.J. Cook: None. **D.H. Hall:** None. **O. Hobert:** None.

Poster

116. Axon and Dendrite Development: Axon Growth and Guidance: Adhesion and Cytoskeletal Dynamics

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 116.20/C15

Topic: A.05. Axon and Dendrite Development

Support: Canadian Institutes of Health Research (MOP142215)
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Canada Foundation for Innovation (29462)
the BC Knowledge Development Fund (804754)

Title: New insights into Pannexin 1-Crmp2 regulation of neurite formation

Authors: X. XU, J. C. SANCHEZ-ARIAS, L. E. WICKI-STORDEUR, M. LIU, *L. SWAYNE
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Abstract: Neurite extension is an important initial step in axon and dendrite formation. Work from our lab has previously shown that the channel-forming protein Pannexin 1 (Panx1) negatively regulates neurite outgrowth. More recently, we showed that an interaction with the microtubule stabilizing protein collapsin response mediator protein 2 (Crmp2) is important for Panx1 regulation of neurite formation (Crmp2; Xu et al. Front. Cell. Neurosci doi: 10.3389/fncel.2018.00124). Briefly, probenecid uncoupled Crmp2 from Panx1, increased the concentration of Crmp2 at the distal ends of growing neurites, and promoted tubulin polymerization and microtubule stability in Neuro2a cells. Together these data suggested Panx1 negatively regulates Crmp2 and microtubule stability to control neurite formation. Building on these findings, here we more closely examine different aspects of the Panx1-Crmp2 interaction and its regulation of the cytoskeleton in the context of neurite formation. Using live cell confocal imaging of GFP-tagged EB3, a microtubule plus-end binding protein, we examine the impact of Panx1 and the Panx1-Crmp2 interaction on microtubule dynamics in Neuro2a cells. We also explore the relationship between the Panx1-Crmp2 interaction and other members of the Panx1 interactome with relevance to neurite formation. For example, Crmp2 and Panx1 have been

reported to interact with actin by others and us. We therefore use a combination of imaging and biochemical techniques to better understand the spatial and functional relationships connecting Panx1 and Crmp2 with the actin and microtubule cytoskeletons in Neuro2a cells and primary cortical neuron cultures. Furthermore, because Crmp2 function is regulated by phosphorylation, we also examine how this post-translational modification relates to Panx1-Crmp2 crosstalk and its regulation of neurite outgrowth. Together this work reveals new details underlying Panx1-Crmp2 crosstalk in the regulation of neurite outgrowth.

Disclosures: X. Xu: None. **J.C. Sanchez-Arias:** None. **L.E. Wicki-Stordeur:** None. **M. Liu:** None. **L. Swayne:** None.

Poster

116. Axon and Dendrite Development: Axon Growth and Guidance: Adhesion and Cytoskeletal Dynamics

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 116.21/C16

Topic: A.05. Axon and Dendrite Development

Title: Structural changes in axon initial segment in diabetic brain

Authors: *L. M. YERMAKOV[1], D. E. DROUET[1], R. B. GRIGGS[1], K. M. ELASED[2], K. SUSUKI[1]
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Abstract: Axon initial segment (AIS) is the excitable axonal domain that regulates initiation of action potential propagation along axons. Structural changes at the AIS have been linked to numerous neurological disorders. One such change is decrease of AIS length, which has been shown to reduce neuronal excitability. Type 2 diabetes is known to cause cognitive and mood impairment in patients as well as in animal models such as *db/db* mice. We hypothesized that type 2 diabetes affects the structures of the AIS. To test this hypothesis, we used male and female *db/db* mice and littermate non-diabetic controls at different phases of diabetes (n=3-4 per group). By immunohistochemical approach targeting βIV spectrin, a specific structural marker found at the AIS, we analyzed the brain regions known to be affected in type 2 diabetes, the medial prefrontal cortex and hippocampus. Measurement of AIS was performed by researchers blinded to animal identity. At 5 weeks of age—no or minimal diabetic signs present—we found normal AIS in *db/db* mouse brains. At 10 weeks of age—after development of severe hyperglycemia—AIS was significantly shorter in *db/db* mice compared to non-diabetic controls in both medial prefrontal cortex and hippocampus. We observed no AIS shortening after exercise treatment from 5 to 10 weeks of age, which attenuated the development of type 2 diabetes. We did not see apparent changes in the nodes of Ranvier, another excitable axonal domain that is

structurally very similar to AIS. In conclusion, this is the first report of altered AIS morphology in the type 2 diabetic brain. Shortened AIS, which has been shown to reduce neuronal excitability, could account for neuropsychiatric complications associated with type 2 diabetes and become a novel treatment target in this disease.

Disclosures: L.M. Yermakov: None. **D.E. Drouet:** None. **R.B. Griggs:** None. **K.M. Elased:** None. **K. Susuki:** None.

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116. Axon and Dendrite Development: Axon Growth and Guidance: Adhesion and Cytoskeletal Dynamics

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 116.22/C17

Topic: A.05. Axon and Dendrite Development

Title: The diabetes-related metabolite methylglyoxal induces axon initial segment shortening

Authors: *R. B. GRIGGS, L. M. YERMAKOV, D. E. DROUET, J. M. JABER, D. V. M. NGUYEN, K. SUSUKI
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Abstract: The axon initial segment (AIS) is a specialized structural and functional domain that regulates neuronal output. We recently discovered that the development of type 2 diabetes in db/db mice is associated with decreased AIS length in brain regions important for cognitive function. Other studies report that shortened AIS decreases neuronal excitability. These results suggest that AIS shortening may disrupt neuronal network function, contributing to neurological complications in type 2 diabetes. To determine the mechanisms of AIS shortening in type 2 diabetes, we cultured postnatal neurons from the mouse cortex, exposed them to various diabetic conditions, and measured AIS length under observer-blinded conditions using immunofluorescent imaging with antibodies against the AIS protein ankyrin G. We found that mimicking the *in vivo* diabetic condition of hyperglycemia *in vitro*, by elevating the glucose concentration in culture media, did not change AIS length when initiated during either the growth (days *in vitro* 0-10) or maintenance (days *in vitro* 10-13) periods. Another potential mediator of AIS shortening in diabetes is methylglyoxal (MG), a reactive metabolite of glucose that is elevated in diabetes and implicated in neurological dysfunction. Compared to control cultures exposed to media only for 24 hours during the maintenance period (ranging from days *in vitro* 8-14), MG (1, 10, 100, 1000 uM) exposure resulted in dose-dependent reduction of AIS length without overt signs of neuronal damage. In subsequent 24-hour exposure experiments we used 100 uM MG, which is consistent with purported *in vivo* levels in type 2 diabetes patients. MG shortened the AIS in both Ca2+/calmodulin-dependent kinase II positive and negative neurons, suggesting that MG shortens the AIS in both excitatory and inhibitory neurons. Our

previous study showed that MG activates the calcium-dependent protease calpain, which plays a key role in AIS dismantling. MG-evoked AIS shortening was ameliorated by co-exposure to the calpain inhibitor calpeptin (100 uM). Finally, preliminary electrophysiology recordings using a multi-electrode array system indicate that 24-hour exposure to MG (100 uM) depresses spiking frequency. These results suggest that MG-evoked shortening of the AIS leads to altered neuronal activity, and therefore could be a potential mediator of neurological dysfunction in type 2 diabetes.

Disclosures: R.B. Griggs: None. **L.M. Yermakov:** None. **D.E. Drouet:** None. **J.M. Jaber:** None. **D.V.M. Nguyen:** None. **K. Susuki:** None.

Poster

116. Axon and Dendrite Development: Axon Growth and Guidance: Adhesion and Cytoskeletal Dynamics

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 116.23/C18

Topic: F.01. Neuroethology

Support: MEXT/JSPS KAKENHI 15H04255
MEXT/JSPS KAKENHI 18J10483

Title: Formation of the neuronal connectivity that regulates divergent action selections in *Drosophila* larvae

Authors: *S. TAKAGI[1], A. NOSE[2]
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Abstract: Adaptive behavior of an animal is underpinned by the complex yet precise connectivity of its nervous system. The connectivity of a neuron within the network is established through precise neurite guidance and synapse formation mechanisms, which give rise to the unique topological morphology of the neuron. Although much work has revealed the molecular and cellular basis of neurite guidance and synapse formation, the significance of stereotypic network formation on behavioral regulation of an animal remains largely elusive. We use *Drosophila* larvae as a model to study the circuit basis of action selection. We have previously identified a segmentally-repeated command-like interneurons, that we named Wave, which drive distinct escape behaviors in a segment-specific manner (Takagi et al., 2017). Activation of an anterior Wave elicits backward locomotion, whereas activation of a posterior Wave promotes forward locomotion. Wave neurons exhibit distinct dendritic and axonal polarities in a segment-specific manner, which are closely correlated to their divergent functions in behavioral regulation. Thus, Wave neurons serve as an excellent model to study the role of

neurite guidance in dictating animal behavior.
In the current study, we first observed the neurite extension by Wave neurons during embryonic development. We found that Wave neurons initially project simple neurites that run along the anterior-posterior axis, then form the mature pattern of dendritic and axonal morphology characteristic to the neurons. We next analyzed the function of Hox genes in shaping the Wave neuronal morphology and revealed potential roles of Abd-B in this process. We are now studying the role of candidate neurite guidance cues and their receptors in shaping the Wave neuronal morphology. We would like to discuss how the appropriate wiring of a command system is regulated so that specific sensory inputs are adaptively relayed to motor outputs.

Disclosures: A. Nose: None.

Poster

117. Axon and Dendrite Development: Dendritic Growth and Branching

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 117.01/C19

Topic: A.05. Axon and Dendrite Development

Title: In dysbindin, a schizophrenia susceptibility factor, knock down mouse neuroblastoma cells treated with antipsychotic medications demonstrate alterations in neuronal morphology

Authors: *J. L. LARIMORE[1], S. M. CORDERO ROMERO[2], J. WEBSTER[2], A. CROCKETT[2], R. DZVURUMI[2]
[1]Biol. Dept., [2]Agnes Scott Col., Decatur, GA

Abstract: Schizophrenia is a neurodevelopmental disorder characterized by hallucinations, paranoia, reduced motivation and alterations in learning and memory. Individuals with schizophrenia have reduced levels of dystrobrevin-binding protein 1, or dysbindin, in the prefrontal cortex and hippocampus. Dysbindin is a subunit of the BLOC-1 complex that regulates endosomal trafficking of key membrane proteins and receptors, including the D2 dopamine receptor. Reduction of dysbindin has been implicated in abnormal neuronal morphology and synaptic function. In this study, antipsychotic medications used in the treatment of schizophrenia were tested on a mouse neuroblastoma cell line with reduced dysbindin protein levels to determine their role in neurite outgrowth and cell morphology. These data will lead to a better understand of how antipsychotic medications impact neuronal morphology in patients with schizophrenia.

Disclosures: J.L. Larimore: None. **S.M. Cordero Romero:** None. **J. Webster:** None. **A. Crockett:** None. **R. Dzvurumi:** None.

Poster

117. Axon and Dendrite Development: Dendritic Growth and Branching

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 117.02/C20

Topic: A.05. Axon and Dendrite Development

Title: Neuronal activity-dependent formation of ER-PM contact sites in spines regulates spine formation and dendritic extension via local manipulation of unfolded protein response

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Abstract: Endoplasmic reticulum (ER) stress transducers IRE1, PERK and ATF6 recognize the alternation of ER luminal environments and transduce signals from ER to cytoplasm or nucleus (unfolded protein response: UPR) to maintain ER functions. Recent studies have uncovered novel UPR functions not only in dealing with unfolded proteins but also in regulating cellular homeostasis. The dendritic ER network is complexly extended from cell soma to distal dendrites of neurons, indicating that dendritic ER functions and UPR signaling may orchestrate local events contributing to dendritic capabilities. We focused on the machinery for dendritic functions manipulated by ER-derived signaling including UPR. To assess the induction of UPR in response to neuronal activities, primary cultured mouse hippocampal neurons were pretreated with tetrodotoxin subsequently the washout to induce spontaneous excitatory synaptic activities. The phosphorylation levels of IRE1 and PERK were transiently upregulated at spines after the washout. The phosphorylation levels were reduced by inhibiting calcium ion (Ca^{2+}) release from ER, suggesting that Ca^{2+} release and its depletion in ER lumen by the excitatory synaptic activation triggers the induction of UPR at spines. The ER in the spines formed contact sites with plasma membrane (PM) (ER-PM contact sites) after the excitatory synaptic activation. These contact sites were composed of Stim1 localizing at ER membrane and Orai1 localizing at PM, which are responsible for Ca^{2+} entry from extracellular spaces to ER lumen. The knockdown of Stim1 extended the UPR activation after the excitatory synaptic activation followed by inhibiting spine formation and dendritic extension. These results suggest that the synaptic activation-dependent ER-dynamics including the formation of ER-PM contact sites in spines may fine-tune the development of dendritic spines and intricately branched dendrites through the regulation of UPR signaling derived from spines.

Disclosures: A. Saito: None. **K. Imaizumi:** None.

Poster

117. Axon and Dendrite Development: Dendritic Growth and Branching

Location: SDCC Halls B-H

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Program #/Poster #: 117.03/C21

Topic: A.05. Axon and Dendrite Development

Support: JSPS KAKENHI Grant 17H01890
JSPS KAKENHI Grant 16K18391
JSPS KAKENHI Grant 16J03827

Title: Smads-dependent TGF-β signaling negatively regulates neuronal morphogenesis through TGIF/Smad complex-mediated CRMP2 suppression

Authors: *H. NAKASHIMA[1], K. TSUJIMURA[2], K. IRIE[1], M. ISHIZU[1], M. PAN[1], K. NAKASHIMA[1]
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Abstract: Functional neuronal connectivity requires proper neuronal morphogenesis and its dysregulation causes neurodevelopmental diseases. Transforming growth factor-β (TGF-β) family cytokines play pivotal roles in development, but little is known about their contribution to morphological development of neurons. Here we show that the Smad-dependent canonical signaling of TGF-β family cytokines negatively regulates neuronal morphogenesis during brain development. Mechanistically, activated Smads form a complex with transcriptional repressor TG-interacting factor (TGIF), and downregulate the expression of a neuronal polarity regulator, collapsin response mediator protein 2. We also demonstrate that TGF-β family signaling inhibits neurite elongation of human iPSC-derived neurons. Furthermore, the expression of TGF-β receptor 1, Smad4 or TGIF having mutations found in patients with neurodevelopmental disorders, disrupted neuronal morphogenesis in both mouse and human neurons. Taken together, these findings suggest that the regulation of neuronal morphogenesis by an evolutionarily conserved function of TGF-β signaling is involved in the pathogenesis of neurodevelopmental diseases.

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Poster

117. Axon and Dendrite Development: Dendritic Growth and Branching

Location: SDCC Halls B-H

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Program #/Poster #: 117.04/C22

Topic: A.05. Axon and Dendrite Development

Support: BT/PR8723/AGR/36/776/2013

Title: Translational regulation of actin modulators fine-tune dendritic development in young neurons

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Abstract: Dendritic patterning in neurons is a critical stage during the development of nervous system wherein the basic cellular computational machinery is laid out. Although initially considered as a cell autonomous process, studies have now shown that dendritic morphology is significantly affected by external cues including trophic factors and synaptic inputs. Such cue induced morphological changes are known to be predominantly driven by actin cytoskeletal rearrangement mediated by various actin-binding proteins. Brain Derived Neurotrophic Factor (BDNF) is an important trophic factor involved in neuronal survival, dendrite growth and synaptic plasticity. BDNF has been shown to induce local protein synthesis of some key actin modulators in mature dendritic spines. But, the role of BDNF mediated protein synthesis in the growth and branching of immature dendrites is not well studied. Defect in protein synthesis is thought to be a primary cause of many neurodevelopmental disorders. Our study aims to identify the importance of activity mediated protein synthesis in the early stages of neuronal development.
We studied BDNF induced translational changes of actin modulator proteins in DIV5 rat cortical neurons in culture. Our data shows that BDNF regulates translation of actin modulators in young neurons. We found that BDNF induced robust translational up regulation and increased protein levels of LIMK1. Immunofluorescent studies showed that increase in LIMK1 protein level is present in the dendritic compartment with corresponding increase in phosphorylation of its substrate Cofilin1. Cofilin1 is an actin binding protein which dictates actin polymerization kinetics. Interestingly, total cofilin levels were unchanged on BDNF stimulation confirming the role of its upstream kinase (LIMK1) which is translationally regulated in this process.
BDNF has a significant effect on dendritic arborization which requires extensive actin rearrangements. We also observed a significant increase in total dendrite length and number of primary branches in response to BDNF treatment. We are currently investigating how translation

induced actin dynamics is mediating this physiological outcome. Our findings point out that dendrite patterning is a highly regulated process that also requires fine-tuning at the translational level. This study will also help to better understand the molecular aspects of defective translation in many neurodevelopmental disorders.

Disclosures: S. Ravindran: None. **R.S. Muddashetty:** None.

Poster

117. Axon and Dendrite Development: Dendritic Growth and Branching

Location: SDCC Halls B-H

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Program #/Poster #: 117.05/C23

Topic: A.05. Axon and Dendrite Development

Support: NSFC Grant 91232303

Title: Neuroligin-3 regulates dendritic development in cultured neuron by modulating Akt/mTOR signaling

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Abstract: Neuroligins (NLs) are a group of postsynaptic cell adhesion molecules that function in synaptogenesis and synaptic transmission. Genetic defects in neuroligin-3 (NL3), a member of the NL protein family, are associated with autism. The mammalian target of rapamycin (mTOR) signaling pathway plays a crucial role in regulating gene transcription, mRNA translation, and cell growth and proliferation. In the central nervous system, the mTOR signaling pathway is involved in many mental disorders including autism, epilepsy, and neurodegenerative diseases. In our study, we found that knockdown of NL3 in cultured rat neurons up-regulated the activation of mTOR signaling, resulting in increased protein synthesis and increased dendritic length. The activity of AKT signaling, upstream of the mTOR pathway, was also elevated by NL3 knockdown. Treating these neurons with rapamycin to inhibit the mTOR and LY294002 to inhibit the AKT pathway rescued the developmental defects resulting from their lack of NL3 protein. Similarly, cultured cortical neurons from NL3 knockout mice exhibited enlarged somata, prolonged dendritic length, and increased dendritic complexity. These morphological abnormalities in NL3 knockout neurons were also improved by either rapamycin or LY294002 treatment. Our results suggest that NL3 regulates the development of neurons, especially dendritic development, by modulating the mTOR signaling pathway. Therefore, this study provides a new drug target for treating autism associated with NL3 defects.
Key words:neuroligin-3,mTOR,dendritic development

Disclosures: R. Zheng: None. **J. Xu:** None. **J. Xu:** None.

Poster

117. Axon and Dendrite Development: Dendritic Growth and Branching

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Topic: A.05. Axon and Dendrite Development

Support: NIH Grant R01NS058721
UCSF Discovery Fellowship

Title: C12ORF57 is a novel gene crucial to brain development and neuronal excitability

Authors: *R. JIANG[1], C. MOEY[3], L. J. RICHARDS[4], E. H. SHERR[2]
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Abstract: *C12ORF57* is a novel highly conserved gene (98% amino acid identity with its mouse homologue *Grcc10)* encoding a 126 amino acid protein with no known structural homology or function that is highly expressed in the developing or mature central nervous system. Our collaborative team has identified patients with homozygous loss of function mutations in this gene with clinical findings of agenesis of the corpus callosum (ACC), microphthalmia, optic coloboma, severe intellectual disability and intractable seizures; a recent study suggests 1%+ of all intellectual disability with genetic cause in Arabic populations are due to mutations in *C12ORF57* (PMID:29383837). Based on this scientific and clinical interest, we sought to determine the molecular function of C12ORF57. *Grcc10 -/-* mice show severe preweaning mortality with 90%+ mice dying before P3 as well as a shortened corpus callosum phenotype when compared to WT mice (770 uM, 637 uM p=.0052) reminiscent of the phenotype seen in human patients. Using the yeast-two-hybrid platform we demonstrated that C12ORF57 binds to the Calcium Calmodulin Kinase IV (CAMK4). We further strengthened this association through multiple functional and biochemical approaches showing decreased signaling through the CAMK4 pathway in the absence of C12ORF57; Loss of C12ORF57 causes a decrease in 30% phosphorylation at CAMK4 primary phosphorylation site, Thr200 (p=0.0182). We also see considerable disruption of *Grcc10 -/-* neuronal function in vitro. We see a decrease in the ramification index, a measure of dendrite complexity, by a factor of 2.41 ($p<0.0001$) in *Grcc10-/-* mouse neurons (n=60) compared to heterozygote controls (n=41). Furthermore, we have found an increase in the number of AMPA positive synapses in *Grcc10 -/-* neurons (57% vs 30% $p<0.00001$). Our data further show an increased seizure susceptibility to the pro-convulsant kainic acid in *Grcc10 -/-* mice compared to their control littermates, with *Grcc10 -/-* mice proceeding through all 6 Racine stages in 29% of the time of their control littermates(15 min, 48 min, p=0.0003). This finding may indeed correlate with the epilepsy found in human patients and

with the electrophysiologic homeostasis role previously reported for CAMK4. These robust findings in human and mouse C12ORF57/*Grcc10* gene absence suggest a previously unappreciated and crucial role for this new gene in neuronal biology.

Disclosures: R. Jiang: None. **C. Moey:** None. **L.J. Richards:** None. **E.H. Sherr:** None.

Poster

117. Axon and Dendrite Development: Dendritic Growth and Branching

Location: SDCC Halls B-H

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Program #/Poster #: 117.07/C25

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant NS090030
NIH Grant NS055272

Title: Genetic dissection of the role of gamma-protocadherin isoform diversity in neurodevelopment using CRISPR/Cas9 genome editing

Authors: *A. M. GARRETT[1], J. A. WEINER[2], R. W. BURGESS[3]
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Abstract: The mammalian *Pcdhg* gene cluster consists of 22 variable exons and 3 constant exons, which encode for the gamma-protocadherins (γ-Pcdhs), a family of 22 distinct cadherin superfamily cell adhesion molecule isoforms. The majority of each isoform is encoded by a single variable exon, while a common cytoplasmic C-terminus is encoded by the constant exons. The γ-Pcdhs are expressed broadly throughout the nervous system, with individual cells expressing ~7 of the 22 isoforms, including ~4 of the stochastically expressed gamma-A and –B subfamily genes plus the 3 ubiquitously expressed gamma-C subfamily genes. The proteins dimerize promiscuously in *cis*, but the binding specificity across membranes is strictly homophilic. This is thought to result in a zipper-like lattice of dimers in *trans*. More isoforms in common between membranes will allow a larger lattice to form. Indeed, in cell aggregation studies, subtle differences between cells in isoform ratios greatly affect cell binding. Thus, through isoform combination, the γ-Pcdhs, alone or in complex with the α- and β-Pcdhs, could generate many thousands of distinct cellular recognition units. The γ-Pcdhs critically regulate multiple neurodevelopmental processes, including synapse formation, neuronal survival, dendrite self-avoidance, and dendrite arborization, but the requirement for isoform diversity in these functions is still unclear. Previous experiments testing isoform variety used the relatively blunt tools of complete cluster knockouts and single isoform transgene overexpression. To more precisely ask whether molecular diversity is essential for normal γ-Pcdh functions and to determine if any isoform(s) is critically important, we used CRISPR/Cas9 genome editing to

generate an array of mutations in the *Pcdhg* locus in mice. Single guide RNAs were designed to target sequences near the start codon of each of the 22 *Pcdhg* variable exons, and were injected together as a mixture into fertilized eggs. We established 30 new lines with between 1 and 19 disrupted variable exons, with disruptions ranging from small indels to large rearrangements between guide sites. Multiple lines with substantially reduced isoform diversity are viable as homozygotes, despite the neonatal lethality of *Pcdhg* cluster null mice. Furthermore, we have preliminarily identified that specific isoforms are more essential for cell survival in the retina and spinal cord than others, and disruption of these isoforms results in perinatal lethality. Currently, we are generating homozygous mutants for additional lines to analyze the necessity for isoform diversity in neurodevelopmental processes known to require Pcdhg function.

Disclosures: A.M. Garrett: None. **J.A. Weiner:** None. **R.W. Burgess:** None.

Poster

117. Axon and Dendrite Development: Dendritic Growth and Branching

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 117.08/C26

Topic: A.05. Axon and Dendrite Development

Title: Neuronal nitric oxide synthase in the cerebellum: Implications on development of the parallel fiber-Purkinje neuron synapses in mice

Authors: V. TELLIOS, M. J. E. MAKSOUD, Y.-Y. XIANG, *W.-Y. LU
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Abstract: Parallel fibers (PFs) arising from granule cells express vesicular glutamate transporter 1 (vGluT1) and establish excitatory synapses with dendritic spines of Purkinje neurons (PNs). In response to burst action potentials occurring at PFs, PNs generate a fast excitatory postsynaptic current (EPSC) mediated by ionotropic glutamate receptors, followed by a slow EPSC that results from metabotropic type 1 glutamate receptor (mGluR1) activation, stromal interaction molecule 1 (STIM1) oligomerization in the endoplasmic reticulum (ER) and cation (Ca^{2+}/Na^{+}) entry through transient receptor potential C3 (TRPC3) channels. Intracellular Ca^{2+} dynamics in PNs is essential for PF-PN synaptic plasticity and hence motor learning during development. Neuronal nitric oxide synthase (nNOS) is abundantly expressed in PFs and critically regulates PF-PN synaptic plasticity, and nNOS-deficient ($nNOS^{-/-}$) mice exhibit motor deficits. We recently demonstrated a significantly larger slow EPSC in PNs of young $nNOS^{-/-}$ mice in comparison to age-matched WT controls. Given that intracellular Ca^{2+} homeostasis is critical for PN dendritic development, this project was set to explore the role of nNOS/NO signaling in PN morphological development. Cerebellar tissues were collected from wild type (WT) and $nNOS^{-/-}$ mice at postnatal 7- and 14-days and 7-weeks. Immunohistochemistry and image analyses using ImageJ showed that PN dendritic growth was significantly reduced and dendritic spine

morphology was notably abnormal in nNOS$^{-/-}$ cerebella during postnatal development. Immunofluorescence clusters of vGluT1 and mGluR1 were significantly reduced in adult nNOS$^{-/-}$ cerebella. Immunoblotting assays demonstrated significantly lower expression of vGluT1 and mGluR1 in nNOS$^{-/-}$ cerebella, while the expression of STIM1 and calpain-1, a Ca^{2+}-dependent protease that inhibits neurite growth, was drastically increased. To explore the specific role of NO in PN development, *ex vivo* cerebellar slice cultures were established and treated with the NO-donor NOC-18 and the NOS-inhibitor N-nitro-L-arginine methyl ester (L-NAME), as well as the mGluR1 agonist dihydroxyphenylglycine (DHPG) and antagonist (LY367385). In nNOS$^{-/-}$ cerebellar tissues NOC-18 largely ameliorated the delay of dendritic growth while WT tissues treated with L-NAME showed dendritic deficits and higher expression of calpain-1. Together, these novel data indicate that nNOS/NO signaling in the cerebellum plays a pivotal role in PN development by regulating calcium homeostasis during PF-PN synaptic transmission.

Disclosures: V. Tellios: None. **M.J.E. Maksoud:** None. **Y. Xiang:** None. **W. Lu:** None.

Poster

117. Axon and Dendrite Development: Dendritic Growth and Branching

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 117.09/C27

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant 5R21 NS088062-02
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CONACYT, Postdoctoral fellowship, CVU-267642

Title: Krüppel-like factors 9 and 13 block neurite outgrowth by cAMP pathway inhibition

Authors: *J. AVILA MENDOZA, A. SUBRAMANI, C. SIFUENTES, R. J. DENVER
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Abstract: The ability of neurons to eloborate projections declines during postnatal development, in part due to the establishment of a new genetic program. Krüppel-like factors (Klfs) have been identified as important regulators of neuronal differentiation and regeneration. They comprise a family of 17 zinc finger transcription factors grouped into 3 subfamilies based on the sequences of their N-terminal domains. Our previous work focused on Klf9, a member of subfamily 3, which promotes and maintains the differentiated state of neurons, and is thus implicated in the loss of regenerative capacity of adult mammalian neurons. We recently identified Klf9 target genes in mouse hippocampal neurons, and discovered that Klf9 functions predominantly as a transcriptional repressor. One of the most strongly regulated genes by Klf9 is Klf13, also a member of subfamily 3. Although all 17 of the Klfs are expressed in the central nervous system, very little is known about their molecular mechanisms of action. Here we analyzed and

compared the cellular functions, and genomic targets of Klf13 and Klf9 to test the hypothesis that these two closely related Klfs inhibit neurite outgrowth by similar mechanisms. We engineered the adult mouse hippocampus-derived cell line HT22 to control Klf9 or Klf13 expression by addition of doxycycline. We also used CRISPR/Cas9 genome editing to generate Klf9 and Klf13 knock out HT22 cell lines. To induce neurite outgrowth, we treated cells with forskolin(FK)+IBMX, which increases cAMP; elevated cAMP is a hallmark of regenerative responses of neurons to injury. Our results show that elevated cAMP reduced mRNAs for Klf9 and Klf13. Treatment with FK+IBMX increased neurite length in the parent cell line, and this effect was strongly enhanced in Klf9 and Klf13 knock out cell lines. Conversely, the stimulatory effect of FK+IBMX on neurite outgrowth was blocked by simultaneous forced expression of Klf9 or Klf13. This effect on neurite outgrowth was confirmed in primary hippocampal neurons where electroporation of Klf9 or Klf13 expression plasmids resulted in significantly shorter neurite length after FK treatment compared with control transfected cells. Analysis of RNAseq data obtained from HT22 cells following 8 hr of induced Klf9 or Klf13 expression showed that both proteins perturb the cAMP signaling pathway, suggesting that, in part, these transcription factors inhibit neurite outgrowth by inhibiting this pathway. Our results support that cAMP-dependent neurite outgrowth in hippocampal neurons can be blocked by Klf9 and Klf13. These two Klfs of subfamily 3 cooperate to maintain neuronal differentiation, and to inhibit regeneration.

Disclosures: J. Avila Mendoza: None. **A. Subramani:** None. **C. Sifuentes:** None. **R.J. Denver:** None.

Poster

117. Axon and Dendrite Development: Dendritic Growth and Branching

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 117.10/C28

Topic: A.05. Axon and Dendrite Development

Title: Analysis of dendritic development and morphological phenotype in primary olfactory cortex

Authors: *L. MORENO VELASQUEZ[1,2], J. TEFERA[1], D. SCHMITZ[1,3,4,5,6], F. W. JOHENNING[1,2,3]
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Abstract: The piriform cortex (PC) is a three-layered paleocortex and the first cortical relay for olfactory processing. Current descriptions of PC infer a convergence of sensory and intracortical

inputs onto principal cells in layer II. They receive monosynaptic sensory inputs from the olfactory bulb via the lateral olfactory tract (LOT), as well as local and long-range intracortical connections. Additionally, two morphologically distinctive glutamatergic cell types predominate in layer II, with strikingly different properties: superficial pyramidal and semilunar cells. Superficial pyramidal cell establish synaptic connections with the LOT in layer Ia and with intracortical association fibers in layer Ib, II and III. Semilunar cells are a specific population of neurons located in the most superficial aspect of layer II and, in contrast to pyramidal cells, their basal dendritic tree is less complex and does not form strong associative synapses. While the structure of the adult PC is well characterized, postnatal development and maturation of this cortex is undetermined. In this study, electrophysiology and morphometry were combined in order to identify developmental emergence of these differences between semilunar and pyramidal cells. Topological and geometric morphometrics were used to describe the morphological profile, including elongation, branching, density and complexity of the dendritic arborizations. Insights into the circuit-specific dendritic development of the PC establish a foundation for analyzing circuit specific alterations of dendrite development in neurodevelopmental disorders such as Fragile X Syndrome.

Disclosures: L. Moreno Velasquez: None. **J. Tefera:** None. **D. Schmitz:** None. **F.W. Johenning:** None.

Poster

117. Axon and Dendrite Development: Dendritic Growth and Branching

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 117.11/C29

Topic: A.05. Axon and Dendrite Development

Support: CONICET
FONCyT
UNC

Title: Rab11 endosomes regulate both the dendrite morphology and the specific positioning of dendritic proteins

Authors: *C. B. CONDE[1], S. O. SIRI[1], V. ROZES SALVADOR[1], E. ARTUR DE LA VILLARMOIS, 5000[2], M. S. GHERSI[2], M. F. PÉREZ[2]
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Abstract: The correct development of the dendritic arbor requires a proper spatiotemporal distribution of the elements of the secretory and endocytic pathways, as well as a dynamic and tightly regulated communication between them. One of the main players in these processes is the

Rab family of small GTPases. The members of this family of proteins not only regulate endocytic trafficking but also act as specific markers for different types of endosomes. Emerging evidence shows that Rab11 play a role in several neuronal processes is required for the proper functioning of the growth cone and regulation of the synapse architecture. However, less is known about the specific function of Rab11 in the development of the dendritic arbor. Thus, we have focused our work on characterizing the role of the Rab11-positive (Rab11+) recycling endosomes (REs) during neuronal development using a model of primary culture of embryonic rat hippocampal (HP) neurons. Furthermore, we aimed to characterize the functional role of Rab11 in HP synaptic plasticity and its correlate in an HP-dependent behavior in adult rats. First, we determined the endogenous localization of the Rab11+ REs at different stages of the neuronal development in vitro. The immunostaining experiments showed that during the first 24 hours and up to 3 days in vitro (DIV), Rab11 is mainly distributed in the neurites tips of the minor processes and redistributed to a juxtanuclear position at later stages. Moreover, suppression of Rab11 resulted in a shortening of the main neurite and increased number of dendritic branches. These alterations were accompanied by a significant miss-distribution of dendritic proteins, which were found not only in dendrites but also in axons. For example, we observed that the reduction of Rab11 expression selectively alters the traffic and delivery to the plasma membrane of Transferrin receptor (TfR) and AMPA receptor subunit (GluR1). In addition, the in vivo Rab11 suppression by injecting animals with lentiviral particles in the CA1 region led to decrease sensitivity to induce HP long-term potentiation and to a reduced performance in memory-related behaviors. Taken together, our experimental results indicate that Rab11 follow a spatial pattern of distribution that changes with the neuronal stage development. The specific REs localization at each stage seems to be a requirement, not only for the elongation and branching of dendritic processes at early stages but also to control key aspects of neuronal development as maturation and proper function of the neuronal dendritic tree. At later stages, in mature neurons, Rab11 would play a role in the maintenance of synaptic transmission and in memory formation.

Disclosures: C.B. Conde: None. **S.O. Siri:** None. **V. Rozes Salvador:** None. **E. Artur de la Villarmois:** None. **M.S. Ghersi:** None. **M.F. Pérez:** None.

Poster

117. Axon and Dendrite Development: Dendritic Growth and Branching

Location: SDCC Halls B-H

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Program #/Poster #: 117.12/C30

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James Madison University Second Century Scholarship
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James Madison University Department of Biology

Title: Control of dendritic arborization of layer II/III pyramidal neurons in developing cerebral cortex by integrin beta 3

Authors: Z. L. HOLLEY, K. M. BLAND, Z. O. CASEY, C. J. HANDWERK, *G. S. VIDAL Biol., James Madison Univ., Harrisonburg, VA

Abstract: Integrin subunits have been implicated in axonal and dendritic outgrowth, as well as dendritic spine plasticity. In particular, a strong positive association has been found between mutations in integrin beta 3 (Itgb3) and autism spectrum disorder, but little is known about neuronal Itgb3 function in vivo. Many forms of autism spectrum disorder are thought to arise from dysfunctional dendritic arborization and synaptic pruning. Global knockout of Itgb3 in mice leads to autistic-like behaviors. Itgb3-/- mice also have reduced callosal volume, a key neuroanatomical correlate of autism. Here, we test the hypothesis that Itgb3 is required for normal dendritic arborization in layer II/III pyramidal neurons of mouse neocortex. This was achieved by causing Itgb3 loss of function through Cre-lox-mediated excision of Itgb3 in a subset of layer II/III cortical neurons. Layer II/III cortical neurons were targeted for excision via in utero electroporation of GFP/Cre DNA constructs to the ventricular zone of developing telencephalon of mice in which exon 1 of Itgb3 is flanked by loxP sites. Laminar positioning, regional targeting, and dendritic arborization of targeted neurons in juvenile mice (P23-P30) were analyzed. Male and female mice were used for the study and analysis was done blind to genotype. Results point to normal laminar positioning but aberrant dendritic arborization of mutant neurons, when compared to C57BL6/J controls. Thus, integrin beta 3 appears to regulate dendritic arborization of layer II/III pyramidal neurons in the developing neocortex.

Disclosures: Z.L. Holley: None. **K.M. Bland:** None. **Z.O. Casey:** None. **C.J. Handwerk:** None. **G.S. Vidal:** None.

Poster

117. Axon and Dendrite Development: Dendritic Growth and Branching

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 117.13/C31

Topic: A.05. Axon and Dendrite Development

Support: National Institute on Aging (5R01AG047296)

Louisiana Board of Regents RCS (LEQSF(2016-19)-RD-A-24) to RM

Title: The absence of the tumor suppressor p53 causes dendritic spine instability in the primary somatosensory cortex of juvenile and young adult mice

Authors: *T. LIU[1], J. WU[2], S.-C. PENG[2], A. DAVIDSON[3], S. ZENG[4], H. LU[4], M. RICARDO[5]
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Abstract: Aim: The tumor suppressor *p53*, the 'guardian of the genome', is modulated in response to cellular stress, including DNA damage, ischemic stress, and neuronal excitotoxic stress. Previous studies show that *p53* is necessary for neurite outgrowth and axon regeneration in cultured cells (Di Giovanni et al., 2006, Tedeschi et al., 2009), but the role of *p53* in intrinsic dynamics of dendritic spines in vivo as well as its role in sensory-evoked synaptic plasticity is not known. Methods: Thy1-eGFP-M transgenic mice were crossed to *p53* heterozygous mice, both withC57BL/6 genetic backgrounds, to generate the p53-KO:Thy1-eGFP-M (KO) mice and Thy1-eGFP-M wildtype mice (WT, control). Thy1-eGFP-M mice were used because they sparsely express GFP and thus allow for repeated in vivo imaging of apical tuft dendrites of layer V pyramidal neurons within the whisker barrel cortex. Chronic cranial window surgeries and long-term in vivo two photon microscopy imaging were performed as previously described (Mostany et al., 2013; Alexander et al., 2018) from two age groups: juvenile (aged 3 weeks) and young adult (aged 3-5 months). Imaging sessions were carried out every 4 days (from days 0 - 8 and days 0 - 16, for juvenile and young adult mice, respectively) to observe changes in spine dynamics. Adult mice were imaged before and following sessions of induction of sensory-evoked plasticity, occurring from days 8 - 11, and that consisted of continuous stimulation of the whiskers contralateral to the cortical area imaged with a piezoelectric actuator at 8 Hz for 10 minutes a day over these 4 days. Reults: We found that the baselines of spine density were significantly decreased in both juvenile (0.38 ± 0.08 spines/μm) and adult (0.23 ± 0.04 spines/μm) KO mice compared with WT (0.48 ± 0.05 and 0.32 ± 0.06 spines/μm, respectively). In the absence of whisker stimulation, dendritic spine turnover is not altered in juvenile mice ($P > 0.05$). However, we observed a decrease in turnover ratio from 0.12 ± 0.01 (WT) to 0.09 ± 0.01 (KO) spines/μm in young adult mice ($P < 0.05$). Furthermore, in the presence of whisker stimulation, we observed an increased turnover ratio in WT ($P < 0.05$) but not in KO ($P > 0.05$) adult mice. Conclusions: These results indicate that the loss of *p53* gene function leads to the abnormal dendritic spine dynamics, suggesting an important functional implication of *p53* in neurodevelopmental disorders.

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Poster

117. Axon and Dendrite Development: Dendritic Growth and Branching

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 117.14/C32

Topic: A.05. Axon and Dendrite Development

Title: Erk-dependent phosphorylation regulates NeuroD1 activity

Authors: *T. LEE, N. BASHYAL, J.-M. CHOI, S.-S. KIM, H.-Y. SUH-KIM
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Abstract: NeuroD1 is a transcription factor with a basic-helix-loop motif, which is known to regulate differentiation and survival of neuronal cells, enteroendocrine cells, and pancreatic beta cells. Previous reports showed that the overexpression of NeuroD1 increased the expression of Rab3 interactive molecule-1 RIM1, RIM2, and Munc18-1. These proteins are known to play critical roles in exocytosis of secretory vesicles in neuronal and endocrine cells in which NeuroD1 may function as a master transcription factor. Furthermore, NeuorD1 is related with dendrite outgrowth and dendritic spine development. To test whether the transcription activity of NeuroD1 is regulated by intracellular signaling molecules, we investigated the effect of Erk on NeuroD-mediated gene expression. Several NeuroD mutants were tested for their protein stability, transactivation in the presence of MEK inhibitors. We will discuss how Erk regulates the functionality of NeuroD1.

Disclosures: T. Lee: None. **N. Bashyal:** None. **J. Choi:** None. **S. Kim:** None. **H. Suh-Kim:** None.

Poster

117. Axon and Dendrite Development: Dendritic Growth and Branching

Location: SDCC Halls B-H

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Program #/Poster #: 117.15/C33

Topic: A.05. Axon and Dendrite Development

Support: CIHR to J.L. Lefebvre
University of Toronto Fellowship to J. Marocha

Title: Clustered protocadherins regulate Purkinje cell development and motor function

Authors: *J. MAROCHA[1,2], J. L. LEFEBVRE[1,2]
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Abstract: Cerebellar Purkinje cells (PCs) display highly elaborate dendrites which are stereotypically patterned by a process called dendrite self-avoidance. This process establishes the mature arbor where dendrites of individual neurons are evenly spaced with minimal self-overlap. Such a pattern maximizes the receptive field of a neuron for effective sampling of inputs. We have shown that members of a large group of neuronal surface recognition molecules called the clustered Protocadherins (Pcdhs) regulate PC dendrite self-avoidance. Pcdhs have the potential to generate enormous molecular diversity through combinatorial expression of 58 genes which are divided into three gene clusters, *alpha* (*a*), *beta* (*b*) and *gamma* (*g*). PCs randomly express distinct subsets of Pcdh isoforms from each cluster, bestowing them with unique cell-surface identities. In a past study, we found that deletion of *Pcdhg* genes in PCs leads to dendrite self-overlaps observed as early as P15 (Lefebvre et al., *Nature* 2012), indicating that PC dendrite self-avoidance is a developmental process requiring Pcdhgs. However, the role of other Pcdh members in PCs and the cellular mechanisms of dendrite self-avoidance were unknown. Here, we first tested whether members of the *Pcdha* cluster functionally interact with *Pcdhgs* in regulating PC dendrite self-avoidance. Deletion of *Pcdha* genes led to dendrite self-avoidance defects to a similar extent to loss of *Pcdhgs*, suggesting that they are equally required. Next, using CRISPR technology, we generated a novel mouseline in which PCs lack both *Pcdha* and –*g* genes (Ing-Esteves et al., *J. Neuro.* 2018). Dendrite crossing defects were far more severe in double mutant PCs than in either single *Pcdha* and *Pcdhg* mutants, revealing that the clustered Pcdhs interact in an additive manner. Secondly, we characterized the cellular basis of dendrite self-avoidance by studying branching patterns during a time of dynamic branch growth, sampling, and retraction. Developing Pcdh mutant PCs (≤P12) displayed a higher density of dendrites with reduced arbor size, suggesting a failure to retract. To determine whether Pcdh-dependent patterning of cerebellar neurons have implications on motor outputs, we are testing *Pcdh* mouse mutants in motor behavioral assays. *Pcdha/g* mouse mutants perform more poorly on the rotarod and gait assays. In conclusion, our results show that *Pcdha* and *-g* cluster diversity is required for PC dendrite self-avoidance beginning early in development and that they contribute to proper motor function in mice.

Disclosures: J. Marocha: None. **J.L. Lefebvre:** None.

Poster

117. Axon and Dendrite Development: Dendritic Growth and Branching

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 117.16/C34

Topic: A.05. Axon and Dendrite Development

Title: The importance of actin binding for the plasma membrane localization and the ability to induce neurite outgrowth of DGKβ

Authors: R. TSUMAGARI, T. KANO, S. UEDA, M. YAMANOUE, *Y. SHIRAI
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Abstract: Diacylglycerol kinase (DGK) is an enzyme converting DG to phosphatidic acid (PA). So far, 10 subtypes of mammalian DGKs have been identified. Among them, DGKβ is abundantly expressed in neuron, especially cerebral cortex, hippocampus and striatum. Unlike other DGKs, DGKβ shows a unique localization of plasma membrane. We previously reported that plasma membrane localization of DGKβ via C1 domain and a cluster of basic amino acids at C-terminus is necessary for its ability to induce neurite outgrowth. In addition, DGKβ has a recoverin homology (RVH)-EF motif domain (RVH-EF), which is responsible for Ca^{2+} binding. However, it remains to be uncover whether Ca^{2+} influences localization and the function of DGKβ. Therefore, we first investigated the effect of Ca^{2+} on localization of DGKβ. We found that ionomycin induced translocation of DGKβ to the cytoplasm and constitutive active mutant (CA) lacking RVH-EF and kinase negative mutant (KN) also showed ionomycin-induced cytosolic localization, indicating that it independent of RVH-EF domain and enzymatic activity. It is well known that Ca^{2+} influx induced rearrangement of actin and DGKβ has putative actin binding site (ABS). Hence, we examined interaction between DGKβ and actin. Immunofluorescent staining showed co-localization of DGKβ and actin at plasma membrane in resting state and they co-localized in the cytoplasm after ionomycin treatment. Furthermore, we proved that DGKβ directly bound to actin via ABS, and the ABS deletion mutant (ΔABS) abolished the neurite outgrowth ability. These results suggested that the plasma membrane localization of DGKβ via actin binding is important for its localization and the ability to induce neurite outgrowth.

Disclosures: R. Tsumagari: None. **T. Kano:** None. **S. Ueda:** None. **M. Yamanoue:** None. **Y. Shirai:** None.

Poster

117. Axon and Dendrite Development: Dendritic Growth and Branching

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 117.17/C35

Topic: A.05. Axon and Dendrite Development

Title: The role of pea3 protein in neurite extension and neuroregenerative approaches

Authors: *B. KANDEMIR[1], G. GULFIDAN[2], K. Y. ARGA[2], B. YıLMAZ[3], I. KURNAZ[1]
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Abstract: Pea3 proteins are a subfamily of the ETS transcription factor superfamily, consisting of Pea3, Erm and Er81. These proteins, which are expressed in different tissues exhibiting branching, play a role in a variety of events such as the formation of motor neuronal circuits in the nervous system, retinal differentiation, neurite extension. These proteins, which have been working for many years in our laboratory, soon began to be determined in our group by deciphering axon elongation mechanisms. The purpose of this present study is to understand the mechanisms of axon elongation through Pea3, to identify the genes which are regulated by Pea3 and to use this information in neuroregenerative approaches. For this, novel target gene expression levels were investigated by microarray analysis and Real-time PCR in Pea3 overexpressed various neural cell lines. The genes were classified by KEGG analysis, the pathways associated with neurons (neurotrophin signaling pathway, axon dynamics, etc.) were selected and the relationship between the genes in these pathways was examined and mapped by informatics analysis. Our results showed that the members of Pea3 family regulate the expression of both common and unique genes in neuron-specific pathways at similar and / or different levels. In addition, the interaction mapping was created as a result of the informatics analysis. In order to elucidate which of these identified genes play a role in the selectivity of the motor neuron - sensory neuron circuits in Pea3 - overexpressed cells, studies on the relationship between different Pea3 family members in the co-culture system have been continued and the role of the Pea3 in neurite extension and circuit formation system in the direction of the obtained data is clarified.

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Poster

117. Axon and Dendrite Development: Dendritic Growth and Branching

Location: SDCC Halls B-H

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Program #/Poster #: 117.18/C36

Topic: A.05. Axon and Dendrite Development

Support: NIH R01 NS086082
GSU Brains & Behavior Seed Grant

Title: The PP2A serine/threonine phosphatase complex functions in regulating dendritic diversification

Authors: *S. BHATTACHARJEE[1], A. GOLSHIR[1], D. N. COX[2]
[2]Neurosci. Inst., [1]Georgia State Univ., Atlanta, GA

Abstract: The vast morphological diversity exhibited by neurons is critical in specifying patterns of synaptic connectivity. Thus, elucidating the molecular mechanisms that regulate dendritic

diversification is essential to understanding the formation and modulation of functional neural circuitry. While transcription factors (TFs) have been implicated in regulating dendritic diversity, the molecular mechanisms by which this is achieved remain incompletely understood. In *Drosophila*, recent studies have demonstrated that the TFs Cut and Knot regulate cell-type specific dendritogenesis in multidendritic (md) sensory neurons via cellular pathways that converge on cytoskeletal architecture. Neurogenomic analyses identified the highly conserved PP2A serine/threonine phosphatase complex as a putative downstream effector of both Cut and Knot. The PP2A complex is composed of a catalytic subunit encoded by *microtubule star* (*mts*), a scaffolding subunit encoded by *PP2A-29B* and one of four alternate regulatory subunits encoded by *widerborst* (*wdb*), *twins*, *PP2A-B'* and *CG4733*. Mutant analyses of *mts* and *PP2A-29B* reveal severe reductions in dendritic arborization with *wdb* appearing to function as the relevant regulatory subunit in CIV neurons. In contrast, mutations in *mts* and *PP2A-29B* leads to increased dendritic complexity via *de novo* filopodia formation with *CG4733* appearing to function as a relevant regulatory subunit in CI neurons. Furthermore, Mts expression can rescue *cut* mutant defects in CIV dendritic complexity. Cellularly, live imaging reveals that *mts* and *wdb* mutations lead to MT destabilization, whereas the effects of these genes on F-actin are distinct, with Mts restricting F-actin levels and Wdb promoting F-actin levels. Loss of Mts also leads to defects in both mitochondria and Golgi localization in CIV neurons. Moreover, overexpression analyses reveal dendritic shape is sensitive to expression levels of different complex components, which likewise have distinct impacts on the cytoskeleton. Mechanistically, while Mts and PP2A-29B are also part of the STRIPAK complex with Cka as the regulatory subunit, our data suggest that the PP2A and STRIPAK complexes function in parallel to regulate dendritic architecture. At a regulatory level, phenotypic analyses suggest that the translational repressor FMR1 may be a functional target of the PP2A complex in CIV neurons, whereas the transcription factor Foxo may be a functional target of this complex for in CI neurons. Collectively, these analyses provide novel insights into the roles of PP2A phosphatase function in promoting dendritic diversity.

Disclosures: S. Bhattacharjee: None. **A. Golshir:** None. **D.N. Cox:** None.

Poster

117. Axon and Dendrite Development: Dendritic Growth and Branching

Location: SDCC Halls B-H

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Topic: A.05. Axon and Dendrite Development

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The Charles Hood Foundation

Title: Rem2 is a cell-autonomous negative regulator of dendritic complexity in the mouse visual cortex

Authors: *S. E. RICHARDS[1], A. R. MOORE[3], S. SAXENA[4], S. PARADIS[2], S. D. VAN HOOSER[2]
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Abstract: The critical period in mouse visual cortex is an epoch of heightened plasticity at the systems, circuit, and cellular levels. However, little is known about the changes in dendritic morphology that occur in the mammalian cortex during this time or about the molecules that may regulate these changes. Our recent work establishes for the first time that visual experience is necessary during the critical period for the proper development of basal arbors of L2/3 pyramidal neurons in the mouse. Through these experiments, we also identified that the small GTPase Rem2, a member of the RGK family, is an experience-dependent negative regulator of basal arbor development. To better understand the role of Rem2 in establishing and maintaining dendritic complexity, we used viral methods to achieve sparse knockout of Rem2 in the visual cortex of mice. Sparse loss of Rem2 resulted in significant dendritic growth in just 7 days, indicating that an endogenous, cell-autonomous function of Rem2 is to oppose this growth. We also explored how Rem2 may impact dendritic macro-architecture, such as basal arbor polarity. Additionally, we present here a new analysis package for assaying over a dozen parameters of dendritic complexity from reconstructed neurons. Taken together, this work characterizes novel functions of Rem2 in regulating dendritic architecture and provides an analysis infrastructure for efficient identification and quantification of future molecular targets.

Disclosures: S.E. Richards: None. **A.R. Moore:** None. **S. Saxena:** None. **S. Paradis:** None. **S.D. Van Hooser:** None.

Poster

117. Axon and Dendrite Development: Dendritic Growth and Branching

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 117.20/C38

Topic: A.05. Axon and Dendrite Development

Title: Effect of d^9(-) THC (tetrahydrocannabinol) exposure during gastrulation on neuronal development in Zebrafish

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Abstract: Cannabis is one of the most popular illicit recreational drugs, after alcohol and tobacco. δ^9-tetrahydrocannabinol (δ^9-THC) is the main psychoactive ingredient in marijuana. It has two stereoisomers, δ^9(+) THC and δ^9(-) THC, of which the (-) enantiomer is the more active form. Several lines of evidences suggest that the interaction of cannabinoids with their receptors (CB1Rs and CB2Rs) depend upon the steriospecificity of the compounds. In this study we wanted to determine the effect of brief exposure of δ^9(-) THC during early development. To do this, we exposed zebrafish embryos to δ^9(-) THC for 5 hours during a stage of development known as gastrulation (5.25 hr-10.75 hr). δ^9(-) THC exposed embryos exhibited a dose-dependent reduction in survival and a delay in hatching. Further, δ^9(-) THC treated embryos showed reduced body length, axial malformation and slower heart rates. Exposed embryos were less active than vehicle controls and rested on their side by 5 dpf as opposed to vehicle controls and wild type embryos that were upright in the water column and that exhibited short bouts of swimming. Treated fish were less responsive to touch whereas controls exhibited robust escape responses upon touching the head or tail with forceps. Because the movement of treated fish was severely limited, we examined motor neuron (MN) development and activity at the neuromuscular junction (NMJ). Fluorescent labelling of primary and secondary MNs indicated a change in branching patterns and a reduction in the number of axonal branches in the trunk musculature. δ^9(-) THC treated embryos exhibited reduced mEPC frequency at NMJs. To further investigate locomotor responses, we tested the ability of treated fish to respond to sound at 5 dpf. We found that larvae exhibited a drastic reduction in the number of C-start escape responses to sound stimuli. Together these findings indicate that zebrafish embryos exposed to δ^9(-) THC during gastrulation exhibited alterations in heart rate, motor neuronal morphology, synaptic activity at the NMJ and locomotor responses. Co-application of CB1 receptor blockers (AM251, CP-9455) with δ^9(-) THC increased the survival and body length of treated embryos. Additionally, the primary and secondary MN branching patterns were largely normal when gastrula were treated with CB1R antagonists plus δ^9(-) THC. However, co-treatment with CB2R antagonists (AM630, JTE907) still resulted in altered MN branching. These findings suggest that δ^9(-) THC may alter early embryonic development via acting through CB1Rs.

Disclosures: M. Amin: None. **K. Ahmed:** None. **D.W. Ali:** None.

Poster

117. Axon and Dendrite Development: Dendritic Growth and Branching

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 117.21/D1

Topic: A.05. Axon and Dendrite Development

Support: NIH/NIMH MH093661
Spastic Paralysis Research Foundation of Illinois-Eastern Iowa District of Kiwanis International

Title: FMRP and RISC-associated RNA helicase MOV10: A novel neuronal helicase required for normal dendrite formation and spine maturation

Authors: *M. C. LANNOM[1], S. CEMAN[2]
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Abstract: Fragile X syndrome (FXS) is the leading cause of inherited cognitive impairment affecting 1/4000 males and 1/8000 females. FXS is caused by loss of expression of the RNA binding protein FMRP and it is unknown how FMRP regulates translation of its bound targets. We identified RNA helicase MOV10 as an FMRP interactor. MOV10 is highly expressed in brain during late embryonic and early postnatal stages, a crucial time for neurogenesis and synaptic spine growth and remodeling. Based on cross-linking immunoprecipitation (CLIP) and RNAseq experiments, FMRP and MOV10 bind a common set of primarily cytoskeleton-related mRNAs. We hypothesize that both proteins act together to modulate neuronal morphology. Our objective was to characterize the neuronal phenotype of the Mov10 +/- mouse, since the full knockout is embryonic lethal. We also tested our hypothesis using a mouse neuroblastoma cell line (N2A) that extends neurite-like projections, where we fully ablated Mov10 using CRISPR-Cas9. We cultured hippocampal neurons from male and female WT, Fmr1 KO mice and Mov10 +/- mice, stained for MAP2 at DIV14 and performed Sholl analysis (n > 50 neurons for all three genotypes) to characterize neuronal morphology. Our analysis uncovered severe defects in the Mov10 +/- neurons including reduced dendritic complexity, fewer and wider branches, smaller soma size and an increase in the overall number of immature spines—the latter of which we identified using Golgi impregnation. We hypothesize that reduced Mov10 leads to increased spine elimination rate. We also recapitulated some of these phenotypes in our N2A cells, where loss of Mov10 leads to shorter neurites. Interestingly, the RNA and protein levels of some of the cytoskeletal MOV10 and FMRP targets decrease in N2A cells in the absence of Mov10, suggesting that MOV10 protects them from degradation. To validate targets, we will over-express them in N2A and examine whether the shortened neurite phenotype is reversed suggesting a co-regulation by MOV10 and FMRP on their common mRNA targets. We are now attempting to uncover the mechanism behind this regulation and since FMRP and MOV10 exhibit a protein-protein interaction with AGO2, we're exploring the role of miRNAs and translational suppression/degradation in neurons leading to changes in cellular morphology. MOV10 KO is embryonic lethal and when 50% of it is gone as in the Mov10+/- mice, neuronal architecture is dramatically altered causing the neurons to exhibit an intermediate phenotype between WT and Fmr1 KO mice which we predict will have a profound impact on normal neuronal function.

Disclosures: M.C. Lannom: None. **S. Ceman:** None.

Poster

117. Axon and Dendrite Development: Dendritic Growth and Branching

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 117.22/D2

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant EY-011912
NSF GRFP DGE-1321846

Title: Dscam differentially shapes dendritic and axonal arbor morphology in the developing visual system

Authors: *R. A. SANTOS, A. FUERTES, H. SHAO, B. VO, R. ARIAS, G. SHORT, S. COHEN-CORY
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Abstract: Retinal ganglion cells (RGCs) and bipolar cells (BCs) are two key cell types that process visual information in the vertebrate retina. Proper design of dendritic and axonal arbors from both cell types is critical for information to be efficiently carried throughout the visual circuitry. Developing neurons rely on an array of molecular cues to shape arbor morphology, but the underlying mechanisms guiding the differentiation of dendritic and axonal arbors from the same retinal neuron remains unclear. Here we explore how Down Syndrome cell adhesion molecule (DSCAM) differentially shapes the dendritic and axonal morphology of RGCs and BCs in the *Xenopus* visual system. Our previous work showed that lowering DSCAM expression in RGCs impacts axon branching in the midbrain of *Xenopus* embryos. RGC axon arbors with DSCAM knockdown had a similar initial number of terminal axon branches as controls, but over 48 hrs of imaging failed to significantly increase their number of branches. Our results suggest that DSCAM has a cell-autonomous role in facilitating axonal arbor development. Because DSCAM also localizes to the dendrites of RGCs, altering DSCAM levels in RGCs may influence dendritic arbor development as well. To determine effects of DSCAM downregulation on dendritic arbor development in RGCs, we measured the total number of branches and branch length of RGCs electroporated with either control anti-sense oligonucleotide morpholino (MO) or DSCAM MO. Confocal microscopy of retinal sections showed that the number of branches and the total length of the dendritic arbors of RGCs with DSCAM MO-mediated knockdown were not significantly different from those of control MO transfected RGCs. In contrast, analysis of BCs revealed that downregulation of DSCAM in retinal BCs resulted in significant morphological changes, with neurons possessing a significantly higher number of dendritic branches and longer total dendritic arbor length when compared to control MO transfected BCs. To further evaluate potential effects of DSCAM downregulation on dendritic arbor morphology on developing *Xenopus* retinal cells, we quantified the number of dendrite crossings and of

dendrites that overlap in both RGCs and BCs with DSCAM MO-mediated knockdown. Only BCs showed deficits in dendrite self-avoidance, therefore demonstrating differential effects of DSCAM downregulation that depend on the cell type. Together, these results indicate that in the vertebrate visual system, endogenous DSCAM acts at multiple levels along the visual pathway and independently modulates dendrite and axon arborization, where cell-autonomous roles vary depending on the neuronal population.

Disclosures: R.A. Santos: None. **A. Fuertes:** None. **H. Shao:** None. **B. Vo:** None. **R. Arias:** None. **G. Short:** None. **S. Cohen-Cory:** None.

Poster

117. Axon and Dendrite Development: Dendritic Growth and Branching

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 117.23/D3

Topic: A.05. Axon and Dendrite Development

Support: NIH intramural support

Title: Regulation of dendritic branch dynamics by visual experience and amphiphysin in developing *Drosophila* larval visual circuit

Authors: *C. SHENG, U. JAVED, M. GIBBS, C. LONG, B. QIN, J. YIN, Q. YUAN
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Abstract: Dynamic dendritic branches exhibit rapid extensions and retractions in young neurons, sampling the environment and initiating new contacts with potential synaptic partners. Previous studies have shown that synaptic contacts stabilize the dynamic branches through locally induced calcium signaling and the motility of dendritic branches decreases once high synapse density is established. Although dynamic dendritic branches are clearly linked to synapse formation, molecular mechanisms regulating their prevalence during development are not well understood. We used genetic approaches to study dendritic branch dynamics in the developing Drosophila larval visual circuit, where dendritic arbors of ventral lateral neurons (LNvs), the postsynaptic targets of larval photoreceptors, exhibit robust homeostatic structural plasticity when the animal is subjected to different visual experiences.
Using high-resolution time-lapse imaging studies on single labeled LNvs in the intact larval brain, we observed temporal correlation between heightened dendrite dynamics and synaptogenesis. In addition, constant light exposure reduces dendrite dynamics and synaptogenesis, while constant darkness generates opposite effects. Subsequent in vivo RNAi screens identified Amphiphysin (Amph), a BAR-domain containing protein, that is specifically required for cell autonomous regulation of dendrite dynamics without affecting synaptogenesis. We further investigated how Amph exert its effects by examining its interaction with

postsynaptic proteins. We found that Amph regulates dendrite maturation and synaptic functions through modulating the localization of Fas2, a cell adhesion molecule involved in synapse organization and function. Taken together, our data provide additional evidences to support the synaptotrophic model, in which dynamic dendritic branches promote synaptogenesis. In addition, we demonstrate that synapse number is not the sole determining factor for dendrite dynamics. Instead, we found that the prevalence of dynamic dendritic branches is regulated by the maturation state of the dendrite, which is collaboratively determined by synaptic inputs and the internal developmental program.

Disclosures: C. Sheng: None. **U. Javed:** None. **M. Gibbs:** None. **C. Long:** None. **B. Qin:** None. **J. Yin:** None. **Q. Yuan:** None.

Poster

117. Axon and Dendrite Development: Dendritic Growth and Branching

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 117.24/D4

Topic: A.05. Axon and Dendrite Development

Support: NSF IOS 1354913
NSF STC CBET 0939511

Title: High-resolution studies of miR-125b in filopodial structure and dynamics

Authors: *R. IYER[1], M. E. KANDEL[2], Y.-S. KIM[3], G. POPESCU[2], M. U. GILLETTE[4]
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Abstract: Dendritic filopodia are long, thin, highly dynamic structures that play a critical role in establishing the dendritic arbor and spinogenesis. They interact with the microenvironment and relay information that is used for local decision-making with respect to growth and synapse formation. The mechanisms by which filopodia are briefly maintained during development, and how their function is regulated, are critical to understanding the wiring of the brain.Recent studies have pointed to microRNAs (miRNA), small noncoding RNAs about 22 nucleotides long, as potential sites for integration of external stimuli to changes in local protein expression. This can lead to rapid and local changes in filopodial and dendritic structures, a key requirement to navigate the spatio-temporally variant signals dendrites receive. Here we investigate the brain-abundant miRNA, miR125b, to elucidate its role in the dynamics of filopodia and the corresponding changes in filopodial and dendritic structure during development. We inhibit its activity in cultured hippocampal neurons as dendritic filopodia explore their microenvironment. Using a combination of confocal microscopy and high resolution image analysis we study the

effect of miR125b inhibition on filopodial structure and density, dendritic outgrowth, as well as the expression and perifilopodial localization of a confirmed target of miR125-b: the GluN2A subunit of the NMDA receptor. Using whole cell patch clamp recordings, we find that miR125b inhibition changes the neuronal response to diffusive glutamate signals due to increased GluN2A expression. Using microfluidic devices, we deliver a cell permeant inhibitor of miR125b to isolated dendrites, and study local changes in filopodial structure and GluN2A incorporation. To understand the effect of miR125b in the dynamics of filopodia, we use Spatial Light Interference Microscopy (SLIM), an interferometry-based, label-free, live imaging system. Using SLIM, we characterize the rate of filopodial extension and retraction, and stability in response to miR125b inhibition. These high-resolution analyses reveal fresh insights into the process by which neurons integrate multiple external signals to establish the correct connections. Such insights are critical to understanding the implicated role of miR125b in various neurological disorders, e.g., Fragile X Syndrome and Alzheimer's Disease.

Disclosures: R. Iyer: None. **M.E. Kandel:** None. **Y. Kim:** None. **G. Popescu:** None. **M.U. Gillette:** None.

Poster

117. Axon and Dendrite Development: Dendritic Growth and Branching

Location: SDCC Halls B-H

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Program #/Poster #: 117.25/D5

Topic: A.05. Axon and Dendrite Development

Support: NSF 1640885
F32MH110184

Title: Dendritic spine pruning on corticostriatal intratelencephalic (IT) type neurons in the dorsomedial prefrontal cortex during adolescence

Authors: N. OKADA, K. DELEVICH, C. D. HALL, *L. E. WILBRECHT
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Abstract: The prefrontal cortex (PFC) exhibits delayed maturation, evidenced by grey matter thinning and dendritic spine pruning that extends late into adolescence. The protracted maturation of the PFC may support the maturation of higher order cognitive functions but might also confer heightened vulnerability to psychiatric disease during adolescence. However, little is known about how different cell types in the PFC mature during adolescence or whether puberty plays a causal role. Pyramidal Tract (PT) and Intra-Telencephalic (IT) cells are two dominant classes of excitatory neurons that reside in layer 5 of PFC but exhibit distinct anatomical and electrophysiological properties. Relative to PT-type neurons which are labeled by Thy1-YFP reporter mouse lines, IT-type neurons are more difficult to access experimentally, and therefore

less is known about their structural maturation. Here, we leveraged retro-AAV with two-photon microscopy to investigate spine pruning in cross-corticostriatal IT (CCstr-IT) neurons in the dorsomedial prefrontal cortex (dmPFC) of mice. C57Bl6 mice were injected unilaterally into dorsomedial striatum (DMS) with retrograde pAAV-CAG-GFP at postnatal day 21 (P21) or 52 (P52). We allowed 8 days for viral expression before perfusing mice at P29 (early adolescent) or P60 (early adult) timepoints. Fixed tissue was then processed to amplify GFP signal and spines were imaged on a 2 photon microscope. Microinjection (50 nL) of dilute retro-GFP virus into DMS produced sparse labeling of neurons in the contralateral dmPFC. We confirmed that GFP+ neurons in the contralateral dmPFC exhibited IT-like intrinsic properties, including spike frequency adaptation and limited voltage sag. We found main effect of age and sex in apical dendritic spine density on CCstr-IT neurons, but no significant interaction. Spine density decreased during adolescence between P29 and P60. In early adulthood (P60), apical dendrite spine density on CCstr-IT neurons in males was significantly lower than in females of the same age. Sex differences in spine density in early adulthood have not been observed in PT-type neurons. Ongoing work is examining the influence of pubertal hormones on spine pruning in CCstr-IT neurons by comparing spine density in sham or prepubertally gonadectomized mice at adulthood (P60). In addition we are comparing intrinsic electrophysiological properties of CCstr-IT neurons between early adolescent and adult timepoints. Together, our preliminary findings suggest major cortical cell types undergo differential adolescent maturation in the PFC in a sex-specific manner.

Disclosures: N. Okada: None. **K. Delevich:** None. **C.D. Hall:** None. **L.E. Wilbrecht:** None.

Poster

118. Synaptogenesis and Activity-Dependent Development: Synapse Formation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 118.01/D6

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

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Title: Impairment of inhibitory synapse formation and motor behavior in mice lacking the NL2 binding partner LHFPL4/GARLH4

Authors: *M. WU[1], H.-L. TIAN[1], X. LIU[1], J. LAI[1], S. DU[2], J. XIA[1]
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Abstract: Normal brain functions depend on the balanced development of excitatory and inhibitory synapses. Our knowledge of the molecular mechanisms underlying inhibitory synapse formation is limited. Neuroligin-2 (NL2), a transmembrane protein at inhibitory postsynaptic sites, is capable of initiating inhibitory synapse formation. In an effort to search for NL2 binding proteins and the downstream mechanisms responsible for inhibitory synapse development, we identify LHFPL4/GARLH4 as a major NL2 binding partner that is specifically enriched at inhibitory postsynaptic sites. LHFPL4/GARLH4 and NL2 regulate the protein levels and synaptic clustering of each other in the cerebellum. *Lhfpl4/Garlh4*$^{-/-}$ mice display profound impairment of inhibitory synapse formation as well as prominent motor behavioral deficits and premature death. Our findings highlight the essential role of LHFPL4/GARLH4 in brain functions by regulating inhibitory synapse formation as a major NL2 binding partner.

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Poster

118. Synaptogenesis and Activity-Dependent Development: Synapse Formation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 118.02/D7

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

Support: NIH R01 NS055272
March of Dimes FY11-456

Title: Physical and functional interaction between gamma-protocadherins and neuroligin-2 in the development of inhibitory synapses

Authors: D. M. STEFFEN, C. G. MARCUCCI, M. J. MOLUMBY, *J. A. WEINER
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Abstract: The mammalian *Pcdha, Pcdhb, and Pcdhg* gene clusters encode a diverse group of cadherin superfamily adhesion molecules, the α-, β-, and γ-Protocadherins, respectively. The 22 γ-Protocadherins (γ-Pcdhs) are combinatorially expressed in the brain, and play critical roles in synaptogenesis, dendrite arborization and patterning, and the survival of subsets of neurons *in vivo*. The γ-Pcdhs can interact promiscuously with each other, and with other clustered Pcdhs, in *cis*, but interact strictly homophilically in *trans*. Mice lacking the γ-Pcdhs in the cerebral cortex *in vivo* exhibit severely reduced dendrite arborization (Garrett, et al., *Neuron*, 2012). Recently,

we further demonstrated that these cortical mutants exhibit a significant increase in the density of dendritic spines and excitatory synapses, and found that the γ-Pcdhs physically interact in *cis* with neuroligin-1, a postsynaptic adhesion molecule implicated in autism and schizophrenia that is important for the maturation of excitatory synapses. In an "artificial synapse assay" *in vitro*, γ-Pcdhs could inhibit the ability of neuroligin-1 to induce presynaptic differentiation (Molumby et al., *Cell Reports*, 2017). While this study identified a new mechanism through which γ-Pcdhs regulate excitatory synapses, their effect on inhibitory synapse development has not yet been examined. Here, we provide evidence that the γ-Pcdhs can also negatively regulate inhibitory synapse development in forebrain neurons. Using co-immunoprecipitation assays, we find that γ-Pcdhs interact both *in vitro* and *in vivo* with neuroligin-2, which is found at inhibitory postsynaptic sites and promotes inhibitory synapse maturation. Utilizing the artificial synapse assay, we find that multiple γ-Pcdhs can, when co-expressed in COS cells, strongly inhibit the ability of neuroligin-2 to promote presynaptic clustering of synaptic vesicle proteins synapsin and VGAT in contacting axons. To ask whether the γ-Pcdhs negatively regulate inhibitory synapse density *in vivo*, we analyzed mice in which a conditional *Pcdhg* allele has been excised in excitatory cortical neurons (the postsynaptic sites of many inhibitory synapses) using *Emx1-Cre*. Using immunostaining for synaptic markers, we find that inhibitory synapse density is, indeed, significantly increased in the absence of γ-Pcdhs. Together, these data suggest that γ-Pcdhs interact with neuroligin-2 in *cis* at inhibitory postsynaptic sites to negatively regulate the formation and/or maturation of inhibitory synapses.

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Poster

118. Synaptogenesis and Activity-Dependent Development: Synapse Formation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 118.03/D8

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

Support: MH086032

Title: Leptin increases GABAergic synaptogenesis via β-pix in hippocampal neurons

Authors: *G. S. SAHIN[1], I. MEDINA[3], J.-L. GAIARSA[3], S. M. APPLEYARD[2], G. A. WAYMAN[1]
[1]Integrative Physiol. and Neurosci., [2]Neurosci. Program, Washington State Univ., Pullman, WA; [3]Inst. de Neurobiologie de la Mediterranee, Marseille, France

Abstract: Leptin is a hormone whose function is well-characterized in energy homeostasis; however, less is known about its roles in the hippocampal physiological processes, while leptin

receptors are widely expressed in this brain region. During rodent development, leptin surges during postnatal day 7-14, which is equivalent to third trimester in human fetal development. Importantly, leptin deficiency in this period is associated with cognitive defects, anxiety, and depression, and leptin replacement alleviates these symptoms, suggesting that leptin is a crucial factor in development and mental health. In the hippocampus, mice expressing the signaling deficient LepRb mutant (db/db), have reduced number of glutamatergic and GABAergic synaptic connections. While several studies have addressed the molecular mechanisms in which leptin acts through to control glutamatergic synaptogenesis, leptin-mediated GABAergic synaptogenesis is less understood. In the present study, we aimed to determine the molecular pathways through which leptin controls GABAergic synaptogenesis during neonatal development of the hippocampus. Using primary hippocampal cultures, we did live staining and showed that acute leptin treatment increases surface expression of $GABA_A$ receptors. Using whole-cell voltage-clamping in organotypic hippocampal cultures, we recorded mini IPSCs and showed that acute leptin treatment increases frequency of mini IPSCs, which is blocked by targeted knockdown of the long form of the leptin receptor, LepRb. Taken together, these observations suggest that acute leptin increases GABAergic synaptogenesis. Furthermore, using live staining on primary hippocampal cultures, we found that Rho Guanine Nucleotide Exchange Factor 7 (β-pix) controls leptin-dependent surface expression of $GABA_A$ receptors; specifically, targeted knockdown of β-pix blocks the effects of leptin. Importantly, via co-immunoprecipitation followed by western blotting in cell lines, we showed that β-pix forms a complex with leptin receptors, and their interaction increases with leptin treatment. Based on previous studies reporting that β-pix acts as a scaffolding protein for $GABA_A$ receptors on cell membrane, our findings suggest that the leptin receptor might be in a complex with $GABA_A$ receptors in hippocampal neurons. Our study is first to identify molecular mechanisms underlying leptin effects on GABAergic synaptogenesis during hippocampal development. Ultimately, our study is expected to provide critical insights into the role of leptin not only in the hippocampus but also in other brain regions.

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Poster

118. Synaptogenesis and Activity-Dependent Development: Synapse Formation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 118.04/D9

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

Support: Charles E. Kaufman New Investigator Award, The Pittsburgh Foundation

Title: The synaptic adhesion protein Slitrk2 interacts with MAGUKs via a carboxy terminal PDZ binding motif

Authors: C. LOOMIS, P. SPRINGMANN, R. JANICOT, L. DREBUSHENKO, *J. E. ROUND
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Abstract: Slitrks are a family of leucine-rich repeat containing single-pass transmembrane proteins that promote synaptogenesis in the developing nervous system. Slitrks localize to the postsynaptic fraction, where they induce synapse formation via trans-synaptic interactions with the LAR family of receptor protein tyrosine phosphatases. While trans-synaptic binding partners of Slitrks have been reported, little is known about the intracellular proteins that interact with Slitrk's cytoplasmic domain. It is also unknown whether these intracellular binding partners influence the ability of Slitrks to localize to synaptic sites or induce postsynaptic differentiation. Here we report an interaction between Slitrks and multiple members of the MAGUK scaffold family. Coimmunoprecipitation from postnatal mouse forebrain indicates that MAGUKs bind to Slitrk family members that contain a consensus Type I PDZ-binding motif at their carboxy terminus. Mapping analysis in yeast confirms that the C-terminal PDZ binding motif of Slitrk2 and the PDZ domains of MAGUKs are required for the interaction. We also show that MAGUKS induce robust clustering of Slitrk2 in 293T cells, and the C-terminal PDZ binding motif of Slitrk2 is necessary for this clustering. These data suggest that Slitrk-MAGUK interactions may mediate localization of Slitrks to synaptic sites and may recruit additional intracellular signaling molecules involved in postsynaptic differentiation.

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Poster

118. Synaptogenesis and Activity-Dependent Development: Synapse Formation

Location: SDCC Halls B-H

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Program #/Poster #: 118.05/D10

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

Title: mechanism of intracellular signal transduction of synapse formation

Authors: *X. JIANG, R. SANDO, T. SUDHOF
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Abstract: In the brain, neurons are joined into a network by connections called synapses, which ensure the neurons to transmit signals with high speed and spatial precision. The formation of the synapses is coordinated by an array of pre-and post-synaptic adhesion molecules such as

neurexins, neuroligins, PTPRs, LRRTM, NPR, etc. The roles of these synaptic adhesion molecules in the formation of synapses has been extensively investigated, however, what are the intracellular signal transduction pathways that mediate synaptogenesis is still largely unknown. To address this question, we took advantage of a simplified system of synapse formation, the artificial synapse formation assay. In this assay, we express the synaptogenic protein, for example neurexin or neuroligin, in the HEK293 cells, and then co-culture them with mature neuronal cells. In about 24 hrs, pre- or post-synaptic markers can be detected on the surface of the non-neuronal HEK293 cells, which reflects the pre- or post- synaptic specialization/differentiation induced by this molecule. We did a small scale chemical screen in this reduced system and found that inhibitors for JNK or PKA pathway blocked both pre- and post- synaptic specialization and the inhibitors for the PI3 kinases specifically blocked post-synaptic specialization. Also inhibition of protein synthesis did not block the artificial sysnapse formation. Furthermore, expression of a post-synaptic targeting form of PDE (PDE_homer) inhibited NRXN1β induced post synaptic specialization but not NL1 induced pre-synaptic specialization. Moreover, JNK and PKA inhibitors also blocked LRRTM2 induced pre-synaptic specialization and NPR induced post-synaptic specialization. Thus our data indicate that JNK and PKA signaling pathways play important roles in both pre- and post- synaptic specialization while the PI3 kinase pathway functions specifically in the post-synaptic specialization.

Disclosures: X. Jiang: None. **R. Sando:** None. **T. Sudhof:** None.

Poster

118. Synaptogenesis and Activity-Dependent Development: Synapse Formation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 118.06/D11

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

Support: NRF Grant 2014R1A1A2058234
KBRI Grant 18-BR-01-04

Title: A new *in vitro* and *in vivo* genetic tools to study synapse formation mechanism through semaphorin 3E signaling

Authors: *M.-H. JUN[1], R. YU[2], D.-G. KIM[2], W.-J. OH[2]
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Abstract: Precise synaptic connections are essential for the proper formation of functional circuits and the generation of complex behavior and cognitive function. During development, axons first navigate long distances to reach their target regions and then select an appropriate

synaptic partner as well as a specific location on this postsynaptic cell to establish a synapse. However, very little is currently known about the precise mechanisms underlying synaptogenesis in the complicated neuronal circuit of the mammalian brain. Previously, we discovered that *in vivo* interaction between traditional axon repulsive cue, Semaphorin 3E (Sema3E), and its receptor, Plexin-D1, determines synaptic specificity in thalamo-striatal circuits of basal ganglia system. Specifically, Sema3E-Plexin-D1 signaling normally restricts the number of thalamo-striatal synapses formed onto direct pathway medium spiny neurons (MSNs). However, its molecular mechanism at the subcellular level has still not been revealed due to the lack of proper experimental tools. In this study, we successfully established the *in vitro* thalamo-striatal co-culture system and confirmed the expression of PlexinD1 and GluR1 along the neuronal differentiation stages in this system. We also observed that overexpressed Plexin-D1 is discretely located with GluR1 sites, indicating that Sema3E-Plexin-D1 signaling axis regulates excitatory synapse formation sites. In addition to the co-culture system, we also developed new knock-in mouse model using the CRISPR-Cas9 technique, in which 3xFlag-tag is attached at the N-terminus of the endogenous Plexin-D1 to easily detect and analyze the Plexin-D1 in the subcellular level. We verified that the Flag-tagged Plexin-D1 behaves like the normally expressing-Plexin-D1 using histological and biochemical methods. Taken together, this mouse line and *in vitro* thalamo-striatal co-culture system will be useful genetic tools for the mechanistic study of synaptic specificity through the Sema3E-PlexinD1 signaling.

Disclosures: M. Jun: None. **R. Yu:** None. **D. Kim:** None. **W. Oh:** None.

Poster

118. Synaptogenesis and Activity-Dependent Development: Synapse Formation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 118.07/D12

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

Support: NIH Grant R01 MH082808
NIH Grant R01 NS082266

Title: Axo-axonic innervation of neocortical pyramidal neurons by GABAergic chandelier cells requires ankyrinG-associated L1CAM

Authors: *N. B. GALLO[1], Y. TAI[1], J.-R. YU[2], L. VAN AELST[1]
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Abstract: Proper cortical network development and function is reliant on the generation, maturation, and activity of numerous cell types in addition to their complex cell-cell interactions. Essential to this process is the output of glutamatergic pyramidal neurons (PyNs), which is

highly modulated by inhibitory GABAergic interneurons. One subset of interneurons that exerts powerful control over PyN spiking is the chandelier cell (ChC), which forms connections specifically at the site of action potential initiation in PyNs, referred to as the axon initial segment (AIS). Due to the unique connections formed between the terminals of ChC axonal arbors and the AISs of large populations of spiking PyNs, ChCs are physiologically poised to regulate the output of excitatory cortical networks. As a result, it is not surprising that disruptions in ChC biology have been linked to autism spectrum disorder (ASD) and schizophrenia, debilitating mental health disorders resulting from aberrant neuronal network activity. Despite the importance of ChCs, virtually nothing is known about the molecular factors governing their selective innervation at the AIS of neocortical PyNs. By performing a novel, *in vivo* RNA interference screen against PyN AIS-specific and -enriched adhesion molecules, we intriguingly revealed an essential role for the axonal cell adhesion molecule L1CAM in ChC/PyN AIS innervation. Specifically, L1CAM knockdown in neocortical PyNs was found to significantly reduce PyN AIS innervation by ChCs, thus identifying L1CAM as the only known molecule to date to regulate this process. To further elucidate how L1CAM governs selective ChC/PyN AIS innervation, we used molecular tools to perturb L1CAM/AIS cytoskeleton interactions and to manipulate PyN L1CAM levels at different developmental time points. Our results indicate that the AIS cytoskeletal proteins ankyrinG and βIV-spectrin are essential for proper ChC/PyN AIS innervation and demonstrate a dual requirement for L1CAM during both the establishment and maintenance of neocortical ChC/PyN AIS innervation. Together, our findings provide vital information on the mechanisms governing PyN AIS subcellular innervation by ChCs and, as such, shed new light on the connectivity defects underlying debilitating mental disorders.

Disclosures: N.B. Gallo: None. **Y. Tai:** None. **J. Yu:** None. **L. Van Aelst:** None.

Poster

118. Synaptogenesis and Activity-Dependent Development: Synapse Formation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 118.08/D13

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

Support: CIHR Grant MOP142215
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Canada Foundation for Innovation (29462)
University of Victoria-Division of Medical Sciences
University of Victoria Fellowship Graduate Award
Key Laboratory of Neuroregeneration of Jiangsu and Ministry of Education, Co-innovation Center of Neuroregeneration, Nantong University, China

Title: Pannexin 1 regulates somatosensory pyramidal neuron dendritic spine density and sensorimotor function

Authors: *J. C. SANCHEZ-ARIAS[1], M. LIU[1,2], O. SHEVTSOVA[1], L. A. SWAYNE[1,3]
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Abstract: Pannexin 1 (Panx1) forms channels permeable to ions and metabolites and is enriched in the central nervous system. We have previously demonstrated that Panx1 disruption increased neurite formation in neural precursors *in vitro*. Our new findings demonstrate that Panx1 protein levels drop dramatically between P14 and P30, corresponding to the critical period for cortical dendritic spine and synapse formation. Moreover, we observed increased levels of PSD-95, a scaffolding protein important for dendritic spine functional development, in Panx1 knockout (KO) cortical synaptosome preparations. Based on these findings, we hypothesized that Panx1 "keeps the brakes" on dendritic spine formation in the cerebral cortex. To test this hypothesis, we examined the impact of disrupting Panx1 (KO or block with probenecid) on primary somatosensory cortex layer 5 dendritic spine density and length in the postnatal period (P14 and P29) using diolistic labeling. Panx1 KO, as well as systemic treatment with the Panx1 blocker probenecid, increased spine densities at P14 and P29. Similarly, disruption of Panx1 in primary cortical neuronal cultures also led to increased density of dendritic spines, the majority of which were positive for PSD-95, suggesting the additional spines were functional. Panx1 disruption in cortical cultures also increased the number of groups of spontaneously co-active neurons, known as network ensembles, as assessed using Ca^{2+} imaging with Fluo-4AM, suggesting the increase in active spines affected network properties. At the behavioural level, Panx1 KO mice performed significantly better in forelimb sensorimotor tests compared to WT counterparts. Together, these results support a role for Panx1 as a negative regulator of dendritic spines with implications for network connectivity and sensorimotor function.

Disclosures: J.C. Sanchez-Arias: None. **M. Liu:** None. **O. Shevtsova:** None. **L.A. Swayne:** None.

Poster

118. Synaptogenesis and Activity-Dependent Development: Synapse Formation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 118.09/D14

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

Support: GM055145
NS089578

Title: The role of MARK1 in synaptic plasticity and cognitive functions

Authors: *E. C. KELLY[1], M. SUN[2], H. ZHANG[2]
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Abstract: Dendritic spines are dynamic postsynaptic structures that play an important role in cognitive functions, such as learning and memory. Abnormalities in size, shape and density of dendritic spines may lead to learning and memory deficiencies and neurological disorders such as autism spectrum disorders (ASD). We previously found that partitioning defective 1 c (Par1c), also known as microtubule affinity regulating kinase 1 (MARK1), regulates dendritic spine morphogenesis and plasticity in cultured hippocampal neurons. Furthermore, studies have found multiple SNPs of MARK1 are associated with ASD, and MARK1 has also been implicated in bipolar disorder. However, the role of Par1c/MARK1 in synaptic plasticity and cognitive functions *in vivo* is still unknown. Therefore, we developed a conditional knockout (cKO) MARK1 mouse model, in which MARK1 is depleted postnatally from pyramidal neurons of the forebrain including the hippocampus and the cerebral cortex. Proteomic analyses show MARK1 targets synaptic proteins as well as proteins regulating cytoskeletal dynamics. In addition, we conducted behavioral analyses of the MARK1 cKO mice. Preliminary data suggest that MARK1 regulates cognitive functions such as learning and memory. Together, our studies point to an important role for MARK1 in regulating synaptic and cognitive functions *in vivo*.

Disclosures: E.C. Kelly: None. **M. Sun:** None. **H. Zhang:** None.

Poster

118. Synaptogenesis and Activity-Dependent Development: Synapse Formation

Location: SDCC Halls B-H

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Program #/Poster #: 118.10/D15

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

Support: NIH Grant 5R01GM120519

Title: Midazolam sedation causes cognitive dysfunction in mice and alters synapse formation and mtor signaling *in vivo* and *in vitro*

Authors: *J. XU, R. P. MATHENA, C. D. MINTZ
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Abstract: Introduction: A recent safety advisory from the FDA has raised concerns that sedative drugs may have lasting, harmful effects on cognitive functions[1]. Longer duration of exposure is an important risk factor for anesthetic toxicity. It is of great importance to determine whether commonly used sedative drugs have neurotoxic properties during development and the underlying mechanism. Here we test the hypothesis that midazolam shares a mechanism of

action with many general anesthetic agents, causes lasting cognitive deficits in mice when administered in a sedative paradigm to early postnatal mice. We further investigated the effects of midazolam sedation on key developmental processes and the pathologic activation of the mechanistic target of rapamycin (mTOR) pathway. Methods: For *in vivo* experiments, C57BL/6 mice aged P18 to P22 were intraperitoneally injected using 10mg/kg or 20mg/kg midazolam per hour for 12 hours a day, 5 days in total to create a sedation model. The control groups consisted of naïve littermates which stayed with the dam. Behavioral tests were conducted at P60 using Y maze and fear conditioning test. 50mg/kg BrdU was injected from P57 to P59 at 24-hour intervals before transcardial perfusion. Immunohistochemistry (IHC) was conducted using antibodies against BrdU, synapsin-1, homer-1, phosphorylated S6 (pS6), parvalbumin (PV). For *in vitro* experiments, dissociated primary neuronal cultures obtained from E18 rat neocortex plated on glass coverslips were used. Exposure to 50nM, 100nM, and 150nM midazolam was conducted for a continuous 72hrs from 7 DIV to 10 DIV. IHC using anti-synapsin-1 were performed and analysis via fluorescence microscopy was conducted. Results: The midazolam treated groups showed memory loss in Y maze and fear conditioning test compared to the control group. We found less BrdU positive neurons in the dentate gyrus after midazolam while the synapsin-1 intensity was decreased but the homer-1 intensity increased, pS6 positive interneurons were significantly increased after the midazolam treatment. *In vitro,* exposure to 100nM and 150nM midazolam for 72hrs significantly decreased the intensity of synapsin-1.Conclusion: Midazolam would cause long-term learning deficiency and memory loss in mice after continuous injections at the young age. Neuroproliferation and neurogenesis were involved, and mTOR pathway might be a critical mechanism to explain these changes. References:1. FDA Drug Safety Communication: FDA approves label changes for use of general anesthetic and sedation drugs in young children. Available online: https://www.fda.gov/Drugs/DrugSafety/ucm554634.htm (Accessed on April 28th, 2017).

Disclosures: J. Xu: None. **R.P. Mathena:** None. **C.D. Mintz:** None.

Poster

118. Synaptogenesis and Activity-Dependent Development: Synapse Formation

Location: SDCC Halls B-H

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Program #/Poster #: 118.11/D16

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

Support: MEXT

Title: Lemur kinase 1 regulates dendritic spine formation through rab11-dependent endosomal trafficking

Authors: *S.-I. HISANAGA[1], T. SAITO[1], K. ANDO[1], M. TOMOMURA[2], M. FUKUDA[3], H. NISHINO[1]

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Abstract: Synapses are composed of pre- and post-synaptic terminals. A postsynaptic region of excitatory synapses forms a mushroom-like protrusion called dendritic spine. Upon long term potentiation, spines become large and, in contrast at the time of long term depression, spines reduce the size. Thus, the number and size of spines are critically regulated depending on synaptic activity. The process of spine formation is well investigated, and actin cytoskeleton is known to be a major structural component. In contrast, when spines grow and mature, they have to increase their surface area together with synaptic proteins. For the expansion of surface area, membrane components should be supplied. The membrane components are transported by vesicle trafficking system that is regulated by a family of Rab small GTPases. Several previous reports indicate the role of Rab11A in spine formation. Lemur kinase 1A (LMTK1A) is a novel Ser/Thr kinase that regulates axon outgrowth and dendrite arborization negatively via Rab11A-dependent endosome trafficking. We hypothesized that LMTK1A also functions in dendritic spine formation and examined it in vivo in LMTK1 KO mouse brain and in vitro in primary neurons.
First, we compared the number of spines in cerebral cortex of brains between LMTK1+/+ and LMTK1-/- mouse by immunostaining with anti-PSD-95. The number of PSD-95-positive puncta was larger in brain of LMTK1-/- than LMTK1+/+ mouse. Dendritic spines were also increased in neurons of LMTK1-/- mouse in cultures. When we knocked LMTK1 down in hippocampal neurons with miR-LMTK1, the spine density was increased about 36% than control neurons transfected with scramble micro RNA. The effect of knockdown or expression of LMTK1A was also confirmed in developing mouse brains using in utero electroporation. By overexpression of Rab11A mutants in neurons under the LMTK1-knockdown background, we confirmed that LMTK1 regulates spine density through Rab11A. The active form of Rab11A Q70L increased the spine
density whether LMTK1 was knocked down or not, whereas Rab11A S25N decreased the spine density. Taken together, these results indicate that LMTK1 regulates spine formation, maturation and density through Rab11 activity.

Disclosures: S. Hisanaga: None. **T. Saito:** None. **K. Ando:** None. **M. Tomomura:** None. **M. Fukuda:** None. **H. Nishino:** None.

Poster

118. Synaptogenesis and Activity-Dependent Development: Synapse Formation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 118.12/D17

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

Support: the Ministry for Health and Welfare Affairs, HI17C0080

Title: Diverse extracellular and intracellular mechanisms are involved in PTP-σmediated presynaptic assembly

Authors: *T. YOON[1], K. HAN[1], G. PRAMANIK[2], J. UM[3], J. KO[4]
[1]DGIST, Daegu, Korea, Republic of; [2]Shinshu Univ. Sch. of Med., Matsumoto, Japan; [3]Yonsei Univ. Col. of Med., Seoul, Korea, Republic of; [4]Dept. of Brain and Cognitive Sci., Daegu Gyeongbuk Inst. of Sci. and Technolog, Daegu, Korea, Republic of

Abstract: Leukocyte common antigen-receptor protein tyrosine phosphatases (LAR-RPTPs) are hub proteins that organize excitatory and inhibitory synapse development through binding to various extracellular ligands. Here, we report that knockdown (KD) of the LAR-RPTP family member PTPσ reduced excitatory synapse number and transmission in cultured hippocampal neurons, whereas KD of PTPδ produced comparable decreases at inhibitory synapses, in both cases without altering expression levels of interacting proteins. An extensive series of rescue experiments revealed that extracellular interactions of PTPσ with Slitrks are important for excitatory synapse development. These experiments further showed that the intracellular D2 domain of PTPσ is required for induction of heterologous synapse formation by Slitrk1 or TrkC, suggesting that interaction of LAR-RPTPs with distinct intracellular presynaptic proteins drives presynaptic machinery assembly. Consistent with this, double-KD of liprin-α2 and -α3 or KD of PTPσ substrates (N-cadherin and p250RhoGAP) in neurons inhibited Slitrk6-induced, PTPσ-mediated heterologous synapse formation activity. We propose a model in presynaptic neurons involving LAR-RPTP-organized retrograde signaling cascades, in which both extracellular and intracellular mechanisms are critical in orchestrating distinct synapse types.

Disclosures: T. Yoon: None. **K. Han:** None. **G. Pramanik:** None. **J. Um:** None. **J. Ko:** None.

Poster

118. Synaptogenesis and Activity-Dependent Development: Synapse Formation

Location: SDCC Halls B-H

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Program #/Poster #: 118.13/D18

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

Support: NICHD Z1A-HD001205-24
NIGMS PRAT Fellowship

Title: Who versus where: Effects of heterotopia on the development of cellular properties and connectivity of principal cells

Authors: *J. D'AMOUR[1], T. G. EKINS[3], C. J. MCBAIN[2]
[2]Lab. Cell/Molec Neurosci, [1]NIH, Bethesda, MD; [3]NIH/NICHD, Bethesda, MD

Abstract: We are interested in the role of principal cell (PC) identity and positioning in the development of cellular properties and connectivity. PCs of the developing mouse hippocampus arise in waves of cell birth and radial migration in an inside-out manner, over embryonic day 12-17 to ultimately appear as a compacted layer of neurons. This results in a rough correlation between embryonic cellular birthday and radial positioning (earliest born cells are deepest). Here, we aim to dissociate position and identity by working in Lis1 mice ($Lis1^{+/-}$). Lissencephaly is a rare human condition, most commonly due to mutations in the Lis1 gene which is involved in neuronal migration. Lis1 mice display ectopic cellular positioning in a variety of brain structures, particularly in area CA1 of the hippocampus where-in two or more principal cell layers(PCLs) develop; we refer to these as deep and superficial PCLs. While in normal mice calbindin expressing PCs localize to the superficial side of the PCL, we find that in Lis1 mice calbindin positive PCs primarily occupy the deeper heterotopic band. Importantly, preliminary birth dating experiments suggest this reflects an inverted layering of the same calbindin cell population, rather than a new PC population adopting calbindin expression. Therefore, the Lis1 mouse gives us the ability to examine the same PC sub-population, uncoupled form its typical positioning.
We find that this cell population in Lis1 mice retains a complex apical branch morphology, hyperpolarized resting membrane potentials, and high amounts of sag current; characteristic features of calbindin expressing PCs in normal animals. In examining synaptic inputs, staining experiments indicate that calbindin positive cells in both normal type and Lis1 mice are preferentially targeted by cholecystokinin expressing interneurons - which in normal mice has been suggested to contribute to greater relative excitability of calbindin positive PCs (low I:E ratios). Interestingly, despite being mispositioned, calbindin expressing PCs in Lis1 mice retain this heightened excitability phenotype. Taken together, these observations indicate that developmental mechanisms are able to substantially compensate for and overcome the mis-lamination present in the Lis1 animal. Additionally, it suggests that layering is of secondary importance to genetic identity in the determination of synaptic partners. These findings will contribute to our understanding of cellular layers, how developmental disturbances perturb intrinsic cellular properties and extrinsic synaptic connectivity, and possibly reveal circuit motifs between interneuron and pyramidal cell sub-populations.

Disclosures: J. D'Amour: None. **T.G. Ekins:** None. **C.J. McBain:** None.

Poster

118. Synaptogenesis and Activity-Dependent Development: Synapse Formation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 118.14/D19

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

Title: Two pathways of synaptic outgrowth in *Drosophila* neuromuscular junction

Authors: *A. VASIN[1], C. L. TORRES FERRERIS[2], M. BYKHOVSKAIA[1,2]
[1]Neurol., [2]Anat. and Cell Biol., Wayne State Univ. Sch. of Med., Detroit, MI

Abstract: We investigated the initial steps of synaptic differentiation and maturation during activity-dependent growth at the *Drosophila* larval neuromuscular junctions (NMJ). We took advantage of larvae with GFP-tagged neuronal membranes (CD8-GFP) and mCherry-tagged synaptic vesicle markers Synaptogyrin (SG-mCherry). The activity-dependent outgrowth *in vivo* in intact larvae was observed following either intense locomotion or seizure activity. Locomotion (3 hours) was induced by either exposing larvae to elevated temperatures or to high salt solutions. To induce seizure activity, we took advantage of the *sei* fly line, which shows neuronal hyperexcitability at restrictive temperatures. The wild type (WT) line at restrictive temperatures was used as control. We found that both paradigms produced robust outgrowth of new synaptic structures, including filopodia, synaptic boutons devoid of vesicles, as well as more mature synaptic boutons containing vesicles. The experiments at the *sei* line demonstrated that the mature boutons filled with vesicles can be formed rapidly, within one minute. The experiments at dissected preparations demonstrated two different pathways of the formation of new boutons: 1) the growth of filopodia, sometimes followed by the formation of less mature boutons devoid of vesicles; and 2) budding of more mature boutons filled with vesicles. We found that the activity selectively promoted the second pathway. To elucidate the molecular mechanism of the activity-induced outgrowth, we treated the preparations with a protein-kinase A (PKA) activator forskolin. We found that forskolin treatment selectively promoted the activity-dependent outgrowth of more mature boutons filled with vesicles. Since synaptic growth depends on a protein Synapsin (Syn), which is a PKA target, we investigated the synaptic growth in Syn(-) preparations and found that the activity-dependent budding of more mature boutons is selectively inhibited in Syn(-). Altogether, out results demonstrate that the synaptic growth occurs via two different pathways, which produce boutons at different stages of the maturation. One of the pathways involves the activity-dependent budding of more mature boutons filled with vesicles, and this pathways is regulated by intense activity via the PKA/Syn dependent mechanism.

Disclosures: A. Vasin: None. **C.L. Torres Ferreris:** None. **M. Bykhovskaia:** None.

Poster

118. Synaptogenesis and Activity-Dependent Development: Synapse Formation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 118.15/D20

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

Title: Probing the role of *Drosophila* thrombospondin in larval NMJ formation

Authors: *N. VELAZQUEZ ULLOA, E. LOWENSTEIN
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Abstract: Thrombospondin (TSP) is an extracellular matrix glycoprotein that has been shown to have a role in synaptogenesis in the mammalian brain. In mammals, TSP is released by astrocytes at glutamatergic synapses. The TSP family of glycoproteins is composed of 5 members. There is a single homologous protein in *Drosophila melanogaster* (D-TSP) and there is conservation in the protein domains that are involved in TSP's function in synaptogenesis. In *Drosophila*, D-TSP has mostly been studied in the myo-tendinous junction at embryonic stages. Not much is known about the role of D-TSP at larval stages nor whether it plays a role in synaptogenesis. Hence, we set out to determine if D-TSP functions at glutamatergic synapses in the larval NMJ. We hypothesized that D-TSP would be necessary for normal NMJ formation. To test this hypothesis, we used immunohistochemistry to visualize larval NMJ structure at the 6/7 muscles in segments A3 and A4. We quantified established NMJ markers (muscles with phalloidin, presynaptic axons with HRP, and post-synaptic density with DLG) in flies with normal expression of D-TSP and flies with decreased expression of D-TSP. Decreased expression was achieved by RNAi, and we knocked down D-TSP in muscle (C600-GAL4 or C600-DCR2), pan-neuronally (elav-GAL4), or in motor neurons (D42-GAL4 or OK6-GAL4). Our preliminary results suggest a change in NMJ morphology in the knockdowns. For example, there appear to be differences in muscle coverage of the innervation, NMJ area and number of branches. We are also quantifying muscle area, number of boutons, branching pattern, and the number of islands. In addition, we noticed that the HRP axons of the knockdown looked thinner overall and did not have distinct boutons. We are in the process of validating the knockdown. Our results suggest that D-TSP plays a role in synaptogenesis at the larval NMJ.

Disclosures: N. Velazquez Ulloa: None. **E. Lowenstein:** None.

Poster

118. Synaptogenesis and Activity-Dependent Development: Synapse Formation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 118.16/D21

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

Support: R01 DC007695
CoBRE P30 GM103503
John W. and Jeanette S. Straton Endowed Chair

Title: Exploring the cellular ecosystem of a developing neural system through single cell RNA-Sequencing

Authors: *A. BRANDEBURA[1,2], D. R. KOLSON[1], P. STOILOV[2], P. H. MATHERS[2,1,3], G. A. SPIROU[1,3]
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Abstract: Neural circuit formation is guided by a complex regulatory program of gene expression and signaling interactions among neurons and glia. Innervation of the Medial Nucleus of the Trapezoid Body (MNTB) is used as a model system to study cell type-specific gene expression during formation of a large nerve terminal, the calyx of Held (CH), because of its precise and rapid timeframe for development. The CH terminal grows between postnatal day (P)2 to P4 and removal of supernumerary inputs is mostly completed by P6. In synchrony with this process, glia have close physical contacts with the developing terminal and the postsynaptic neuron. Little is known about the signaling interactions between neurons and glia in the MNTB and how these signals contribute to formation of the mature neural tissue are as of yet unknown. We utilized single cell RNA-Sequencing (scRNA-Seq) to reveal cell type-specific gene enrichment in order to understand intercellular signaling interactions during the development of this neural system.
Microdissected MNTB tissue was enzymatically dissociated into a single cell suspension and loaded into the C1 microfluidics chip for single cell capture. Over 300 single cells at P3 were captured and analyzed. cDNA libraries were of high quality, resulting in the detection of between 5,000 and 10,000 genes per single cell library. A hierarchical clustering approach yielded five main cell clusters, including vascular-associated cells, oligodendrocytes, astrocytes and two distinct clusters of neurons. Through this process the cell type-specific transcriptional profiles for these clusters were identified. Transcripts encoding for proteins related to perineuronal net formation were assigned to specific cell types based on enrichment, which will allow for targeted genetic knockout studies in the future. Single molecule fluorescent *in situ* hybridization is being used to investigate cell type-specificity of transcripts encoding for cell adhesion proteins and ion channels in neurons and glial cells, which will shed light on the function of glial process organization at developing synaptic sites in a maturing neural tissue. In conclusion, the scRNA-Seq database provides a platform for the development of new tools, such as conditional Cre lines and genetic knockout mice, which can be used to investigate the functional role of neuron-glia communication and how this signaling guides growth of the CH terminal.

Disclosures: A. Brandebura: None. **D.R. Kolson:** None. **P. Stoilov:** None. **P.H. Mathers:** None. **G.A. Spirou:** None.

Poster

118. Synaptogenesis and Activity-Dependent Development: Synapse Formation

Location: SDCC Halls B-H

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Program #/Poster #: 118.17/D22

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

Support: P20GM121310

Title: A role for presenilin in synapse formation

Authors: *K. G. PRATT, Z. LIU
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Abstract: Presenilin (PS) is a molecule that was first identified, and named, in the context of Alzheimer's disease, but is now known to carry out a myriad of functions that are important during development. Here, we characterize the role of PS in the developing Xenopus tadpole retinotectal projection, the major component of the amphibian visual system, comprised of the retinal ganglion cell (RGC) axons that project from the eye and synapse directly onto neurons in the optic tectum. Western blot studies confirmed that PS is expressed in the tadpole optic tectum during the time when the retinotectal circuit is forming. To test the role of PS we inhibited it by electroporating a PS morpholino (PS-MO) or a control morpholino (Ctrl-MO) into the postsynaptic tectal neurons at developmental stage 45, the time when retinotectal synapse formation is at its peak. We then tested the circuit at stage 48, when the circuit is more mature and supporting visual avoidance behavior. We found that this manipulation greatly compromised visual avoidance behavior, suggesting that inhibiting PS in the tectal neurons during synapse formation disrupts proper function of the visual system. To directly test this, light-evoked responses were measured in PS-MO and Ctrl-MO neurons by projecting a whole field flash of light onto the retina while recording from single tectal neurons in whole cell configuration. We found that PS-MO neurons displayed significantly smaller light-evoked currents. Minimal stimulation experiments, designed to quantify the synaptic strengths of *individual* RGC axons contacting a given PS-MO or Ctrl-MO neuron, indicate that RGC axons form weaker synapses onto PS-MO neurons compared to Ctrl-MO neurons, and, further, that NMDA currents are reduced in PS-MO neurons. The reduction in NMDA receptor-mediated currents observed in the PS-MO neurons is an important result because it implicates a role for PS in the initial stages of glutamatergic synapse formation, when NMDA receptors are being recruited to nascent synaptic sites. Piecing together previous reports from the Robakis, Dalva, and Greenberg labs about the relationship between PS and EphB receptors, and the intracellular domain of EphB receptors and NMDA receptors, led to our hypothesis that PS is involved in early synapse formation by cleaving EphB receptors, which liberates the intracellular domain of EphB so that it can then recruit or up-regulates NMDA receptors. We are currently testing this hypothesis by expressing the already-cleaved EphB2 intracellular domain in PS-MO neurons to determine if it will rescue the reduced NMDA currents. Preliminary data appear to support this hypothesis.

Disclosures: K.G. Pratt: None. **Z. Liu:** None.

Poster

118. Synaptogenesis and Activity-Dependent Development: Synapse Formation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 118.18/D23

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

Support: NSERC Grant 2016-04695

Title: Neuronal development in zebrafish is altered by brief exposure (5-hr during gastrulation) to cannabidiol (CBD)

Authors: *D. W. ALI[1], M. AMIN[2], K. T. AHMED[3]
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Abstract: Marijuana is one of the most commonly used illicit recreational drugs and is widely used for medicinal purposes. Cannabidiol (CBD) is the major non-psychoactive ingredient in marijuana, whereas δ^9-Tetrahydrocannabinol (δ^9-THC) is the main psychoactive ingredient. Several studies indicate that cannabis use during pregnancy is on the rise due to the ease of access and that it is considered to be relatively harmless. It is reported that up to 14% of pregnant females aged between 12 to 44 have used cannabis during their first trimester. Epidemiological studies suggest that children exposed to cannabis prenatally exhibit neurocognitive deficits, aggressive behavior and attention disorders. In contrast to δ^9 THC, a limited number of studies have focused on the role of embryonic CBD exposure on organismal development. In this study, we exposed zebrafish embryos to CBD (1, 2, 3 and 4 mg/L of CBD) for 5 hours during the critical stage of development known as gastrulation, allowed them to develop and then examined the embryos and larva for a range of features. CBD treated embryos exhibited dose dependent reduction in survival, reduced heart rates, increased incidences of axial malformations and shorter trunks. CBD treatment also altered synaptic activity at neuromuscular junctions (NMJs), and fluorescent labelling of primary and secondary motor neurons indicated a change in branching patterns and a reduction in the number of axonal branches in the trunk musculature. Furthermore, there were alterations in the α-bungarotoxin labelling of nicotinic acetylcholine receptors at NMJs. Locomotion studies showed that larvae that had been exposed to CBD during gastrulation exhibited drastic reductions in the number of C-start escape responses to sound stimuli. Taken together these findings indicate that zebrafish embryos exposed to CBD during gastrulation exhibited alterations in heart rate, motor neuronal morphology, synaptic activity at the NMJ and locomotor responses to sound. Co-treatment of embryos with pharmacological antagonists of either CB1 (AM251, CP-9455) or CB2 receptors (AM630, JTE907) plus CBD, resulted in improved morphology and increased survival rates. These results indicate that both CB1 and CB2 receptors are involved in mediating the detrimental effects of CBD exposure during gastrulation.

Disclosures: D.W. Ali: None. **M. Amin:** None. **K.T. Ahmed:** None.

Poster

118. Synaptogenesis and Activity-Dependent Development: Synapse Formation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 118.19/D24

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

Title: Investigating the role of a *Drosophila* tRNA methyltransferase in neurons

Authors: *C. HOGAN, J. J. BRUCKNER[1], S. GRATZ[2], X. HE[2], K. M. O'CONNOR-GILES[3]
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Abstract: tRNAs are ubiquitous adaptor molecules that decode mRNAs through codon-anticodon base pairing and insertion of the appropriate amino acid into growing polypeptide chains. tRNAs undergo extensive posttranscriptional modifications that affect stability, efficiency, and fidelity. Although once thought to function as simple adaptor molecules, it is becoming apparent that tRNAs can function as signaling molecules in the dynamic regulation of translation. Importantly, disruptions to the posttranscriptional processing of tRNAs have recently been linked to neurological disease.
We identified a neuronal tRNA methyltransferase as a novel regulator of synaptic growth through a genetic screen in Drosophila. This tRNA methyltransferase is one of two metazoan paralogs of a yeast enzyme, TRM9, that methylates uridines found in the wobble position of the anticodon loop to increase translational efficiency.
For comprehensive analysis of TRM9L's neuronal role, we generated a null allele using CRISPR-Cas9. Consistent with our previous RNAi results, TRM9L mutants exhibit significant synaptic overgrowth at the neuromuscular junction. Despite ectopic synapse formation, synaptic transmission is significantly impaired in TRM9L mutants, demonstrating that TRM9L is necessary for proper synaptic function as well as growth. Because yeast TRM9 mutants are sensitive to a variety of stressors, we examined the role of TRM9L in stress response and found that loss of TRM9L increases sensitivity to paraquat, a toxin that induces oxidative stress. This suggests that translational regulation of stress response genes may be a conserved function of TRM9-family enzymes. Through genetic and biochemical analyses we are dissecting the mechanism through which TRM9L regulates synaptic function and stress responses.

Disclosures: J.J. Bruckner: None. **S. Gratz:** None. **X. He:** None. **K.M. O'Connor-Giles:** None.

Poster

118. Synaptogenesis and Activity-Dependent Development: Synapse Formation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 118.20/D25

Topic: A.05. Axon and Dendrite Development

Support: NIH intramural grant

Title: Understanding the molecular mechanism of synapse development in cultured rat cortical neurons mediated by TFP5 a peptide derived from CDK5 activator p35

Authors: *S. P. YADAV[1], M. BHASKAR[2], N. AMIN[2], S. SKUNTZ[2], C. WINTERS[2], H. PANT[2]
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Abstract: Cyclin-dependent kinase 5 (CDK5) plays critical role in neuronal development and synapse formation. Phosphorylation activity of Cdk5 on target proteins in post-mitotic neurons is regulated by its activators p35 and p39. In pathological conditions CDK5 activity gets deregulated due to increased calcium level that leads to calpain mediate cleavage of p35 into p25. CDK5/p25 becomes deregulated and hyperactive, hyperphosphorylates its substrates including neurofilament (NF), tau and amyloid precursor protein (APP) leading to formation of toxic aggregates of neurofilaments, phosphorylated tau and amyloid beta plaques in cell soma resulting in neuronal death. To protect the neurons from deregulated CDK5/p25 multiple inhibitors have been developed however most of these inhibitors are ATP analogues, nonspecific and toxic. Therefore, a better hyperactive CDK5/p25 specific inhibitor needed. To this end we have developed a 24 amino acid peptide TFP5 derived from CDK5 regulator p35 that specifically inhibits the hyperactive CDK5/p25 in *in vitro* as well as in mice models. Previously we have shown that TFP5 can rescue Alzheimer's disease phenotype in mice model after intraperitoneal injections. Therefore, we decide to find out the signaling pathways affected by TFP5 in cultured rat cortical neurons. Here we report that treatment of cultured rat cortical neurons with 500nM TFP5 and scrambled peptide leads to their increased uptake by microglia. We also noted increased transformation of the rounded microglia into ramified microglia in TFP5 treated cultures. Ramified microglia have been associated with neuronal pruning and synapse development. Further analysis of the day *in vitro* (DIV) 1, 2 and 3 cultured neurons stained with acetylated alpha tubulin and phalloidin revealed enhanced neurite growth in TFP5 treated neurons. Staining with axon initial segment (AIS) enriched protein AnkG revealed well developed AIS in the TFP5 treated cultured neurons compared to that of scrambled peptide. Moreover, we noted increased dendritic arborizations and enhanced synapse formation in TFP5 treated cortical neurons by DIV17. Our data suggest that TFP5 plays a critical role in the neurite development, AIS formation and synaptogenesis. Future studies using next generation

sequencing technology (RNA-Seq) of the DIV6, 12 and 17 cultured cortical neurons are under progress and will allow us to find out the signaling pathways affected by TFP5 in different cells types.

Disclosures: S.P. Yadav: None. **M. Bhaskar:** None. **N. Amin:** None. **S. Skuntz:** None. **C. Winters:** None. **H. Pant:** None.

Poster

119. Developmental Disorders: Autism: Genetic Models

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 119.01/D26

Topic: A.07. Developmental Disorders

Support: SFARI Award #: 368406

Title: Deciphering autism spectrum disorder-associated variants of uncertain significance in human PTEN

Authors: *T. MCDIARMID[1], K. POST[2], R. DINGWALL[2], P. GANGULY[2], M. BELMADANI[2], M. EDWARDS[2], F. MIELI[2], W. MEYERS[2], B. YOUNG[2], S. ROJIC[2], C. LOEWEN[2], D. W. ALLAN[2], S. X. BAMJI[3], T. P. O'CONNOR[5], P. PAVLIDIS[6], K. HAAS[4], C. H. RANKIN[5]
[1]Grad. Program in Neurosci., [3]Cell. and Physiological Sci., [4]Brain Res. Ctr., [2]Univ. of British Columbia, Vancouver, BC, Canada; [6]Psychiatry, [5]Univ. British Columbia, Vancouver, BC, Canada

Abstract: The application of whole exome and whole genome sequencing has dramatically increased the pace at which we implicate rare genetic variants in the etiology of Autism Spectrum Disorder (ASD). However, our ability to sequence genomes has vastly surpassed our ability to interpret the genetic variation we discover, resulting in thousands of variants of uncertain significance. This creates a challenging situation that requires direct assessment of the functional effects of disease-associated variants *in vivo*. To determine the functional effects of ASD-associated missense variants we developed a novel strategy based on genome engineering in the high-throughput genetic model organism *Caenorhabditis elegans*. We have begun by focusing on the high-confidence ASD-associated gene *PTEN*. The sole *C. elegans* ortholog of *PTEN*, called *daf-18*, regulates naïve positive NaCl chemotaxis such that worms harbouring reduction-of-function mutations in *daf-18* actively avoid normally appetitive NaCl concentrations. Using a machine vision-based chemotaxis paradigm we have shown that either directly replacing *daf-18* with a single copy of human WT *PTEN* using CRISPR or nervous system specific overexpression of WT *PTEN* is able to substitute for complete deletion of *daf-18* and rescue this behavioural deficit. Surprisingly, all ASD-associated missense mutations in

PTEN assessed resulted in partial- or complete loss-of-function and failed to rescue this sensory deficit. Collaborative complementary *in vivo* functional assays in yeast, and fly as well as *in vitro* assays in HEK293 cells and rat neural culture directly corroborate our finding that ASD-associated *PTEN* variants are loss-of-function. The wealth of in vivo empirical data from this research will improve algorithms that estimate the pathogenicity of missense mutations, improve diagnostic accuracy, and further precision medicine efforts to treat ASD.

Disclosures: K. Post: None. **R. Dingwall:** None. **P. Ganguly:** None. **M. Belmadani:** None. **M. Edwards:** None. **F. Mieli:** None. **W. Meyers:** None. **B. Young:** None. **S. Rojic:** None. **C. Loewen:** None. **D.W. Allan:** None. **S.X. Bamji:** None. **T.P. O'Connor:** None. **P. Pavlidis:** None. **K. Haas:** None. **C.H. Rankin:** None.

Poster

119. Developmental Disorders: Autism: Genetic Models

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 119.02/D27

Topic: A.07. Developmental Disorders

Support: ERA-NET Neuron - Autisyn
BSF
AMN Foundation
ISF
Mafat
Gildor Chair
Elton Laboratory

Title: The autism-mutated ADNP spleen/serum expression is correlated with cognition: Rescue by intranasal PACAP or NAP

Authors: *I. GOZES[1], S. SRAGOVICH[2], G. HACOHEN KLEIMAN[3]
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Abstract: Activity-dependent neuroprotective protein (ADNP) discovered and first characterized in our laboratory as vital for mammalian brain formation is one of the leading genes mutated *de novo*, driving the autistic ADNP syndrome (1). ADNP is regulated by vasoactive intestinal peptide (VIP), as well as by pituitary adenylate cyclase-activating peptide (PACAP), which change its content toward neuroprotection. ADNP contains a microtubule-dynamic enhancing motif, the neuroprotective drug candidate NAP (davunetide, CP201). NAP enhances ADNP's binding to microtubule end binding proteins, thereby augmenting ADNP's neuroprotection. In the current study, the impact of the Adnp genotype (homozygous or heterozygous) and the

efficacy of PACAP and NAP were tested in a unique mouse model of Adnp-haploinsufficiency. As observed before in the object recognition test that evaluates learning and memory, the Adnp haploinsufficient mice, unlike Adnp-intact littermates preferred the familiar object in contrast to the novel object (2). Here, intranasal treatment with either PACAP or NAP ameliorated these cognitive deficits, suggesting regulation at the level of ADNP, and enhancement of expression and function, respectively. Interestingly, a significantly positive correlation was discovered between spleen Adnp mRNA expression and object recognition performance in males ($r=0.637$, $*p<0.05$), implicating a biomarker for cognitive function and corroborating previous results showing that serum ADNP levels correlate with IQ tests in the elderly population (3). Together, these results suggest a surrogate blood marker for cognition, emphasize the suitability of the Adnp haploinsufficient mouse for translational research and promote two ADNP-related drug candidates. 1] Transl Psychiatry. 2017 Feb 21;7(2):e1043. doi: 10.1038/tp.2017.27; Biological Psychiatry DOI: 10.1016/j.biopsych.2018.02.1173, in press. 2] Mol Psychiatry. 2016 Oct;21(10):1467-76; Transl Psychiatry. 2015 Feb 3;5:e501. 3] J Alzheimer's Dis. 2016;50(1):249-60.

Disclosures: I. Gozes: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Coronis Neurosciences. **S. Sragovich:** None. **G. Hacohen Kleiman:** None.

Poster

119. Developmental Disorders: Autism: Genetic Models

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 119.03/D28

Topic: A.07. Developmental Disorders

Support: 5R01NS058721-09

Title: SUPT16H de novo mutations in patients with neurodevelopmental disorders

Authors: *R. BINA[1,2], J. TARSITANO[2], B. FREGEAU[2], R. JIANG[2], J. BARKOVICH[3], G. HOUGE[4], R. BEND[5], H. WARREN[5], R. STEVENSON[5], E. SHERR[2]
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Abstract: Whole-exome sequencing studies have identified a number of genes with damaging *de novo* mutations in children with neurodevelopmental disorders (NDD) and Autism Spectrum Disorder (ASD). Many of these identified genes are involved in transcription regulation, specifically chromatin remodeling pathways. *SUPT16H* encodes a subunit of Facilitates chromatin transcription (FACT), a heterodimer protein complex that has been implicated in DNA replication, transcription, and repair. FACT has been shown to interact with H2A-H2B

dimer from the nucleosome during transcription, allowing RNA polymerase access to the previously coiled DNA. Previously, deletions and duplications in 14q11.2 involving the *CHD8* and *SUPT16H* genes have been reported in patients with NDD and ASD. However, the exact role of *SUPT16H* in human neurodevelopment remains elusive.
Here we describe novel *SUPT16H* mutations in patients with neurodevelopmental disorders, including agenesis of the corpus callosum and ASD. We identified seven individuals with *de novo* mutations in *SUPT16H*. Five of these individuals had *de novo* missense mutations, one had an InterVening Sequence (IVS) and one had a 2.05 deletion that included *SUPT16H*. All the missense variants occurred at evolutionarily conserved amino acids. Three of the missense variants were located in the middle domain of SPT16, the larger subunit of FACT encoded by *SUPT16H.* This domain has shown to associate with the H2A-H2B dimer from nucleosome. These variants were not previously reported in The Genome Aggregation Database (gnomAD) and Exome Aggregation Consortium (ExAC) browser. In silico prediction of effects on protein function was assessed. When applicable, the protein products were predicted to be damaging. Clinical symptoms in this cohort included global developmental delay, autistic behavior, speech delay, epilepsy, macrocephaly, and craniofacial dysmorphic features. Callosal abnormalities were seen in all patients with Brain MRIs available.
In conclusion, our findings suggest that *de novo* mutation in the chromatin regulator gene *SUPT16H* can contribute to a genetic syndrome characterized by neurodevelopmental defects, behavioral problems and structural brain abnormalities.

Disclosures: R. Bina: None. **J. Tarsitano:** None. **B. Fregeau:** None. **R. Jiang:** None. **J. Barkovich:** None. **G. Houge:** None. **R. Bend:** None. **H. Warren:** None. **R. Stevenson:** None. **E. Sherr:** None.

Poster

119. Developmental Disorders: Autism: Genetic Models

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 119.04/D29

Topic: A.07. Developmental Disorders

Support: DFG Grant WO1732/1-1

Title: Social communication deficits in mice lacking profilin1: Implications for autism spectrum disorder?

Authors: *A. Ö. SUNGUR[1], L. STEMMLER[1], M. KOROTIN[1], M. WÖHR[1,3], M. B. RUST[2,3,4]
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Abstract: The neuronal actin cytoskeleton is essential for development and function of the nervous system. Human genetic studies revealed that deficits in neuronal actin dynamics can impair the function of nerve cells, thereby causing neurodevelopmental disorders such as autism. Members of the ADF/cofilin and profilin family are key regulators of actin dynamics. Recently, we have shown that mice with a specific deletion of cofilin1 in excitatory neurons of the postnatal telencephalon are impaired in paradigms of associative learning such as Morris water maze, conditioned place preference or contextual and cued fear conditioning. They also display deficits in novel object recognition, in the absence of any social and communication deficits and repetitive behavior, thereby depicting cofilin1's role in non-social cognition. To further investigate the contribution of actin dynamics to autism-like phenotypes, in this study we have compared juvenile mice with a brain-specific deletion of profilin1 (Pfn1) and their control littermates in behavioral assays developed to detect social communication deficits and aberrant cognitive phenotypes. When assessing direct reciprocal social interactions, we observed a clear reduction in time spent interacting in female but not male Pfn1 mutant pairs. Reduced social interaction in female mutants was paralleled by fewer ultrasonic vocalizations (USV) emitted during the entire social interaction. Detailed temporal analysis revealed that, in female Pfn1 mutants only 40% of USV occurred while mice were engaging in active social behaviors such as anogenital sniffing and social grooming, while this ratio was ~63% in control mice, with the total number of USV emitted during active social behavior also differing between the genotypes. The social deficits in female mutants persisted across different tests, as they showed intact social approach but impaired social recognition in the three-chambered box assay. Furthermore, novel object recognition was affected by Pfn1 deficiency. Female, as well as male Pfn1 mutants failed to discriminate between novel and familiar objects, and they spent equal time exploring both objects. In summary, the present findings indicate that lack of Pfn1 leads to sex-dependent autism-like phenotypes and an aberrant cognitive phenotype.

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Poster

119. Developmental Disorders: Autism: Genetic Models

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 119.05/D30

Topic: A.07. Developmental Disorders

Support: Lou Lou Foundation ODC-CDKL5
NIH Grant NS080565-04

Title: Functional consequence and therapeutic implications of increased hippocampal CP-AMPARs in a mouse model of CDKL5 deficiency disorder

Authors: *R. S. WHITE[1], M. YENNAWAR[3], F. E. JENSEN[2]
[1]Neurol., [2]Dept Neurol, Univ. of Pennsylvania Perelman Sch. of Med., Philadelphia, PA;
[3]Pharmacol., Univ. of Pennsylvania, Philadelphia, PA

Abstract: CDKL5 Deficiency Disorder (CDD) is a rare neurodevelopmental disease resulting in early-life epilepsy, intellectual disability, and autistic behaviors. It is caused by pathogenic mutations in the gene for cyclin-dependent kinase-like 5 (CDKL5). Using the CDKL5 R59X knock-in (CDKL5 R59X) mouse model of CDD, we previously reported that the mutant mice exhibit deficits in learning and memory and social interaction along with lowered seizure threshold. Furthermore, our Western blot analysis of glutamate receptor subunits showed significantly decreased levels of AMPA receptor (AMPAR) subunit GluA2 in the hippocampus, resulting in decreased GluA2:GluA1. Decreased GluA2:GluA1 is consistent with the literature of excitatory/inhibitory (E/I) imbalance and suggests that there is an increase in the number of GluA2-lacking, Ca2+ permeable (CP)-AMPARs at the membrane, which have a significant role in neuronal plasticity, excitability, and disease. Indeed, we observed that CDKL5 R59X mice have significantly elevated early-phase long-term potentiation. We now report that the functional contribution of CP-AMPARs is significantly greater in CDKL5 R59X mice than WT littermates, indicated by the characteristic inward rectification of the current voltage (I-V) relationship ($p<0.01$ at +20mV and +40mV, n=5). An increase in sEPSC frequency are observed with a decrease in amplitude in the CDKL5 R59X mice when compared to WT at p28 (KI:3.4±0.4pA vs WT:2.1±0.3pA; $p<0.05$, n=5). This suggests that the changes in neuronal activity in the hippocampus that lead to E/I imbalance in CA1 neurons. Additionally, we show that deficits in working memory, social interaction, and seizure threshold are rescued with acute treatment of CP-AMPAR open-channel blocker IEM1460 ($p<0.0001$, $p<0.0001$, and $p<0.05$ respectively, n=10-12 for all behavioral experiments). Acute treatment with IEM1460 partially rescued deficits in fear conditioning. These novel results indicate that increased levels of CP-AMPARs in the hippocampus underlie alterations in plasticity, social behavior and hyperexcitability in the CDKL5 R59X model, and may be a therapeutic target in patients with CDD.

Disclosures: R.S. White: None. **M. Yennawar:** None. **F.E. Jensen:** None.

Poster

119. Developmental Disorders: Autism: Genetic Models

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 119.06/D31

Topic: A.07. Developmental Disorders

Support: University of Louisville 21s Century Initative

Title: Imaging genetics analysis for autism spectrum disorders based on functional and structural brain imaging modalities

Authors: *G. N. BARNES[1], M. ELMOGY[2], A. SWITALA[3], E. ROUCKA[4], M. GHAZAL[2], R. KEYNTON[2], A. EL BAZ[2]
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Abstract: Introduction: Autism spectrum disorder (ASD) is a complex condition that can be defined as a group of diverse neurodevelopmental disorders which are identified by communication and social development deficits. ASD can be affected by environmental and genetic factors. Here, we applied imaging genetics approaches to link genetic changes to functional and structural variations in the brain. **Methods:** Imaging and genomics were obtained from the NDAR. 200 individuals (80 with ASD and 120 control) were identified as having T1-weighted MRI, resting-state functional MRI (rs-fMRI), and single nucleotide polymorphism (SNP) genotyping. sMRI scans were processed to segment the outer cortical (pial) surface. A "global" descriptor was obtained by approximating the surface with an 81st-order SPHARM model and calculating the power spectrum from the spherical harmonic coefficients. A vector of "local" descriptors was aligned on a map of select 25 Brodmann areas per hemisphere, and taking the average Gaussian curvature of the region surface. Rs-fMRI scans were processed using independent component analysis. The pattern of activity within each component was scored as consistent with ASD using a fuzzy, neural network-based classifier. SNP genotyping had been performed using the Illumina HumanOmni 2.5-8 whole-genome kit. Raw intensity data files were processed with GenomeStudio software to obtain SNP calls. The extracted descriptors from both sMRI and fMRI were used as phenotypic information in the genomic linkage analysis performed using PLINK software to identify associated genomic variants and were cross-referenced with databases. **Results:** Using a false discovery rate (FDR) of 0.1, twelve SNPs were significantly associated with regional brain curvature, including four intron, two coding, and six within intergenic DNA variants. Of the SNPs strongly associated with global brain shape, five were intronic and two intergenic. The top 5 SNPs linked with ASD-related functional connectivity, with uncorrected $p < 10^{-5}$, were intronic or intergenic but none were significant at FDR = 0.1. Implicated genes include several associated with vesicles/vesicle transport, with metal ion transport including calcium ions, and with embryonic development, and neural migration in particular. Other affected genes may have a membrane or chromatin regulatory function or are non-protein coding. **Conclusion:** We present a pipeline that uses imaging genetics approaches to extract the most affected genes by ASD. These analyses may suggest novel genes which impact brain structure and function which differs from traditional autism risk genes and contribute to the heterogeneity in ASD.

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Poster

119. Developmental Disorders: Autism: Genetic Models

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 119.07/D32

Topic: A.07. Developmental Disorders

Support: MOST 105-2311-B-001-061-MY3

Title: Investigation of the role of CTTNBP2 in autism spectrum disorder

Authors: *P.-Y. SHIH[1], B.-Y. HSIEH[2], Y.-P. HSUEH[3]
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Abstract: Dysregulation of the neural development leads to several psychiatric diseases such as autism spectrum disorder (ASD). Previously we reported that cortactin-binding protein 2, CTTNBP2, a brain specific actin-associates protein, regulates neuronal morphogenesis through its interaction with F-actin or microtubules. CTTNBP2 oligomer associates and bundles microtubule in premature neuron. The interaction with CTTNBP2 stabilizes microtubules and controls dendritic arborization. While in mature neuron, CTTNBP2 becomes highly concentrated in dendritic spine and colocalized with PSD95. In dendritic spines, CTTNBP2 modulates the mobility of cortactin and thus regulates local actin dynamics. The interaction between CTTNBP2 and cortactin is critical for dendritic spine formation and maintenance, because depletion of CTTNBP2 or expression of CTTNBP2 mutant that loss the interaction with cortactin decreases the density of dendritic spine and the width of spine head. Recently, *CTTNBP2* has been reported as a high risk candidate gene in ASD patients. The goal of the current study is to investigate how CTTNBP2 deficit causes ASD phenotypes. We generated CTTNBP2 knockout mice and ASD mutation knock-in mice by genome-editing methods. We analyzed the brain anatomy, dendritic spine morphology and ASD-related behaviors such as social interaction and anxiety in our mutant mice. Our study is expect to provide the biological insights of the mechanism of ASD.

Disclosures: P. Shih: None. **B. Hsieh:** None. **Y. Hsueh:** None.

Poster

119. Developmental Disorders: Autism: Genetic Models

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 119.08/D33

Topic: A.07. Developmental Disorders

Support: SFARI pilot grant 401457
CIHR postdoctoral fellowship to KYC

Title: Oxytocin normalizes altered social circuit connectivity in the Cntnap2 knockout mouse

Authors: *K. Y. CHOE[1], M. SAFRIN[2], R. A. BETHLEHEM[4], N. G. HARRIS[3], D. H. GESCHWIND[1]
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Abstract: Autism spectrum disorders (ASD) is a prevalent neurodevelopmental disorder with a significant genetic component. Hundreds of ASD risk genes have been identified; how these heterogeneous genetic disruptions lead to common neurobiological mechanisms generating the core behavioral abnormalities, social deficits and repetitive behavior, is poorly understood. A potential mechanism of social dysfunction in ASD is aberrant functional connectivity (FC), which is correlated with the degree of social abnormalities (Supekar et al., 2013). Previously we found that oxytocin (OXT) administration or activation of paraventricular nuclei (PVN) OXT neurons improves social deficits in mice lacking the *Cntnap2* gene (loss-of-function mutation found in recessive forms of ASD; Penagarikano et al., 2011;2015). This coupled with work showing that OXT modulates interneuron function, increasing circuit signal-to-noise (Owen et al., 2013), suggested that OXT might exert its effects via rescuing alterations in FC. To test this, we used high field (7T) resting-state fMRI to assess the effects of OXT on FC in dexmedetomedine-sedated wild-type (WT) and *Cntnap2*KO mice (n=15/group). At baseline in KO mice, we observed significantly lowered mean FC between brain regions with established functions in social behavior (e.g. PVN, septal regions, medial prefrontal cortex; $p<0.001$ vs WT, Monte Carlo exact permutation test). In contrast, the mean FC between these socially-relevant regions and other brain regions that are not typically involved in social functions (e.g. sensory cortices, thalamus, striatum) was significantly higher in KO mice ($p<0.001$), similar to observations in patients (Rudie et al., 2013). OXT administration significantly strengthened the mean FC between the socially-relevant brain regions ($p<0.001$), and weakened connections between social and other regions ($p<0.001$). Application of independent component (IC) analysis to these data revealed significant OXT-induced shifts in relative FC in the following KO IC pairs (paired t-test, FDR=0.1): visual cortex-lateral hypothalamus, anterior cingulate cortex-dorsal striatum, and nucleus accumbens-retrosplenial cortex. These results suggest that the observed social deficits in KO mice are related to lowered FC between social regions, which can be temporarily normalized by OXT administration. They also identify region-specific FC changes that may mediate the OXT-mediated behavioral rescue in these mice.To confirm and expand these findings, we are currently performing pharmacologic and chemogenetic (DREADD) MRI to identify the regional patterns of activity elicited by exogenous or endogenous OXT.

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Poster

119. Developmental Disorders: Autism: Genetic Models

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 119.09/D34

Topic: A.07. Developmental Disorders

Support: Israel Science Foundation 770/17

Title: Social impairments and brain-wide alterations measured with intrinsic functional connectivity MRI in a novel mouse model of autism

Authors: *D. LICHTMAN, A. KAVUSHANSKY, N. COHEN, N. S. LEVY, A. P. LEVY, I. KAHN
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Abstract: IQSEC2 is an X-linked gene which is associated with autism spectrum disorder (ASD), intellectual disability (ID) and epilepsy in humans. IQSEC2 is a postsynaptic density protein, localized on excitatory synapses as part of the NMDA receptor complex and is suggested to play a role in AMPA receptor trafficking and mediation of long term depression (LTD). Here, we present behavioral and brain-wide functional connectivity characterization in a new mouse model with a point mutation in the IQ domain of IQSEC2 (A350V). Animals with A350V mutation displayed impairment in sociability and social novelty when tested in the three-chamber social preference task. Relative to controls, A350V animals displayed equivalent motor performance and anxiety-like behavior measured with the Rotarod and Open field tests, respectively. Several recent studies characterized the changes in the connectivity of brain networks in animal models for ASD, mostly using electrophysiological recordings but also using functional connectivity MRI (fcMRI; also termed resting-state fMRI). We acquired fcMRI in awake head-fixed mice to examine brain-wide functional connectivity changes in A350V relative to controls, correlated individual variability in social behavior and brain functional connectivity and the extent to which previously implicated brain networks are recapitulated in A350V. We specifically sought to examine whether social impairment is consistent with increased corticostriatal connectivity, a finding previously shown in other mice models for ASD. The current novel mouse model will complement work on established mouse models (such as BTBR, CNTNAP2, and SHANK3) to evaluate the behavioral and neural commonalities and differences across ASD models facilitating translation of novel therapeutic approaches in ASD.

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Poster

119. Developmental Disorders: Autism: Genetic Models

Location: SDCC Halls B-H

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Program #/Poster #: 119.10/D35

Topic: A.07. Developmental Disorders

Support: NIH Grant R01-NS091027
OHSU Fellowship for Diversity in Research

Title: Role of PTEN in somatosensory development

Authors: *A. FERNANDEZ, K. ROSETTE, K. WRIGHT
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Abstract: The cellular basis for altered sensory processing in autism spectrum disorders (ASD) is poorly defined. Defects in higher-order regions of the brain are thought to contribute to abnormal sensory processing in ASD. However, abnormal development and function in primary sensory neurons has recently been identified in mouse models of syndromic ASD. Whether defects in primary sensory neurons can be generalized to other models of ASD remains to be determined. The survival, differentiation, target innervation and function of somatosensory neurons is regulated by Neurotrophin/*Trk* and *p75* signaling. Phosphatase and tensin homolog (*PTEN*), an ASD susceptibility gene, functions as an intersection point between these pathways and is involved in differentiation and circuit formation in different neuronal populations in the central nervous system (CNS). However, the role of *PTEN* in the development of the peripheral nervous system (PNS) remains to be determined. We hypothesized that loss of *PTEN* during development in the PNS might affect morphogenesis and innervation patterns of peripheral somatosensory neurons. We generated *PTEN* wild type (WT), heterozygous (F/+) and homozygous (F/F) mutant mice using the *Islet 1 (Isl1Cre)* and *Advillin* (*AvilCreERT)* drivers to specifically focus on the contribution of altered *PTEN* levels on PNS development. We found that loss of *PTEN* increases survival of primary sensory neurons, disrupting the balance of population diversification in the dorsal root ganglia (DRG). Moreover, loss of *PTEN* induces early morphological maturation and increased axonal branching in primary sensory neurons. Defects in the timing of primary sensory neuron maturation due to *PTEN* loss affects central and peripheral innervation patterns, particularly in distal targets. Overall, these data suggest that *PTEN* function is critical for peripheral sensory neuron development, and provides insight into the role of *PTEN* mutations in altered sensory processing in a mouse model of syndromic ASD.

Disclosures: A. Fernandez: None. **K. Rosette:** None. **K. Wright:** None.

Poster

119. Developmental Disorders: Autism: Genetic Models

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Program #/Poster #: 119.11/D36

Topic: A.07. Developmental Disorders

Support: Tuberous Sclerosis Alliance #332884
NIH/NINDS R21 NS089441

Title: Delayed differentiation of neuronal progenitors cells and induced neurons derived from tuberous sclerosis complex patient ipscs

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Abstract: Tuberous Sclerosis Complex (TSC) is a developmental disorder characterized by tumor susceptibility in multiple organs, brain malformations, and neurological manifestations. TSC is a relatively rare genetic disorder affecting approximately 1 in 6,000 individuals. However, it is highly associated with epilepsy, intellectual disability, and autism; therefore, understanding the mechanism of disease in TSC may also provide insights into associated neurological conditions. TSC is an autosomal dominant disorder caused by mutations in either the *TSC1*or *TSC2*gene. Patients are heterozygous for these mutations, however some cells become homozygous likely due to loss of heterozygosity (LOH), and contribute to the formation of tumors or regions of dysplasia, such as cortical tubers in the brain. It is presently unclear whether LOH is required to elicit cognitive dysfunction. Since molecular studies of the human brain are tremendously challenging due to the difficulty of obtaining live cells, we employ a recently developed technique that allows the conversion of somatic cells into induced pluripotent stem cells (iPSCs). For our studies, we used iPSCs derived from TSC patients carrying heterozygous *TSC2*mutations, and unaffected control subjects to generate neural progenitor cells (NPCs), NPC-derived neurons, or induced excitatory neurons (iNs) differentiated directly from iPSCs. We investigated in detail neuronal development and morphology, and signaling pathways relevant to the TSC genes. A delay in neuronal differentiation was consistently identified in all TSC patient-derived cells, and in both NPC- or iPSC-derived neuronal cultures. Furthermore, we investigated molecular mechanisms that underlie this phenotype, and discovered specific abnormalities in the activity of the PI3K-Akt-mTOR signaling pathway that were not previously reported in heterozygous models of TSC. This work will lead to further studies aimed at understanding the functional significance of the observed abnormalities, and identifying

compounds that can correct them. These compounds could then be developed as drug treatments that could potentially benefit not only TSC patients, but also other patients that suffer from epilepsy, intellectual disability, and autism.

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Poster

119. Developmental Disorders: Autism: Genetic Models

Location: SDCC Halls B-H

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Program #/Poster #: 119.12/D37

Topic: A.07. Developmental Disorders

Support: Simons Foundation SFARI #342005
NIH Grant HD042182

Title: Increased cellular stress disrupts migration of cortical interneurons in the LgDel model of 22q11.2 DS

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Abstract: Cortical interneurons have been identified as key pathological targets in behavioral disorders including autism (ASD) and schizophrenia (Scz). We showed previously in the LgDel mouse model of 22q11.2 Deletion Syndrome (22q11.2 DS), a neurodevelopmental syndrome strongly associated with ASD and SCZ, that interneuron migration into the cortex is delayed and disrupted, leading to an aberrant distribution of distinct interneuron subclasses. This disruption is due to dysregulated signaling via the Cxcr4 cytokine receptor, which can influence cell motility via modulation of the mammalian target of rapamycin (mTOR) signaling pathway. Microarray analysis of FACS sorted interneurons shows reduced expression of two key mTOR inhibitors: Ddit4 and Sesn2, consistent with mTOR signaling dysregulation. We used conditional deletion to assess genetic interaction between mTOR signaling and heterozygous 22q11 gene deletion in developing LgDel and WT mice. Heterozygous Dlx5/6-Cre-mediated ablation of the mTOR-repressor Tsc2 in interneuron precursors amplifies migration defects observed in the LgDel cortex. In these LgDel:Tsc2+/- E14 fetuses, unlike their LgDel or Tsc2+/- counterparts, few interneurons migrate into the cortex. mTOR signaling is regulated by cellular stress, including oxidative stress and nutrient availability, and we have previously found that LgDel neurons have abnormally high levels of oxidative stress and disruptions in mitochondrial morphology. We used the antioxidant N-Acetylcysteine given via maternal drinking water to restore cortical interneuron migration in LgDel fetuses, presumably by diminishing oxidative stress due to 22q11

gene/mTOR related dysregulation. Apparently, increased cellular stress, likely via a Cxcr4/mTOR pathway impairs cortical interneuron migration due to diminished 22q11 gene dosage. Increased cellular stress and disrupted mTOR signaling have been associated with several neurodevelopmental disorders. We suggest that that the disruptions in these signaling mechanisms may be a point of convergence between 22q11.2 DS and other syndromic as well as non-syndromic behavioral disorders.

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Poster

119. Developmental Disorders: Autism: Genetic Models

Location: SDCC Halls B-H

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Program #/Poster #: 119.13/D38

Topic: A.07. Developmental Disorders

Support: Clinical Research Associates, L.L.C. (CRA), an affiliate of the Simons Foundation, provided arbaclofen and funded these studies through institutional grants to J.N. C., S.R.D., Y.H., and W.T.O.

Title: Towards preclinical validation of arbaclofen (R-baclofen) treatment for 16p11.2 deletion syndrome

Authors: *A. LUO CLAYTON[1], B. B. GUNDERSEN[1], W. T. O'BRIEN[2], T. ABEL[3], T. TSUKAHARA[4], S. R. DATTA[4], M. SCHAFFLER[5], M. SCHULTZ[6], J. N. CRAWLEY[5], S. MARTIN LORENZO[7], V. NALESSO[7], Y. HERAULT[8]
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Abstract: Human chromosome 16p11.2 microdeletion is one of the most common copy number variants conferring risk for autism spectrum disorder (ASD) and other neurodevelopmental disorders. Animal studies suggest that 16p11.2 deletion may share pathophysiology with Fragile X syndrome (FXS)[1]. Previous clinical trials suggest that the GABA-B receptor agonist, arbaclofen, may improve symptomatology in some individuals with FXS and idiopathic ASD, although the trials did not find statistical significant differences on their primary endpoints. Data from mice support the possibility that arbaclofen may be beneficial for 16p11.2 deletion syndrome[2]. Concerted efforts to validate early scientific findings using rigorous methodological

designs are crucial in addressing the recent paucity of success in translational research[3]. To determine the robustness and reproducibility of arbaclofen's normalizing effects on behavior in 16p11.2 deletion mice, we established a consortium of four academic labs. Three different mouse models of 16p11.2 deletion on three background strains and their wildtype controls were treated for at least 12 days with one of three doses of arbaclofen in their drinking water (0.25, 0.5, 1.0 mg/ml). Mice were tested on open field (OF) activity, novel object recognition (NOR), object location memory, contextual fear conditioning and accelerating rotarod. OF activity was also analyzed using 3D imaging and a machine-learning based algorithm that parcellates behavior to sub-second resolution[4]. HPLC-MS analysis performed by a contract research organization revealed dose-dependent levels of arbaclofen in mouse brain. Behaviorally, deficits in NOR in 16p11.2 deletion mice were rescued by arbaclofen, whereas phenotypes in other behavioral tests were not. Analysis of OF data suggest that arbaclofen does not have sedating effects, although some off-target effects were identified with MoSeq analysis[4]. Our consortium offers an example of how to develop and execute a rigorous test of preclinical efficacy of a potential pharmacological therapy.

[1]Tian D, et al. Contribution of mGluR5 pathophysiology in a mouse model of human chromosome 16p11.2 microdeletion. Nat Neurosci 18, (2015).
[2]Stoppel LJ, et al. R-Baclofen reverses cognitive deficits and improves social interactions in two lines of 16p11.2 deletion mice. Neuropsychopharm 43(3), (2018).
[3]Arrowsmith J. Trial watch: Phase II failures: 2008-2010. Nature reviews Drug discovery 10, 328-9 (2011).
[4]Wiltschko AB, et al. Mapping sub-second structure in mouse behavior. Neuron 88, (2015).

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Poster

119. Developmental Disorders: Autism: Genetic Models

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Topic: A.07. Developmental Disorders

Support: CURE EPILEPSY
SAVOY FOUNDATION
CIHR

Title: Disrupted hippocampal inhibition underlying cognitive and behavioral deficits in a mouse model of MYO9B-related autism

Authors: *P. K. PEDABALIYARASIMHUNI[1,2], X. JIANG[1,2], A. LUPIEN-MEILLEUR[1,2], L. EID[1,2], L. MARCOUX[1], M. LACHANCE[1], J.-C. LACAILLE[2], E. ROSSIGNOL[1,2,3]
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Abstract: Autism spectrum disorders (ASD), characterized by reduced social skills, repetitive behaviors and speech delay with frequent cognitive and epileptic comorbidities are genetically heterogeneous disorders. Genes associated with ASD participate in a variety of cellular processes, including neuronal proliferation, migration, synaptogenesis, neuronal maturation and connectivity. Recent data indicate that reduced network inhibition may underlie a subset of genetically determined ASD. Using whole exome sequencing (WES), we identified *de novo* mutations in *MYO9B* in patients with ASD and epilepsy. *MYO9B* encodes a non-muscular myosin involved in maintenance of cell shape and motility through its actin-based motor functions and its inhibition of the RhoGTPase RhoA. *Myo9b* controls dendritic patterning of pyramidal cells (PCs), but its role in the development of GABAergic interneurons (INs) is unexplored. To determine the requirement of *Myo9b* in the development and maturation of cortical networks, we generated $Nkx2.1^{Cre}$; $Myo9b^{c/c}$ mutant mice carrying a conditional deletion of *Myo9b* specific to MGE-INs. *Myo9b* conditional mutant mice are viable and do not display an obvious epileptic phenotype. At the cellular level, we observe a delay in tangential migration that persists at P3, but that resolves by P21. We also note a reduced frequency of spontaneous inhibitory events (IPSCs), along with a reduced number of GABAergic perisomatic boutons on CA1 hippocampal PCs, suggesting disruption of IN connectivity. As a result, $Nkx2.1^{Cre}$; $Myo9b^{c/c}$ mutant mice display increased anxiety, impaired spatial learning and memory, novelty recognition and reversal learning, and reduced socialization, reminiscent of ASD. Altogether, our data suggest that *Myo9b* is a critical player regulating IN development and that reduced inhibition contributes to the ASD phenotype associated with *Myo9b* loss-of-function mutations.

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Poster

119. Developmental Disorders: Autism: Genetic Models

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Topic: A.07. Developmental Disorders

Support: FAST-Trac Program Grant FT2017-002

Title: Developmental social communication in the Ube3a rat model of Angelman syndrome

Authors: *E. L. BERG[1], M. C. PRIDE[1], R. D. LEE[1], N. A. COPPING[1], L. S. NOAKES[2], B. J. NIEMAN[2], J. ELLEGOOD[2], J. P. LERCH[2], S. HARRIS[3], H. A. BORN[3], A. E. ANDERSON[3], S. V. DINDOT[4], E. J. WEEBER[5], D. J. SEGAL[6], J. L. SILVERMAN[1]
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Abstract: Angelman Syndrome (AS) is a rare neurodevelopmental disorder characterized by developmental delay, impaired receptive and expressive communication skills, ataxia, motor and balance deficits, poor attention, intellectual disabilities, microcephaly, and seizures. The genetic cause of AS is loss of expression of *UBE3A* (ubiquitin-protein ligase E6-AP) in the brain, typically due to a deletion of the maternal 15q11-q13 region. New advances in rat models have allowed for the development and utilization of clinically-relevant assays to measure the sophisticated outcomes of social communication, learning and memory, and other AS symptoms across development. We aimed to utilize our innovative, clinically-relevant outcome measures to identify AS-relevant functional phenotypes in the *Ube3a* mutant rat. Ultrasonic vocalizations (USV) were collected from male and female newborn *Ube3a* m-/p+, m+/p-, and m+/p+ rat pups every other day from postnatal day (PND) 4 through 18. At PND 21, brains were collected via perfusion for MRI analysis. Developmental milestones, including body measurements and reflex development, were assessed along the same PND 4-18 time course, which was followed by the USV playback assay of juvenile social communication. Individual rats were presented with a natural 50-kHz USV and an acoustic control stimulus, and subsequent USV production and approach behavior toward the stimulus source were compared. Motor behavior was measured via the open field assay at early and late juvenile ages, and learning and memory abilities were assessed with the novel object recognition task, touchscreen operant learning platform, and cued and contextual fear conditioning. This study discovered reduced pup call emissions, delayed reflex development, altered social communication, and impaired learning and memory in the *Ube3a* m-/p+ rat model of AS. The results herein lend support for the important role of Ube3a in development, in social communication, and for the use of this rat model as a tool to study the neurobiology underlying the behavioral phenotypes of AS.

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Poster

119. Developmental Disorders: Autism: Genetic Models

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Program #/Poster #: 119.16/D41

Topic: A.07. Developmental Disorders

Support: Foundation for Angelman Syndrome Therapeutics
NIH R01NS097808 (JLS)

Title: Preclinical global electroencephalography and seizure characterization in an Angelman syndrome mouse model

Authors: *N. COPPING[1], J. L. SILVERMAN[2]
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Abstract: Angelman Syndrome (AS) is a rare (1:15,000-20,000) neurologic disorder characterized by a wide range of symptoms including seizures, ataxia, motor deficits, developmental delay, impaired communication skills, intellectual disabilities, and microcephaly. Due to the high prevalence of seizures (>80%) in the AS population, electroencephalography (EEG) data are prominent and have shown abnormalities in epileptiform spike-wake discharges and increased delta waves of AS individuals. The present experiments were designed to build upon previous findings of abnormal epileptiform spike-wake discharges and increased delta waves by applying a global EEG approach in the characterization of excitation/inhibition imbalance and seizure phenotypes. C57Bl/6J males were paired with heterozygous *Ube3a* females, resulting in offspring with the maternal transmission of the *Ube3a* mutant allele (*Ube3a*$^{m-/p+}$) and their wildtype (*Ube3a*$^{m+/p+}$) littermates. At 8 weeks, mice were anesthetized and implanted with a wireless telemetry device designed to measure EEG and EMG in freely moving animals (DSI^{TM}). To capture global EEG, two biopotential leads were attached to surgical screws 1.0mm anterior and 3.0mm posterior to the left and right of Bregma, respectively. EMG activity was measured via two leads rooted in the trapezius muscles. One week post-surgery, subject EEG, EMG, and temperature were recorded for a 24-hour baseline, then seizures were induced with pentylenetetrazole. EEG analysis and seizure characterization were evaluated using Neuroscore software. In support of earlier research efforts, we discovered clear, robust phenotypes of atypical EEG activity in *Ube3a*$^{m-/p+}$ mice, compared to *Ube3a*$^{m+/p+}$ controls. Our results extend earlier brain region specific preclinical studies (Sidorov et al., 2017, Born et al., 2017), with the advantage of a translational approach that will provide critical data necessary to further investigate the possibility of a cross-species EEG biomarker and will serve as a paramount output measure in numerous innovative therapeutic interventions, including antisense oligonucleotides, artificial transcription factors, and stem cell based viral vector deliveries.

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Poster

119. Developmental Disorders: Autism: Genetic Models

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Topic: A.07. Developmental Disorders

Support: NIH Grant R01NS097808
MIND Institute Intellectual and Developmental Disabilities Research Center (IDDRC) Grant HD079125

Title: Haploinsufficiency of the AT-rich interactive domain 1B (ARID1B) causes developmental delay and behavioral and anatomical pathology related to intellectual disability

Authors: *S. P. PETKOVA[1], A. A. WADE[2,3,4], A. S. NORD[2,3,4], J. P. LERCH[5], J. ELLEGOOD[6], J. S. SILVERMAN[1,3,4]
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Abstract: Copy number variants in the chromatin remodeling gene AT-Rich Interactive Domain 1B (ARID1B) is a common genetic variation associated with autism spectrum disorders (ASD), intellectual disability (ID), and Coffin Siris Syndrome (Nord et al., 2011, Halgren et al., 2012, Celen et al., 2017, Jung et al., 2018). Here, we generated a novel mouse at The Centre for Phenogenomics by injecting Cas9 nickase (D10A) and single guide RNAs with spacer sequences resulting in deletion of Chr17 from 5242523 to 5243410. Next, we characterized developmental and adult behavioral phenotypes associated with haploinsufficiency of ARID1B. Behavioral assays relevant to ASD, ID and Coffin Siris Syndrome were conducted from neonatal development to adulthood to evaluate general health, anxiety-like, motor, cognitive, and social behaviors in heterozygous *Arid1b* (+/-) mice compared to littermate wildtypes (+/+). During development, *Arid1b*+/- exhibited robust impairments in ultrasonic vocalizations (USVs) and metrics of developmental growth. As adults, *Arid1b*+/- showed low motor abilities in open field exploration and normal three-chambered approach. Interestingly, *Arid1b*+/- had learning and memory deficits in novel objection recognition. Social interactions in the male-female social dyad with USVs revealed social deficits on some but not all parameters. No repetitive behaviors were observed. Whole brains of adult mice were analyzed by structural MRI to identify volume changes in various brain regions. MRI revealed significant decreases in olfactory bulbs, brainstem and cerebellum in *Arid1b*+/- compared to +/+. Finally, we applied genomic profiling in *Arid1b*+/- mouse brain to identify changes due to haploinsufficiency. This study represents the

first full investigation of *Arid1b*+/- haploinsufficiency on behaviors relevant to development delay, social behavior and learning and memory, with corroborative neuroanatomy and suggests a functional role for *Arid1b*+/- in growth, developmental delay, and impairments in behavior relevant to ASD, ID and Coffin Siris Syndrome.

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Poster

119. Developmental Disorders: Autism: Genetic Models

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Topic: A.07. Developmental Disorders

Support: JSPS KAKENHI Grant Number 15K09873
JSPS KAKENHI Grant Number 18K07610

Title: Characterization of Kirrel3-expressing cells in the mouse substantia nigra and ventral tegmental area

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Abstract: Kin of irregular chiasm-like 3 (Kirrel3), a member of immunoglobulin superfamily, is involved in neuronal functions such as axon pathfinding and synapse formation. In humans, genetic mutations in *KIRREL3* are associated with autism spectrum disorders (ASD). At the 46th annual meeting of Society for Neuroscience, we presented that *Kirrel3*-knockout mice exhibit ASD-like behaviors such as social recognition deficits, communication deficits, repetitive motor behaviors, and sensory abnormality (auditory hypersensitivity). However, the molecular mechanisms underlying the ASD-like behaviors of *Kirrel3*-knockout mice remain unclear. Dopamine (DA) neurons in the substantia nigra (SN) and ventral tegmental area (VTA) form nigrostriatal and mesocorticolimbic dopaminergic systems, respectively. It is well-known that the disruption of these dopaminergic systems leads to ASD-like behaviors. To gain insights into the roles of Kirrel3 in DA neurons of the SN and VTA, we examined the expression patterns of Kirrel3 in these regions by using mice that express the *lacZ* reporter gene under the control of the *Kirrel3* promoter. X-gal staining revealed that *Kirrel3* was expressed in 34.4 ± 3.2% of cells in the SN pars compacta (SNc), 27.0 ± 4.9% of cells in the SN pars reticulata (SNr), and 29.2 ± 2.0% of cells in the VTA. In the SNc and VTA, almost all of *Kirrel3*-positive cells expressed NeuN, a marker of neurons. Neither S100 protein nor Iba-1, markers of astrocytes and microglia, respectively, was observed in *Kirrel3*-positive cells. In addition, most of *Kirrel3*-positive

neurons in the SNc expressed tyrosine hydroxylase, a marker of DA neurons, but not gamma-amino butyric acid (GABA), a marker of GABAergic interneurons. In contrast, *Kirrel3* was expressed in both DA neurons and GABAergic interneurons in the VTA. Thus, *Kirrel3* is expressed in DA neurons in both SNc and VTA, and in GABAergic interneurons in the VTA. Thus, Kirrel3 may play important roles in the development of DA neurons of the SNc and VTA, the defects of which might contribute to ASD-like behaviors in *Kirrel3*-knockout mice.

Disclosures: Y. Shikaze: None. **T. Komori:** None. **T. Hisaoka:** None. **T. Kitamura:** None. **Y. Morikawa:** None.

Poster

119. Developmental Disorders: Autism: Genetic Models

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 119.19/D44

Topic: A.07. Developmental Disorders

Support: JSPS KAKENHI Grant Number 22390036
JSPS KAKENHI Grant Number 15K09873
JSPS KAKENHI Grant Number 18K07610

Title: Abnormal synapse formation of cerebellar pinceau in Kirrel3-knockout mice

Authors: *T. HISAOKA[1], T. KOMORI[1], T. KITAMURA[2], Y. MORIKAWA[1]
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Abstract: A synaptic cell adhesion molecule, Kirrel3, is expressed in the central nervous system, including the cerebellum and plays important roles in various processes of neural circuit formation. Recently, the disruption of *KIRREL3* gene has been reported as a candidate for autism spectrum disorder (ASD). At the 46th annual meeting of Society for Neuroscience, we presented that *Kirrel3*-knockout (*Kirrel3*$^{-/-}$) mice showed ASD-like behaviors such as social recognition deficits, communication deficits, repetitive motor behaviors, and sensory abnormality (auditory hypersensitivity). It has been suggested that the cerebellar abnormalities are involved in ASD-like behaviors, such as the social and communicative deficits and repetitive motor behaviors. In particular, the abnormal development of Purkinje cells (PCs) is well-known to be associated with the ASD-like behaviors. In the present study, we observed that *Kirrel3* was expressed in most of the interneurons of the molecular layer and in subpopulations of Purkinje cells (PCs) and granule cells in the cerebellum. In addition, we found that Kirrel3 protein was intensely expressed in the all PSD95-positive axonal terminals of basket cells, which form pinceau synapse around the axon initial segment of PCs. To investigate the structure of pinceau synapse in the *Kirrel3*$^{-/-}$ mice, we performed immunofluorescence staining for PSD95 in the cerebellum. The PSD95-

positive pinceau areas were increased in *Kirrel3*$^{-/-}$ mice compared to those in wild-type mice. In addition, western blot analysis revealed that the protein level of PSD95 in the cerebellum was higher in *Kirrel3*$^{-/-}$ mice than that in wild-type mice. It has been reported that PSD95 is localized to septate-like junctions between axons of BCs in the pinceau. The enlargement of PSD95-positive pinceau areas and the increase in PSD95 protein expression may reflect abnormal development of BCs axons, including the abnormal axonal branching and pathfinding. The abnormal synapse formation of cerebellar pinceau observed in *Kirrel3*$^{-/-}$ mice may induce abnormal pinceau synaptic activity, which cause ASD-like behaviors, such as the social and communicative deficits and repetitive motor behaviors.

Disclosures: T. Hisaoka: None. **T. Komori:** None. **T. Kitamura:** None. **Y. Morikawa:** None.

Poster

119. Developmental Disorders: Autism: Genetic Models

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 119.20/D45

Topic: A.07. Developmental Disorders

Support: JSPS KAKENHI Grant Number 22390036
JSPS KAKENHI Grant Number 15K09873

Title: Autistic-like behaviors in postnatal and adult Kirrel3-knockout mice

Authors: *Y. MORIKAWA[1], T. HISAOKA[1], T. KOMORI[1], T. KITAMURA[2]
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Abstract: A member of the immunoglobulin superfamily, Kirrel3, is expressed in the brain and involved in the various processes of neural circuit formation. It has been reported that genetic mutations in *KIRREL3* are associated with autism spectrum disorder (ASD). At the 46th annual meeting of Society for Neuroscience, we presented that *Kirrel3*-knockout (*Kirrel3*$^{-/-}$) mice showed social recognition deficits, communication deficits, repetitive motor behaviors, and sensory abnormality (auditory hypersensitivity) as well as hyperactivity, which are relevant to ASD with attention deficit hyperactivity disorder (ADHD). To establish *Kirrel3*$^{-/-}$ mice as a mouse model of ASD with ADHD, we further examined various behaviors related to ASD and other mental disorders comorbid with ASD, including anxiety disorder and intellectual disability. Because ASD symptoms often exist in early childhood, we performed the separation-induced ultrasonic vocalization (USV) recordings in wild-type and *Kirrel3*$^{-/-}$ pups. At postnatal day 7, the number of USVs emitted by *Kirrel3*$^{-/-}$ pups significantly increased than that of wild-type pups, indicating that social communicative abnormalities appeared in early childhood of *Kirrel3*$^{-/-}$ mice. To investigate social recognition memory, we performed olfactory

habituation/dishabituation test. In this test, *Kirrel3*$^{-/-}$ mice showed poor abilities to discriminate different social odors, which may cause social recognition deficits. In both the open-field arena and the home cage, significant increases in rearing were observed in *Kirrel3*$^{-/-}$ mice, indicating that *Kirrel3*$^{-/-}$ mice exhibited repetitive motor behaviors. In the light-dark transition test and the elevated plus maze test, *Kirrel3*$^{-/-}$ mice exhibited hyperactivity and normal anxiety-related behaviors. In the Morris water maze test, *Kirrel3*$^{-/-}$ mice showed normal spatial learning and memory, suggesting normal cognitive ability in *Kirrel3*$^{-/-}$ mice. These findings suggest that *Kirrel3*$^{-/-}$ mouse is a useful animal model for investigating the pathophysiology of ASD comorbid with ADHD.

Disclosures: Y. Morikawa: None. **T. Hisaoka:** None. **T. Komori:** None. **T. Kitamura:** None.

Poster

119. Developmental Disorders: Autism: Genetic Models

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 119.21/D46

Topic: A.07. Developmental Disorders

Support: JSPS KAKENHI Grant Number 22390036

Title: Expression of Kirrel3 in the neurons of dorsal root ganglia and their terminals in the spinal cord

Authors: *T. KOMORI[1], T. HISAOKA[1], T. KITAMURA[2], Y. MORIKAWA[1]
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Abstract: Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by the deficits in social communication, repetitive and stereotyped interests, and sensory abnormalities, including hyper-/hypo-sensitivity to auditory and tactile stimuli. In human, genetic mutation of *Kin of irregular chiasm-like 3* (*Kirrel3*), a synaptic cell-adhesion molecule, has been implicated in ASD. At the 46th annual meeting of Society for Neuroscience, we presented that *Kirrel3*-knockout mice exhibit ASD-like behaviors such as social recognition deficits, communication deficits, repetitive motor behaviors, and sensory abnormality (auditory hypersensitivity). To gain further insights into the functional roles of Kirrel3 in the sensory neurons, we examined the expression of Kirrel3 in the dorsal root ganglia (DRGs) by using heterozygous *Kirrel3*-knockout mice, in which *Kirrel3* gene was replaced with the *lacZ* reporter gene. X-gal staining in adult heterozygous *Kirrel3*-knockout mice revealed that the expression of *Kirrel3* was observed in the DRG neurons (35.4 ± 1.5% of total DRG neurons) and widely distributed throughout all size profiles. Double-immunofluorescence staining demonstrated that

about 80% of TrkB-positive mechanoreceptive neurons expressed *Kirrel3* in the adult DRGs. In addition, Kirrel3 was colocalized with both synaptophysin and vesicular glutamate transporter 3, a synaptic marker of mechanoreceptive neurons in the spinal cord. During the development of the DRGs, the expression of *Kirrel3* was first detected at embryonic day (E) 11.5, gradually increased, and reached the maximum levels (approximately 58% of total DRG neurons) between E17.5 and postnatal day (P) 7. From E13.5 to P7, more than 80% of TrkB-positive mechanoreceptive neurons contained *Kirrel3* in the DRGs of mice. Thus, Kirrel3 may be involved in the development and maintenance of mechanoreceptive neurons by regulating the neuronal functions, including synapse formation. Our results raise the possibility that the functional loss of Kirrel3 in the mechanoreceptive neurons of the DRGs may lead to the sensory abnormalities in ASD.

Disclosures: T. Komori: None. **T. Hisaoka:** None. **T. Kitamura:** None. **Y. Morikawa:** None.

Poster

120. Autism Mechanisms and Therapeutic Development

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 120.01/D47

Topic: A.07. Developmental Disorders

Support: Voelcker Biomedical Research Foundation
NIH Grant HD081261
NIH Grant MH064489
SPUR Program

Title: Effect of maternal PCPA treatment at mid-gestation on adolescent behaviors relevant to autism

Authors: N. A. PATHAPATTI, L. FERREIRA, M. CHACON, P. KAPLAN, A. R. NELSON, V. R. GARBARINO, L. C. DAWS, *G. G. GOULD
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Abstract: Serotonin is a neurotransmitter critical for normal neurodevelopment and behavior. The precursor to serotonin, tryptophan, crosses the placenta to feed fetal needs during pregnancy and is understood to be the main source of serotonin synthesized by the placenta at mid gestation. The experimental hypothesis was that with pharmacological attenuation of maternal serotonin synthesis during pregnancy, more tryptophan would become available for the fetus and this may improve offspring social behavior otherwise impaired. To inhibit maternal serotonin synthesis at mid-gestation, pregnant mice were treated with saline control vehicle or 150 mg/kg of para-chlorophenylalanine (PCPA) by subcutaneous injection daily for 3 days (gestational day 8 - 11). PCPA curbs conversion of tryptophan into serotonin by inhibiting the enzyme tryptophan

hydroxylase. At seven weeks of age, offspring from saline and PCPA treated dams were behavior-tested utilizing three-chamber tests for social interaction and social novelty preference, as well as the marble-burying assay for repetitive behaviors, to measure parallels of the two core clinical features of autism. BTBR mice, with inherent sociability impairments were used to determine if their deficits could be corrected, and sociabile C57BL/6 mice served as a strain control. To ensure scientific rigor each treatment was performed on two cohorts of 5 dams per treatment and strain, and sample size for groups were at least 8 offspring from different dams. There was an interaction between sex and body weight of adolescent offspring, such that males from PCPA dams weighed more than females ($p < 0.05$). In sociability preference tests, female offspring from PCPA-treated dams exhibited greater preference for stranger mice versus novel objects, both with measures of time in chamber and time engaged in social sniffing (Tukey's $p < 0.01$). This effect was also echoed in social sniffing by the adolescent male offspring of C57BL/6 but not BTBR mice. Social novelty preference was greater in the female saline group and in the PCPA-exposed males as seen in time spent sniffing. In females, the PCPA treatment significantly reduced the number of marbles buried, but there was no effect of PCPA treatment on this measure in males. It is possible that while maternal serotonin synthesis was inhibited, the subcutaneous injections of PCPA did not cross the placenta and thus did not impact fetal mice ability to synthesize serotonin, as we did not see major detriments to repetitive or social behavior in the PCPA-treated group, but instead minor enhancements that were more pronounced in C57BL/6 mice.

Disclosures: N.A. Pathapatti: None. **L. Ferreira:** None. **M. Chacon:** None. **P. Kaplan:** None. **A.R. Nelson:** None. **V.R. Garbarino:** None. **L.C. Daws:** None. **G.G. Gould:** None.

Poster

120. Autism Mechanisms and Therapeutic Development

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Topic: A.07. Developmental Disorders

Support: NIH Grant R01 EY007361
NIH Grant R00 HD073270
NIH Grant R01 HD092659
NCATS/NIH Grant UL1 TR001412
Research to Prevent Blindness Unrestricted Grant

Title: Treatment of a mouse photoreceptor cell line with 7-dehydrocholesterol-derived oxysterols induces differential expression of autism spectrum disorder-associated genes and allied pathway elements in an *in vitro* model of Smith-Lemli-Opitz syndrome

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Abstract: Smith-Lemli-Opitz syndrome (SLOS) is caused by mutations in the gene for 7-dehydrocholesterol reductase (DHCR7), catalyzing the final step in cholesterol synthesis. Affected individuals exhibit a range of neurological defects, including autism spectrum disorder (ASD). *DHCR7* is a "syndromic" ASD gene, yet how ASD is linked to deranged cholesterol metabolism is not directly apparent. Accumulated 7-dehydrocholesterol (7DHC)-derived oxysterols affect function and viability of retinal cells in animal models of SLOS. Transcriptomic analysis of a mouse cone photoreceptor cell line exposed to oxysterols showed that loss of viability was correlated with DNA damage/repair and ER stress. Here we further screened our data for expression of ASD genes, and genes for CNS development and synaptic organization imputed in ASD pathophysiology.

661W cells were incubated (n=3) with 5,9-endoperoxy-cholest-7-en-3β,6α-diol (EPCD; specific to SLOS), 7-ketocholesterol (7kCHOL), cholesterol (CHOL, a sterol control), or control vehicle (VC). Oxysterol-treated samples with high RNA integrity were harvested before global loss of cell viability at the doses employed. After conversion of raw data from Affymetrix Mouse Genome arrays, mean fold changes (FC) *vs*. VC, and adjusted P-values (aP) were computed; thresholds for differentially expressed genes (DEG) were $FC \geq 1.5$ and $aP \leq 0.001$. Enrichment analysis was carried out using LRpath.

The EPCD DEG set (in contrast to 7kCHOL and CHOL) was enriched in genes associated with synaptic structure and function (120 total, all down-regulated; $P < 0.05$), with 40/120 among the "top 2500" ASD candidates listed at asd.princeton.edu. Other significantly down-regulated annotations representing ASD-associated attributes, for both EPCD and 7kCHOL, included: cell junctions; cell/matrix adhesion; forebrain development; Wnt pathways; and signaling via channels, kinases, and growth factors. Compilation of relevant DEG suggested contrasting hypermethylating *vs*. demethylating cellular environment outcomes for EPCD and 7kCHOL, respectively. Both oxysterols dysregulated expression of individual ASD signature genes such as: ankyrin-2; ataxin-1; and, putatively, those for V-ATPase subunits. Although reelin, *FMR1*, and synapsins were not directly affected, DEG included signaling elements or transcription/activity regulators for these genes. Oxysterols upregulated genes for heme oxygenase-1, CPOX (heme synthesis), and heme-binding clock protein Rev-Erbβ.

These results support hypotheses of ASD mechanisms incorporating environmental, epigenetic, and epistatic influences, in concert with single gene loss/gain of function.

Disclosures: B.A. Pfeffer: None. **L. Xu:** None. **S.J. Fliesler:** None.

Poster

120. Autism Mechanisms and Therapeutic Development

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 120.03/D49

Topic: A.07. Developmental Disorders

Support: NIAAA: R01 AA026186

Title: Basolateral amygdala-nucleus accumbens circuit activation decreases social interaction via an endocannabinoid-regulated mechanism

Authors: *O. M. FOLKES[1], D. J. MARCUS[2], M. ALTEMUS[3], N. D. HARTLEY[2], J. J. BAECHLE[4], S. PATEL[3]
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Abstract: Social interaction deficits and anxiety are highly comorbid symptoms in several psychiatric illnesses including autism spectrum disorder (ASD). Two brain regions that are involved in the regulation of these behaviors are the basolateral amygdala (BLA), a well-established regulator in the development of anxiety and social interaction, and the nucleus accumbens (NAc), a critical region for regulation of reward and motivation. However, the role of the BLA-NAc circuit in the regulation of social interaction and anxiety-like behaviors has not yet been explored. We used *in vivo* optogenetics to assay the role of the BLA-NAc circuit in social interaction and anxiety-like behaviors in a mouse model. Our research demonstrates that optogenetic activation of the BLA-NAc circuit causes a decrease in social interaction, as assayed by the three chamber social interaction task. Additionally we see increases in negative social behaviors, and decreases in social investigative behaviors in a free social interaction tasks and social conditioned place aversion during stimulation. We do not see an effect on anxiety-like behavior. Additionally, our data shows the glutamatergic BLA-NAc circuit is sensitive to endogenous cannabinoid (eCB) signaling, which has previously been shown to regulate *in vivo* social behavior. We have shown that a pretreatment with JZL-184, a monoacylglycerol lipase inhibitor that increases the eCB 2-arachindonoylglycerol, prevents decreases in social interaction elicited by BLA-NAc activation. We will study the role of this circuit and eCB signaling in genetic mouse models of ASD. These findings could be relevant for elucidating pathophysiological mechanisms and therapeutic approaches for the treatment of psychiatric disorders characterized by increased anxiety and social dysfunction including ASD.

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Poster

120. Autism Mechanisms and Therapeutic Development

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Program #/Poster #: 120.04/D50

Topic: A.07. Developmental Disorders

Support: the Ministry of Health & Welfare, Republic of Korea (HI17C0227)

Title: Ebp1 deficient mice cause Purkinje cells dysfunction and autistic-like behavior

Authors: *I. HWANG[1,2], J.-Y. AHN[1,2]
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Abstract: The autism spectrum disorders (ASD) are characterized by impairments in social interaction and stereotyped behaviors, but the underlying pathogenesis remains poorly understood. Recent studies have suggested that cerebellar abnormalities and Purkinje cell (PCs) loss were implicated in the onset of ASD. Here we report that mice lacking the ebp1 gene (pa2g4) exhibited a severe reduction in the number of PCs and defeats in the migration out of anterior cerebellar lobes results in autistic-like behaviors, including abnormal social interaction, repetitive behavior and open field test.
Moreover, Ebp1 interacts with Ptf1a, a basic helix-loop-helix transcription factor, which is an essential regulator of Lhx1/5 and over-expression of Ebp1 leads to increase luciferase activity of LHX1/5. Here, we show that the Ebp1 prevents ubiquitination and subsequent degradation of Ptf1a by repressing FBXW7 SCF-type ubiquitin ligase and recruiting USP7 deubiquitinating enzyme to cleave the ubiquitin chains. Thus, we propose that Ebp1 is a main controller between an ubiquitin ligase and a deubiquitinating enzyme for a cerebellar contribution in PCs function to cognitive disorders such as autism.

Disclosures: I. Hwang: None. **J. Ahn:** None.

Poster

120. Autism Mechanisms and Therapeutic Development

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 120.05/D51

Topic: A.07. Developmental Disorders

Support: 81771222

Title: Autism-associated SHANK2 mutation impairs neurodevelopment in iPSC-derived neurons

Authors: *X. CHEN[1], G. HUANG[1], P. YU[1], K. WANG[2], S. LINGLING[1]
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Abstract: Autism spectrum disorders (ASDs) are neurodevelopmental disorders characterized by repetitive behavior and severe impairments in social communication and interaction. Loss of function mutations and structural variants in SHANK2 have been associated with susceptibility to ASDs, but the molecular mechanism on how SHANK2 affects neurodevelopment remains poorly understood. We reprogrammed peripheral blood cells from a patient with loss-function mutation in SHANK2 into human induced pluripotent stem cells (hiPSCs) and subsequently differentiated them into functional neurons. We showed that hiPSC-derived neurons had reduced differentiation capability and contribute to diminished differentiation capability of TH neurons. Morphological analysis showed a reduction in dendrite length, dendrite numbers and neuronal soma size, but not axon length and numbers. The neurite length and branch numbers of GABA neurons showed no change. Neurons derived from the ASD patient showed decreased frequency of sodium currents. In summary, we report changes in neuronal phenotypes and electrophysiology in hiPSC-derived neurons from a patient with ASD, and we are in the process of rescuing these phenotypes by CRISPR/Cas9 correction of the point mutation in SHANK2.

Disclosures: X. Chen: None. **G. Huang:** None. **P. Yu:** None. **K. Wang:** None. **S. Lingling:** None.

Poster

120. Autism Mechanisms and Therapeutic Development

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 120.06/E1

Topic: A.07. Developmental Disorders

Support: NIH 1R21NS097899

Title: Human ipsc-derived cerebral organoids reveal increased intrinsic neuronal excitability in an autistic subject harboring cacng2 mutation

Authors: *W. WU[1], H. YAO[2], P. NEGRAES[2], H. ZHAO[2], A. R. MUOTRI[3], J. SEBAT[4], G. G. HADDAD[5]
[2]Pediatrics, [3]Pediatrics/Cellular Mol. Med., [4]Psychiatry, Cell. & Mol. Medicine, and Pediatrics, [5]Pediatrics/Neuroscience, [1]UCSD, La Jolla, CA

Abstract: Loss-of-function mutation in the *CACNG2* gene has been associated with an increased risk for autism spectrum disorder (ASD). Using fibroblasts obtained from a child with ASD carrying in-frame deletion of *CACNG2* and the parent as a control subject, we generated induced pluripotent stem cells (iPSCs) and differentiated them into cerebral organoids to explore the electrophysiological mechanism underlying ASD phenotypes. Whole-cell patch clamp was employed using cerebral organoids in the dish. Neurons and glial cells were functionally distinguished by their characteristic electrophysiological properties: action potential and voltage-gated Na^+ currents in neuron *vs.* Kir currents in glia. We found that neurons with CACNG2 mutation had action potentials with a ~16% higher amplitude and ~33.0% faster upstroke compared to control neurons (n=23-32). The minimum current (rheobase) injected to evoke an action potential in CACNG2 mutation neurons (12.8±1.0 pA, n = 32) was lower than that in control neurons (16.9±1.4 pA, n=22, $p<0.01$). We further discovered that the current densities of voltage-gated Na^+ channels of CACNG2 mutation neurons (-213.8±25.5 pA/pF at -20 mV, n=36) were significantly higher than that from control subject (-96.3±11.7 pA/pF at -20 mV, n=23, $p<0.01$). We also found a hyperpolarizing shift of voltage dependence of Na^+ channel activation in the mutant neurons ($V_{0.5}$ = -35.5±1.3 mV, n=36) compared to control neurons ($V_{0.5}$ = -29.8±1.1 mV, n=23, $p<0.01$). These results suggested an elevated neuronal excitability in CACNG2 neurons in cerebral organoids. In addition, the amplitude and frequency of spontaneous excitatory synaptic currents were significantly lower in mutant neurons (-6.72±0.66 pA and 0.26±0.06 Hz, n=19) compared to control neurons (-13.7±2.8 pA and 0.61±0.31 Hz, n=6, $p<0.05$), indicating an impaired synaptic activity by CACNG2 mutation. The current densities of Kir channel were not significantly different between control (-52.6±13.3 pA/pF at -140 mV, n=8) and mutant (-47.3±13.6 pA/pF at -140 mV, n=12) glial cells. Overall, our study revealed increased intrinsic excitability in CACNG2 neurons of cerebral organoids while the synaptic activity seems disrupted. In the future study, we will focus on investigating alterations of electrophysiological properties of cerebral organoids derived from other ASD mutations. Common electrophysiological changes in ASD neurons could be the potential molecular mechanisms that contribute to dysfunctional cortex processing in autistic individuals.

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Poster

120. Autism Mechanisms and Therapeutic Development

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 120.07/E2

Topic: A.07. Developmental Disorders

Support: CIHR
CGS-Vanier

Title: The effects of prenatal LPS on microglial fractalkine pathway modulated neurodevelopment

Authors: *L. FERNÁNDEZ DE COSSÍO GÓMEZ[1], G. CASTINO[2], C. LACABANNE[3], S. CUESTA[3], G. N. LUHESHI[4]
[1]Douglas Mental Hlth. Univ. Institute, McGil, Verdun, QC, Canada; [2]Ecole Normale Supérieure de Lyon, Lyon, France; [3]Douglas Mental Hlth. Univ. Institute, McGill, Verdun, QC, Canada; [4]Douglas Mental Hlth. Univ. Inst., McGill Univ., Verdun, QC, Canada

Abstract: Background: Microglia, the brain's resident immune-cells, are critically involved in normal brain developmental processes including the shaping of connections between neurons by pruning superfluous synaptic spines. This important task is thought to involve the ligand fractalkine, which is highly expressed by neurons, and its only known receptor (CX3CR1) predominantly expressed on microglia. We previously showed a significant reduction in CX3CR1 mRNA expression and higher spine density in the offspring brain in our prenatal inflammation model. Here we assess immune mediators and expand our investigation on the role of CX3CR1 in our model.
Objective: To identify inflammatory changes and determine the role of CX3CR1 in microglia's spine pruning alterations in our prenatal inflammation model.
Methods: Pregnant mice were injected with a single dose of bacterial Lipopolysaccharide (LPS) at embryonic day 15. An array of immune mediators, cytokines, was measured in maternal plasma, fetal brains and placenta. We are further quantifying microglia density and its co-expression with CX3CR1, as well as assaying levels of post synaptic protein PSD95, as an indicator of pruning alterations. In separate studies, injections were performed on $CX3CR1^{+/-}$ dams mated with $CX3CR1^{+/-}$ males to generate litters with all the different genotypes and we are currently assaying PSD95 expression. Sex effects were considered in all experiments.
Results: Prenatal LPS induced a significant increase in pro-inflammatory cytokines IL-1 beta, IL-6 and TNF alpha and anti-inflammatory IL-10 in the maternal plasma 2hrs post injection, which normalized after 4hrs. Only IL-1 beta and IL-10 were detectable in the fetal brain regardless of treatment and TNF alpha was the only cytokine significantly increased in the placenta of male fetus after 2hrs. These changes are indicators of alterations in the inflammatory milieu during gestation, that we believe are linked to changes in microglial function in general and to the fractalkine pathway specifically, and which we expect to disentangle with our current experimental design.
Conclusions: Previously, our results provided an early indicator that microglial function was altered in the brain of maternally challenged progeny. We show here the inflammatory profile within different tissues and the role of CX3CR1 in our model.

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Poster

120. Autism Mechanisms and Therapeutic Development

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Program #/Poster #: 120.08/E3

Topic: A.07. Developmental Disorders

Support: R21 MH104766
R01 MH109885
R01 MH108528
SFARI Research #345469

Title: Cerebral organoids from autism patients provide insights into dysregulated molecular pathways

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Abstract: The 16p11.2 copy number variant is associated with multiple neurodevelopmental disorders. There is a dosage effect of this CNV on the head size phenotype, with macrocephaly observed in the deletion carriers, and microcephaly observed in the duplication carriers.
To study the molecular pathways impacted by this CNV, we generated human cerebral organoids from iPSCs derived from 16p11.2 DEL/DUP affected individuals. RNA-seq was performed on iPSCs and organoids at 1 month after generation. To process the RNA-seq data, we adopted the "long-rna-seq-pipeline" used by the ENCODE Consortium. The size of the organoids was monitored during their maturation, and cell migration was assessed at 1 month.
We observed that cerebral organoid models recapitulate patients' head size phenotypes. Transcriptomic profiling of iPSCs identified significantly differentially expressed genes with cytoskeletal functions. Gene co-expression network analyses identified co-expression modules that were strongly associated with 16p11.2 copy number status. One module comprised all 16p11.2 genes, and was upregulated in DUPs while downregulated in DELs, confirming strong cis-effect of the 16p11.2 CNV. Another module upregulated exclusively in DUPs was enriched in genes involved in cell adhesion and migration. Finally, the module upregulated exclusively in DELs was enriched in genes with nucleosome and chromatin-related functions. This suggests that dosage changes of the same CNV impact different biological and molecular functions.
We validated some of the molecular findings with regard to the dysregulated gene modules by observing a defect in neuronal migration of cells dissociated from the DEL and DUP cerebral organoids. Furthermore, immunofluorescence demonstrated defects in cell proliferation and differentiation in the mutant organoids. Proteomics studies are ongoing to better understand posttranscriptional impact of the 16p11.2 CNV.

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Poster

120. Autism Mechanisms and Therapeutic Development

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 120.09/E4

Topic: A.07. Developmental Disorders

Support: SFARI grant 513133

Title: The autism-associated gene Scn2a plays an essential role in dendritic excitability, synaptic stability, and learning

Authors: *P. W. SPRATT[1], R. BEN-SHALOM[2], C. M. KEESHEN[2], K. BURKE[2], R. L. CLARKSON[2], S. M. SANDERS[3], K. J. BENDER[2]
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Abstract: Exome sequencing has identified the gene *SCN2A* as a leading monogenetic risk factor for autism-spectrum disorder (ASD) and intellectual disability. *SCN2A* encodes $Na_V1.2$, a voltage-gated sodium channel with a known role in the axon initial segment (AIS), an axonal subcompartment that is the site of action potential initiation. Previously, we found that the majority of ASD-associated *SCN2A* variants result in a loss of channel function (Ben-Shalom et al.), suggesting that $Na_V1.2$ haploinsufficiency bears considerable risk for ASD. How $Na_V1.2$ haploinsufficiency contributes to ASD pathogenicity remains unclear.
Here, we examined the consequences of $Na_V1.2$ haploinsufficiency throughout development using mice with heterozygous loss of one *Scn2a* allele ($Scn2a^{+/-}$). We focused on layer 5 of prefrontal cortex, a region containing neurons implicated in ASD. Within this region, we found that *Scn2a* haploinsufficiency altered excitability and synaptic function within excitatory pyramidal cells and not neighboring inhibitory cells. In pyramidal cells, *Scn2a* loss affected neuronal excitability in two developmentally distinct ways. Early in development, *Scn2a* loss impaired axonal excitability. In more mature neurons, axonal excitability deficits were no longer apparent. Instead, *Scn2a* loss impaired dendritic excitability. Furthermore, we found that excitatory synapse function and the capacity for synaptic plasticity was impaired in mature $Scn2a^{+/-}$ neurons, and that these impairments were associated with behavioral learning deficits. Thus, these results indicate that *Scn2a* expression is critical for maintaining neuronal excitability throughout life, and further identify a role for $Na_V1.2$ channels in the dendrite that is critical for proper synapse stability.

Disclosures: P.W. Spratt: None. **R. Ben-Shalom:** None. **C.M. Keeshen:** None. **K. Burke:** None. **R.L. Clarkson:** None. **S.M. Sanders:** None. **K.J. Bender:** None.

Poster

120. Autism Mechanisms and Therapeutic Development

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Program #/Poster #: 120.10/E5

Topic: A.07. Developmental Disorders

Support: Grant from the Ministry of Health, Labour and Welfare, Japan (to YK)

Title: Electrophysiological and pharmacological evaluation of developmental neurotoxicity using brain slices obtained from juvenile rats prenatally exposed to chemicals

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Abstract: Objective: To evaluate the developmental neurotoxicity of chemicals, we utilized electrophysiological and pharmacological approaches to analyze hippocampal slices obtained from rat pups, prenatally exposed to chemicals of interest, such as valproic acid (VPA) or tributyltin (TBT). VPA is commonly used as an antiepileptic drug and mood stabilizer. However, it is known as a developmental neurotoxicant, because animal models of autism spectrum disorder have been established by prenatal exposure to VPA. **Methods:** Pregnant Wistar rats were exposed to VPA (300 mg/kg) or TBT (20 mg/kg) on day 15 of gestation. Hippocampal slices were prepared from juvenile rats belonging to the VPA or TBT exposed and control groups, at postnatal days (PND) 13-18. We investigated stimulation/response (S/R) relationships of the slope of the field excitatory postsynaptic potential (fEPSP) and the amplitude of the population spikes (PS) evoked in the CA1 area. Data of fEPSP-Spike (E-S) coupling, obtained from each individual slice, were fitted to a logistic curve and the fEPSP slope value at half maximal PS (Eslope50) was compared among the experimental groups. Additionally, effect of ambient γ-aminobutyric acid (GABA) on the PS amplitude was analyzed using bicuculline methiodide (BMI), an antagonist of $GABA_A$ receptors. **Results and discussion:** In the control group, S/R relationships of fEPSP and PS were suddenly enhanced between PND 15 and 16. The effect of BMI on PS amplitude was developmentally altered; BMI enhanced PS amplitude until PND 15, but showed little effect after PND 16. In the VPA groups, S/R relationships gradually enhanced at PND 14 and 15, which was before eye opening. Eslope50 also showed a significant increase on PND 14 and 15. In addition, little effect of BMI on PS amplitude was observed on PND 14 and 15, which was almost identical to that observed on PND 16 and 17. These results

indicate that VPA may hasten the developmental change in hippocampal excitability during the synaptogenic period. In the TBT groups, PS amplitude was decreased without changes of Eslope50 at PND 16, and a slight retardation in developmental change was observed in the effect of BMI on PS amplitude at PND 16, before eye opening in TBT-exposed rat pups. These results indicate that TBT, unlike VPA, may delay development. **Conclusion:** Some developmental neurotoxicity can be detected during the synaptogenic period before neurobehavioral changes become obvious in adolescents and adults, using hippocampal slices obtained from rats prenatally exposed to chemicals. Our approaches may be useful for the earlier prediction of developmental chemical neurotoxicity.

Disclosures: Y. Fueta: None. **S. Yoshida:** None. **Y. Sekino:** None. **Y. Kanda:** None. **S. Ueno:** None.

Poster

120. Autism Mechanisms and Therapeutic Development

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Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 120.11/E6

Topic: A.07. Developmental Disorders

Support: ISRP-240000305

Title: The role of NMDA receptors in the neurodevelopment of chick embryos exposed in ovo to valproic acid

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Abstract: Valproic acid (VPA) is one of the most commonly used antiepileptic drugs. It is also a teratogen that causes birth defects in humans, including neural and neurobehavioral abnormalities in the offspring. However, the underlying molecular mechanisms of these defects remain unclear. N-methyl-D-Aspartate receptor (NMDAR) is one type of glutamate excitatory receptors and is involved in normal neurodevelopment, learning, and memory formation. VPA intervenes in rat synapse formation and suppresses NMDAR-mediated neural response. We aimed to investigate if and how NMDARs play a role in VPA-induced neurodevelopmental anomalies. This study was carried out in fertile White Leghorn chicken eggs. VPA was injected in different dosages (100 μg to 500 μg) into chicken embryos at stage HH4 before the process of neurulation occurred. Embryo development, embryo growth and brain NMDAR expression were analyzed in eggs incubated for 12 days. Our data showed that prenatal exposure of VPA causes failure of embryo development and embryo growth restriction, which are dosage-dependent. NMDARs expression was studied in forebrain of chicken embryos and NMDAR expression increase was found in VPA-treated groups. The potential role of VPA in the functional

regulation of NMDARs during neurodevelopment of chicken embryos will be further studied. And the present findings highlighted that the birth defect model created in this study could be used to test the hypotheses causing birth defects as well as investigate appropriate preventative strategies.

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Poster

120. Autism Mechanisms and Therapeutic Development

Location: SDCC Halls B-H

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Program #/Poster #: 120.12/E7

Topic: A.07. Developmental Disorders

Title: Mechanisms for reduction of microglia in a valproic acid rodent model of autism

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Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental condition affecting approximately 1 in 59 children in North America. Valproic acid (VPA) is a multi-target drug widely used to treat epilepsy. It is also a histone deacetylase inhibitor and fetal exposure to VPA increases the risk of ASD. One important hallmark of ASD is an unusually large brain size that presents at around the age of 2 years. The cause of this enlargement is not known, but a key study revealed that children with autism who show early brain overgrowth have 67 percent more neurons in the prefrontal cortex than controls do. Even though, recent studies suggest that microglia are critical players in regulating neuronal numbers, dendrite and synapse formation during early brain development the effect of prenatal exposure to VPA on microglial survival during embryonic and early postnatal brain development has not been studied and may provide potential hints regarding the etiology of this disorder. We have previously shown that prenatal exposure to VPA significantly reduces the number of microglia in the primary motor cortex in male mice at both postnatal day 6 (P6) and P10. Therefore, in this study, we determined the mechanism for reduction of microglia in a VPA rat model of autism. We injected pregnant rats with 600 mg/kg VPA on embryonic day (E12), harvested the brains on E13, E17, and E20 and double immuno-stained microglia with Ionized calcium binding adapter molecule 1 (Iba1), a microglial marker and cleaved caspase 3 (CC3) a cell death marker. We found increased co-localization of Iba1 and CC3 in VPA treated mice at E17 and E20 in the proliferative zones of the rat brain compared to controls. We confirmed this finding using immunoblot. These data suggest that prenatal exposure to VPA reduces microglial number by inducing caspase 3 mediated apoptosis in these cells. Since microglia are critical players in regulating neuronal numbers, dendrite and synapse formation during early brain development the reduction in the

number of microglia may be partly responsible for mediating the unusual brain size observed in the pathology of ASD.

Disclosures: C. Schram: None. **M. Haney:** None. **P.S. Awale:** None.

Poster

120. Autism Mechanisms and Therapeutic Development

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Program #/Poster #: 120.13/E8

Topic: A.07. Developmental Disorders

Support: Einhorn Family Charitable Trust

Title: Colostrum oxytocin in the newborn gut modulates cellular stress and inflammation in the nucleus tractus solitarius (NTS), an area abnormal in autism

Authors: *M. G. WELCH[1], H. TAMIR[2], M. ANWAR[3], S. GLICKSTEIN[5], R. J. LUDWIG[4], B. Y. KLEIN[3]
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Abstract: Impairment of the nucleus tractus solitarius (NTS) is a potential break-through finding in the understanding of autism spectrum disorder (ASD). Cranial visceral afferents travel via the solitary tract (ST) to contact neurons within the NTS and activate homeostatic reflexes. Hypothalamic projections from the paraventricular nucleus (PVN) release oxytocin (OT) to modulate visceral afferent communication with NTS neurons. However, the cellular mechanisms through which OT acts are poorly understood. We previously showed in newborn rat villi that colostrum OT modulates cellular stress response, inflammation, and autophagy markers impaired in autism. Little is known about whether there are analogous responses in the brain. Here, we tested whether brain regions rich in oxytocin receptors (OTR) mirror the effects previously observed in gut villi. We measured signaling protein markers associated with endoplasmic reticulum stress, including an ER chaperone, BiP, translation initiation factor, eIF2a, as well as two inflammation-signaling proteins, NF-kB and its inhibitor IkB. We measured these markers in newborn NTS stressed by nutrient insufficiency in two conditions: unprimed, the interval between birth and the initiation of nursing; and primed, the interval after the initiation of nursing. Brain tissue from unprimed and primed animals was harvested from the NTS as well as from other brain regions enriched in oxytocin receptors. In contrast to other regions tested, where expression of BiP and NF-kB was the same in the both unprimed and primed conditions, expression of BiP and p-eIF2a in the NTS was upregulated in unprimed and downregulated in primed tissue. Furthermore, we found in the NTS that eIf2a was phosphorylated not by PKR, but by a different kinase, general control nonsuppressed2 kinase (GCN2). These results indicate that

NTS utilizes a different phosphorylation mechanism from the other regions. Collectively, our data shows that brain responses to the stress prior to first feed may be subsequently offset by signaling from colostrum-primed gut, a finding that highlights an important benefit from breastfeeding. Our data also suggest that the newborn gut stress-modulating mechanism we previously observed in enterocytes is paralleled in brain regions rich in OTR. Taken together, our findings in the gut and in the brain, especially NTS, may in part explain both brain and gut problems in cases of autism with abnormalities of OT/OTR function.

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Poster

120. Autism Mechanisms and Therapeutic Development

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Program #/Poster #: 120.14/E9

Topic: A.07. Developmental Disorders

Support: 1R21MH113195-01

Title: Prefrontal network dysfunction in a maternal immune activation model of autism spectrum disorder

Authors: *C. D. MAKINSON[1], T. N. WEERAKKODY[2], J. M. SOROKIN[2], J. R. HUGUENARD[3]
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Abstract: Maternal infection during pregnancy is a strongly implicated environmental risk factor for autism spectrum disorder (ASD) in offspring. Mouse models of maternal immune activation (MIA) corroborate this link and may provide insight into underlying pathogenesis. In particular, prenatal exposure to the viral mimic polyinosinic:polycytidylic acid (poly(I:C)) during mid-gestation (E12.5) produces core behavioral deficits of ASD (i.e. social impairment, abnormal communication, and stereotypy). The medial prefrontal cortex (mPFC), a key regulator in social cognition, is a proposed major locus in ASD pathology; however, its functional role in MIA-related ASD remains unclear. Primarily *histological* abnormalities have been observed in the mPFC of MIA offspring, including reductions in spine density and GABAergic markers. Here, we present a detailed *neurophysiological* examination of mPFC function that points to hypoactivity at the behavioral, network, and cellular levels. Adult MIA offspring displayed resistance to fluorothyl-induced seizures, downregulation of activity-dependent gene transcription, and diminished synaptic transmission. Large-scale synaptic deficits in excitation and inhibition within the mPFC were primarily attributed to reduced presynaptic activation of

layer 5 pyramidal neurons, which corresponded to defective action potential initiation in whole-cell patch clamp recordings. In addition, orthodromic axonal spikes, as detected by axon-attached recordings, were significantly attenuated in MIA offspring and exhibited reduced propagation velocities. Based on these results, we identify presynaptic axonal excitability as a novel contributor to ASD-related mPFC dysfunction.

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Poster

120. Autism Mechanisms and Therapeutic Development

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Topic: A.07. Developmental Disorders

Support: NIH Grant R03 MH 104851
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NSF Grant IOS 1652432

Title: Sensory cortical alterations in mouse models of autism spectrum disorder

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Abstract: Autism spectrum disorder (ASD) is comprised of a group of neurodevelopmental disorders characterized by deficits in social interaction and communication along with restricted interests and repetitive behaviors. Sensory processing abnormalities such as auditory perceptual impairments, atypical visual behavior and over-responsivity to tactile stimuli are frequently observed in autistic individuals and might contribute to the complex behavioral symptoms associated with the disorder. In this study, we examine two genetic mouse models of ASD: one harboring a knockout of the contactin associated protein-like 2 gene (*CNTNAP2*$^{-/-}$), and the other containing a hologous deletion of human chromosome 16p11.2. Using these genetic mouse models, we investigated the neuroanatomical alterations in the primary sensory cortical areas, which might underlie cortical dysfunction during early postnatal ages into adulthood. One of the important neuropathological feature in these genetic mouse models of ASDs is the abnormal developmental migration of neurons destined for superficial cortical layers. In our study, the laminar organization of the primary sensory cortical areas was analyzed by labeling with CUX-1, which is a marker for neurons normally localized to the superficial cortical layers (II-IV). Additionally, we examined changes in the proportion of GABAergic neurons, particularly parvalbumin expressing (PV) interneurons. Deficits in sensory processing relating to

impairments in modulating sensory inputs and changes in synaptic plasticity might also be due to the abnormalities in the extracellular matrix structures, such as the perineuronal nets (PNNs), analyzed by lectin staining in these sensory cortical areas of the mutant and the control animals. By quantifying the laminar distribution of CUX-1 positive cells in the visual, auditory and somatosensory cortical areas of these genetic models of ASDs, our findings show increased number of CUX-1 positive cells mislocalized to the lower layers (V and VI) of these cortical areas as compared to the control animals. Furthermore, our results indicate an increase and a subsequent decrease of perineuronal nets (PNNs) co-localized with parvalbumin expressing interneurons (PVs) in the genetic mouse models in contrast to the controls at early postnatal ages and adulthood, respectively. These results suggest that the neuroanatomical alterations in the auditory, visual and somatosensory cortical areas at various postnatal ages could play a role in the sensory processing impairments that account for some of the core autistic behaviors.

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Poster

120. Autism Mechanisms and Therapeutic Development

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Program #/Poster #: 120.16/E11

Topic: A.07. Developmental Disorders

Support: Supported by a Developmental Start-Up Fund from the University of Iowa, College of Pharmacy

Title: Integrative genomic analysis in autism brain tissue reveals pro-inflammatory signaling

Authors: *A. GNANASEKARAN, R. M. SMITH
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Abstract: Autism spectrum disorder (ASD) is neurodevelopmental disorder that involves a complex interplay of both genetic and environmental risk factors, with possible immune alterations and atypical brain development. Cytokines act primarily as mediators of immunological activity, but they also play a significant role in the nervous system, where they participate in normal neural development and function. Inappropriate cytokine activity can have a variety of neurological implications. Immunological imbalance is proposed as a major etiological component in ASD, taking into account converging evidence of increased levels of pro-inflammatory cytokines observed in autistic patients.
Here, we performed an integrative genomics analysis investigating immune function in ASD, measuring cytokines profiles, transcriptomes, and genetic variation in post-mortem frontopolar brain tissue from individuals with autism and controls. We found increased pro-inflammatory cytokine concentrations and growth factors in ASD, compared with typically developed controls,

including FLT3L ($p<0.05$), PDGF-AB/BB ($p<0.05$), TNF-α ($p<0.1$), and VEGF ($p<0.05$). Our whole transcriptome RNA-seq analysis bolsters evidence for a pro-inflammatory phenotype in ASD, finding enrichment of genes in the TNF-α pathway amongst those most differentially expressed between the groups. Detection of elevated cytokine levels, particularly related to TNF-α signaling in autistic subjects, suggests that activation of pro-inflammatory cytokines may be important in autism.

Disclosures: A. Gnanasekaran: None. **R.M. Smith:** None.

Poster

120. Autism Mechanisms and Therapeutic Development

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Topic: A.07. Developmental Disorders

Support: NSF IRFP 0965110
Whitehall Foundation
GT Neural Engineering Center 1241384
Goizueta Foundation Fellowship
Georgia Tech's President Fellowship

Title: Impaired sensory perception and altered cortical population activity in a mouse model of autism

Authors: *J. DEL ROSARIO, H. ARROWOOD, A. AMER, A. SPEED, S. WATSON, B. HAIDER
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Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental condition that causes significant impairments in sensory processing and behavior. Although many genetic mutations associated with ASD are known, the downstream effects on neural circuits and impaired sensory behavior remain poorly understood. It has been hypothesized that an imbalance between excitatory and inhibitory activity in cortex is a major cause of ASD (Rubenstein and Merzenich, 2003). Strong evidence for this hypothesis entails measuring both cortical excitatory and inhibitory neuron populations during impaired sensory perception.
We directly measured cortical neural activity during impairments of sensory perception in a clinically relevant transgenic mouse model of ASD ($CNTNAP2^{-/-}$). We trained ASD mice (n = 60 sessions) and control mice (C57BL6, n=182 sessions) to detect the appearance of a visual stimulus in the central (binocular) visual field, by licking for water rewards. Mice received rewards only if they licked during the stimulus, and otherwise had to withhold licking for a randomized period of time (0.5-6s) between each trial. We simultaneously monitored pupil

diameter, a known indicator of arousal. ASD mice detected stimuli with significantly longer latencies than control mice (ASD: 0.53±0.06s; control: 0.45±0.09s; $p<0.01$). Signal detection analysis revealed that ASD mice also had a significantly more conservative response criterion (ASD: +0.53±0.39; control: -0.17±0.41; $p<0.01$). Differences in response criterion were not easily explained by low arousal: pupil area and arousal were largest preceding detection failures in both ASD and control mice (8.2±2.5% and 9.6±1.7% larger than average, respectively). Once mice learned the task, we performed multisite electrophysiological recordings in primary visual cortex (V1) to determine if altered cortical activity accompanied impaired sensory perception. Broadband local field potential (LFP) power was reduced in ASD mice (n=8 recordings). Surprisingly, in ASD mice, regular spiking (RS) putative excitatory neurons fired less (ASD: 0.42±0.99 spks/s, n=54; control: 0.71±1.14 spks/s, n=74), while fast spiking (FS) putative inhibitory neurons fired more (ASD: 1.25± 1.95 spks/s, n=18; control: 0.62±0.94 spks/s, n=22). Suppressed excitation and enhanced inhibition may lead to weaker sensory signals, and thus slower and less frequent behavioral responses. Our preliminary results provide direct evidence that imbalanced cortical excitation and inhibition may underlie impaired sensory perception and behaviors in models of ASD.

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Poster

120. Autism Mechanisms and Therapeutic Development

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Program #/Poster #: 120.18/E13

Topic: A.07. Developmental Disorders

Support: R01MH110487

Title: Mapping chromatin dynamics, transcriptional regulation and physiological change after activity-induced circuitry stress in cell models of neuropsychiatric risk genes

Authors: *B. DAVIS[1,2], H. CHEN[5], G. HAMERSKY[5], J. BOHLEN[5], J. SHIN[5], A. JAFFE[5,3,6,7,4], B. MAHER[5,2,3]
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Abstract: Psychiatric genetics continues to reveal the remarkable overlap in the underlying genetic architecture in neuropsychiatric disorders, with transcriptional regulation, chromatin biology and synaptic function among the most significant risk pathways (Cross-Disorder Group

of the Psychiatric Genomics Consortium, 2013; Gandal et al., 2018). These biological pathways are highly interconnected. Neuronal activity drives the developing neural circuit and regulates ongoing synaptic activity, which is dependent on appropriate gene expression. While chromatin is the dynamic substrate on which transcriptional machinery and epigenetic modifications orchestrate the precise patterns of gene expression, essential for regulating neuronal activity. But the dynamic interplay between these functions is not well understood for neuropsychiatric disease despite underlying genetic risk. We studying the dynamic regulation of open chromatin regions (OCRs) after stress induced neuronal activity in 3 cell models that harbor mutation in psychiatric risk genes. TCF4, MBD5 and SCN2A are highly penetrant genes associated with ASD, ID and SCZ. These genes are associated with different aspects of the risk architecture. TCF4 has previously been shown to transcriptionally regulate activity induced gene programs (Rannals et al., 2016; Sepp et al., 2017); SCN2A is an ion channel essential for cortical development (Ben-Shalom et al., 2017; Gazina et al., 2015), and MBD5 co-localizes with heterochromatin (Baymaz et al., 2014; Laget et al., 2010). Primary cortical cultures are generated from the three heterozygous mutant mice. Cultures are treated with KCl to induce excitatory stress, and TTX to reduce synaptic transmission. Changes in OCRs are then assessed using ATAC-sequencing (Buenrostro et al. 2013); biological replicates are used in parallel RNA-sequencing, whole-cell electrophysiology and Ca^{2+} imaging experiments. Differential OCR dynamics and correlational changes in gene expression provide molecular rubrics for interpreting the impact of stress on single-cell physiology and network dynamics in these neuropsychiatric mutant models.

Baymaz, H.I., et al. (2014). Proteomics *14*, 2179–2189. Ben-Shalom, R., et al. (2017). Biol. Psychiatry *82*, 224–232. Buenrostro, J.D. et al., (2013). Nat. Methods *10*, 1213–1218. Cross-Disorder Group of the Psychiatric Genomics Consortium (2013). Lancet *381*, 1371–1379. Gandal, M.J., et al. (2018). Science *359*, 693–697. Gazina, E.V., et al. (2015). Hum. Mol. Genet. *24*, 1457–1468. Laget, S., et al., (2010). PLoS ONE *5*, e11982. Rannals, M.D., et al. (2016). Neuron *90*, 43–55. Sepp, M., et al., (2017). *37*, 10516–10527.

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Poster

120. Autism Mechanisms and Therapeutic Development

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Topic: A.07. Developmental Disorders

Support: Max Planck Society

Title: Deletion of NRXN 1α disrupts specific elements in fear circuit

Authors: D. ASEDE, A. JOSEPH, *M. BOLTON
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Abstract: Neurexins are cell adhesion molecules which interact with their postsynaptic partners to preserve synaptic connections and maintain normal synaptic transmission. Mutations in the gene encoding the alpha isoform of neurexin 1(NRXN 1α) were identified in patients with schizophrenia and autism. Because of the prevalence of cognitive impairment and and emotional dysregulation in these disorders, related brain regions such as the medial prefrontal cortex (mPFC) and amygdala have been in the research spotlight. Although abnormal neural activity patterns in these regions have been implicated in the pathophysiology of these diseases, it is not known whether mutations in a high-confidence risk gene such as NRXN 1α disrupts specific synaptic elements and connections. In this study, we investigated the impact of NRXN 1α deletion on dorsal medial prefrontal cortex (dmPFC) - basal amygdala (BA) connection and relevant behavior. Mice lacking NRXN 1α showed deficit in fear memory retrieval during discriminative fear conditioning paradigm. Probing the putative synaptic circuits in BA, we found a reduction in synaptic strength with a concomitant decrease in AMPA / NMDA ratio at dmPFC - BA synapse but no change in lateral amygdala (LA) input. Activation of either dmPFC or LA afferent inputs resulted in a decreased in feedforward inhibition, suggestive of input-independent impairment in GABAergic transmission within BA. Using circuit mapping technique, we analyzed inhibitory synaptic connectivity in BA and found a reduction in connection probability in BA principal cells. Together, we identify input-specific impairment at dmPFC - amygdala pathway, a defective inhibitory transmission in amygdala microcircuit and impairment in amygdala - dependent behavior in NRXN 1α KO. These synaptic and behavioral phenotypes may contribute to the etiology of emotional dysregulation observed in schizophrenia.

Disclosures: D. Asede: None. **A. Joseph:** None. **M. Bolton:** None.

Poster

120. Autism Mechanisms and Therapeutic Development

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 120.20/E15

Topic: A.07. Developmental Disorders

Title: A novel treatment for autism spectrum disorder: Targeting the cholinergic system

Authors: *S. N. SUDWEEKS[1], S. WERNER[2]
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Abstract: Currently, there is no pharmacological treatment available for autism spectrum disorder (ASD). However, there are significant findings regarding genetic, structural, and behavioral differences in individuals with ASD. Included in these findings is an imbalance in the

cholinergic system. Previously published research has shown that l-carnitine has been found to alleviate symptoms associated with autism in humans, and acetyl-l-carnitine (ALC) has been found to increase the release of acetylcholine in the brain (Geier, 2011; Imperato, 1989; White, 1990). Additionally, in the maternal immune activation (MIA) mouse model, which has been found to induce ASD, maternal choline administration led to pups with less severe symptoms of ASD or typical behaviors when compared with the litters of untreated dams (Wu, 2015). It has also been found that acetylcholine and nicotinic acetylcholine receptors (nAChRs) are deficient in animal models and humans with ASD, specifically a4b2 and a7 nAChRs (Anand, 2011; Dineley, 2015; Martin-Ruiz, 2004; McTighe, 2013). Finally, treatments using acetylcholinesterase inhibitors, which inhibit the enzyme responsible for acetylcholine degradation, and nAChR agonists/positive allosteric modulators (PAMs) have been found to increase nAChR density and alleviate symptoms of ASD (Ghaleiha, 2013; Karvat, 2014; Lopez, 2006; Mostafavi, 2016; Srivastava, 2011; Wang, 2015). As a result of these findings, we believe that a promising pharmacological target to alleviate core symptoms of ASD is the cholinergic system, specifically a4b2 and a7 nicotinic acetylcholine receptors (nAChRs). Our study's purpose is to discover the effects on core symptoms of ASD when the efficacy of those specific nAChR subtypes are increased, thereby increasing cholinergic tone in the brain. To demonstrate these effects, we have selected NS9283 and PAM-II, positive allosteric modulators of a4b2 and a7 nAChRs respectively, to administer to UBE3A and BTBR genetic mice, used to model ASD. We hypothesize that treatment with the selected compounds will alleviate core symptoms of ASD displayed by both animal models.

Disclosures: S. Werner: None.

Poster

120. Autism Mechanisms and Therapeutic Development

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 120.21/E16

Topic: A.07. Developmental Disorders

Support: Robert and Donna Landreth Family Fund
Nancy Lurie Marks Family Foundation

Title: Sex- and region-specific regulation of immune-related genes by maternal and early postnatal immune activation

Authors: *W. KIM[1], G. MISSIG[1], B. C. FINGER[1], S. M. LANDINO[1], A. J. ALEXANDER[1], E. L. MOKLER[1], J. O. ROBBINS[1], Y. LI[1], V. Y. BOLSHAKOV[1], C. J. MCDOUGLE[2], W. A. CARLEZON, Jr[1], K.-S. KIM[1]
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Abstract: Autism spectrum disorders (ASDs) are neurodevelopmental syndrome with significantly higher prevalence in males. Recent emerging clinical and preclinical data suggest that the immune system plays a critical role for etiology of ASDs, as evidenced by its association with autoimmune disorders and maternal infection during pregnancy. To address the hypothesis that ASD's male prevalence is linked to sex-different regulation of immune-related genes, we investigated molecular changes of three categories of inflammatory genes (i.e., pro- and anti-inflammatory genes and neuroinflammation-related marker genes) in male and female mouse brains following treatment of timed-pregnant mice with polyinosinic:polycytidylic acid (Poly I:C) on gestational day 12.5 to produce maternal immune activation (MIA) and/or with lipopolysaccharide (LPS) on postnatal day 9 to produce postnatal immune activation (PIA). Our molecular studies revealed that early immune activation produced prominent sex-specific changes in inflammation-related gene expression in the brain. Both sexes showed increases in pro-inflammatory factors (such as TNFα, iNOS, IL-6 and IL-1β) and neuroinflammation markers (such as Iba-1, GFAP and TSPO) but their increases were in general much higher in male mouse brains, as reflected by levels of mRNA and corresponding proteins. Strikingly, however, we found that expression of anti-inflammatory factors was decreased in male mouse brain regions but increased in female mouse brain regions. Thus, our findings demonstrate that early developmental immune activation can produce sex-specific effects on the function of factors that regulate inflammatory responses in the brain, which may contribute to sex differences in the prevalence of ASD-like behaviors.

Disclosures: W. Kim: None. **G. Missig:** None. **B.C. Finger:** None. **S.M. Landino:** None. **A.J. Alexander:** None. **E.L. Mokler:** None. **J.O. Robbins:** None. **Y. Li:** None. **V.Y. Bolshakov:** None. **C.J. McDougle:** None. **W.A. Carlezon:** None. **K. Kim:** None.

Poster

120. Autism Mechanisms and Therapeutic Development

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 120.22/E17

Topic: A.07. Developmental Disorders

Support: Simons Foundation Pilot Award 515488

Title: Single-cell analysis implicates upper-layer neurons and protoplasmic astrocytes in autism

Authors: *D. VELMESHEV[1], L. SCHIRMER[2], S. MAYER[3], D. JUNG[2], A. BHADURI[4], N. GOYAL[6], M. HAEUSSLER[7], D. ROWITCH[8], A. R. KRIEGSTEIN[5]
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Berkeley, Berkeley, CA; [7]Univ. of California, Santa Cruz, Santa Cruz, CA; [8]Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Autism spectrum disorder (ASD), affecting 1 of 68 children in the US, is heterogeneous in terms of clinical manifestation and associated genetic contributions. Changes in cytoarchitecture and dysfunction of the prefrontal cortex and the limbic system have been consistently reported in ASD patients. These areas are involved in higher-order cognitive functions, processing of social information, emotions and empathy. Although bulk transcriptomic studies of post-mortem tissue from patients with ASD have revealed convergence of disease pathology on common pathways--including splicing of neuronal genes, synaptic signaling, and microglial and astrocyte reactivity—the cell type-specific molecular pathology of ASD is unclear. Here we utilize high-throughput single-nucleus RNA sequencing (snRNA-seq) of snap-frozen post-mortem brain tissue from patients with ASD compared with tissue from neurologically normal age-matched controls to identify ASD-associated molecular changes in specific neural cell types across three cortical regions. Our findings show that synaptic signaling of upper-layer excitatory neurons is especially affected in ASD. Moreover, we find significant dysregulation of grey matter astrocyte-encoded genes responsible for glutamate uptake and modulation of synaptic plasticity. These findings indicate molecular pathological changes in upper-layer cortical neuron circuits and aberrant astroglia-neuronal interactions in ASD and identify potential cellular and molecular therapeutic targets.

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Poster

120. Autism Mechanisms and Therapeutic Development

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 120.23/E18

Topic: A.07. Developmental Disorders

Support: KAKEN 26250024
KAKEN 26290026

Title: Oxytocin secretion and social behavior in mice lacking Ca2+-dependent activator protein for secretion 2 (CAPS2)

Authors: *S. FUJIMA, R. MANIWA, Y. SANO, T. FURUICHI
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Abstract: CAPS2 regulates the exocytosis of dense-core vesicles containing peptidergic neurotransmitters, peptide hormones and monoamine neurotransmitters including catecholamine. We previously reported that CAPS2 knockout mice cause abnormalities in synaptic development and function as well as social behavior. There are some reports suggesting that CAPS2 is a candidate gene associated with autism spectrum disorder (ASD) due to findings in ASD patients with an aberrant increase in a rare CAPS2 alternative splicing variant and with genetic variations including copy number variation and single nucleotide variation in CAPS2 gene. However, it is yet unknown whether the abnormality of this protein is directly associated with autistic-like social disabilities. Interestingly, CAPS2 is expressed in oxytocin (OXT)-producing neurons in the hypothalamic paraventricular nucleus (PVN) of mice. OXT is a neuropeptide to regulate sociality, since administration of OXT could improve symptoms of ASD. Therefore, we got an idea that abnormality of CAPS2 affects OXT secretion, resulting in social disabilities. To address this question, we analyzed distribution of CAPS2-positive in OXT-positive neurons in the PVN. As a result, CAPS2 protein was largely localized in OXT neurons in the PVN and was also concentrated in the posterior pituitary, in which OXT is secreted from axon terminals of OXT neurons to blood vessels. Next, we compared plasma OXT levels between control and CAPS2 KO mice by OXT ELISA. Our preliminary data show that CAPS2 KO mice have a change in plasma OXT levels compared to those of control mice. These results suggest that CAPS2 plays a role in OXT secretion. Moreover, we intraperitoneally infused exogenous OXT to both control and CAPS2 KO mice and tried to compare the effect of OXT administration on the activities of social behavior. Our preliminary data suggest that exogenous OXT administration displayed a tendency to ameliorate impaired social behavior of CAPS2 KO mice. Taken together, we suggest that the CAPS2-mediated OXT secretion mechanism is associated with OXT-related social behavior.

Disclosures: S. Fujima: None. **R. Maniwa:** None. **Y. Sano:** None. **T. Furuichi:** None.

Poster

120. Autism Mechanisms and Therapeutic Development

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 120.24/E19

Topic: A.07. Developmental Disorders

Support: Intramural Program of NINDS, NIH

Title: Mechanistic contribution of defective presynaptic cargo transport to the autism-liked pathogenesis

Authors: G. XIONG, Y. XIE, T. SUN, M.-Y. LIN, X.-T. CHENG, S. LI, *Z. SHENG
Synaptic Function Section, NINDS, NIH, Bethesda, MD

Abstract: The formation, maintenance, and remodeling of synapses require targeted delivery of newly synthesized presynaptic cargoes from the soma to synapses. We previously identified syntabulin as a kinesin KIF5 adaptor that mediates axonal transport of active zone (AZ) precursors from the soma to presynaptic terminals, which is essential for synapse formation in developing neurons and synapse maintenance and remodeling in matured neurons. Autism spectrum disorders (ASDs) are highly inheritable neurodevelopmental disorders characterized by impaired social interaction, reduced communication, and increased repetitive behaviors. Mutations of genes that control synapses formation and maturation are emerging to be an important cellular basis of ASDs. It remains elusive whether impaired axonal transport of presynaptic proteins affects synapse formation and maturation and thus contributes to autism pathogenesis. Interestingly, a genetic study of ASD patients identified a *de-novo syntabulin* variant that changes a conserved residue within the KIF5-binding domain. We confirmed that this mutation impairs the syntabulin-KIF5 interaction. *Syntabulin* gene locates on 8q23.2 within the autism susceptibility loci 8q22-24. Thus, there is an urgent need to establish whether *de-novo syntabulin* variants are associated with the autism-linked phenotypes. To address this, we generated Nestin-Cre; *syntabulin*$^{loxP/loxP}$ conditional knock out (cKO) mice in which *syntabulin* is null in brain since embryonic stage. Time-lapse imaging shows that hippocampal neurons of *syntabulin* cKO mice display impaired axonal transport of AZ precursors from the soma to developing presynaptic boutons and reduced mature synapse density. Electron microscopy study reveals synapse loss of mature excitatory synapses and a decrease in docking vesicles per synapse in cKO hippocampus CA1 neurons. Moreover, *syntabulin* cKO mice show reduced frequency of miniature excitatory postsynaptic currents and impaired long-term synaptic plasticity in hippocampal Schaffer collateral-CA1 synapses. Consequently, the *syntabulin* cKO mice exhibit core autism-like traits including defective social recognition, reduced ultrasonic vocalizations, increased repetitive behavior, and impaired spatial learning and memory. Our study establishes for the first time that defective axonal transport contributes to the pathogenesis associated with synaptic and behavioral abnormalities in mice that bear similarities to autism patients, thus providing new cellular targets for therapeutic intervention. (This work is supported by the Intramural Program of NINDS, NIH)

Disclosures: G. Xiong: None. **Y. Xie:** None. **T. Sun:** None. **M. Lin:** None. **X. Cheng:** None. **S. Li:** None. **Z. Sheng:** None.

Poster

121. Autism Spectrum Disorders: Neural Correlates in Humans

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 121.01/E20

Topic: A.07. Developmental Disorders

Support: KAKENHI No. 17H05923

KAKENHI No. 15H01846

Title: Network analysis of resting-state fMRI from a multi-site database: A comparison between autism spectrum disorder patients and control subjects

Authors: *T. IIDAKA[1], T. KOGATA[2], Y. MANO[2]
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Abstract: Resting-state functional magnetic resonance imaging (rs-fMRI) is a unique tool for analyzing functional connectivity (FC) of brain networks in patients with autism spectrum disorder (ASD). Several network metrics obtained from rs-fMRI and FC have been used in previous studies, to elucidate the differences in network structures between ASD and control subjects (CTL). However, contradictory results have been reported which may be due to the small sample sizes used in these studies. To overcome this issue, we analyzed a large dataset of ASD and CTL obtained from the Autism Brain Image Data Exchange (ABIDE) II database. Only male subjects aged 5-29 years were included in the study. After excluding subjects with excessive head movement during scanning, analysis was conducted using data from 311 ASD and 311 CTL. The mean age±S.D. of ASD and CTL was 13.9±5.2 and 13.3±5.5 years, respectively. The rs-fMRI data were preprocessed using SPM12 and DPARSF software, and signal changes were extracted from 90 brain regions, defined using a standard template. The signal time courses were transformed into a 90 × 90 FC matrix for each subject, using in-house software. Multiple linear regression was used to exclude the effect of site difference in the matrices. The resultant FC matrices were proportionally thresholded from 5% to 50%, in increments of 5%. The average clustering coefficient (*C*), characteristic path length (*L*), global efficiency (*Eg*), modularity (*Q*), and small-worldness (*S*) were computed from the binarized networks using Brain Connectivity Toolbox. A 2-way repeated measures (RM) ANOVA was performed to compare the network metrics between the groups, and multiple linear regression analysis was used to investigate the relationship between the metrics and the subjects' age and IQ. The results of the RM ANOVA showed that the mean values for, *L*, *Eg*, *Q*, and *S* did not significantly differ between CTL and ASD (all p values > 0.05). The group difference in the mean value for *C* was marginally significant ($p = 0.053$, CTL > ASD). Additionally, the results of the regression analysis indicated that *C* at 35% density significantly and positively correlated with age in CTL ($r = 0.17$, $p < 0.01$) but not in ASD ($r = –0.01$, n.s.). The results from this study suggest that altered network structures in ASD as compared with CTL is not a robust finding, when a large dataset obtained from multiple sites is used. The relationship between *C* and age indicates that the network is efficiently clustered as advancing age in CTL, but not in ASD.

Disclosures: T. Iidaka: None. **T. Kogata:** None. **Y. Mano:** None.

Poster

121. Autism Spectrum Disorders: Neural Correlates in Humans

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 121.02/E21

Topic: A.07. Developmental Disorders

Support: Autism Speaks Weatherstone Predoctoral Grant 10616
NIMH Grant R01 MH111599

Title: Physiological response of central and peripheral stress systems during social interaction in children with autism spectrum disorder

Authors: *R. A. MUSCATELLO[1], B. A. CORBETT[2]
[1]Vanderbilt Brain Inst., Vanderbilt Univ., Nashville, TN; [2]Vanderbilt Univ. Med. Ctr., Nashville, TN

Abstract: Autism spectrum disorder (ASD) is characterized by social difficulties as well as frequently increased stress. The hypothalamic-pituitary-adrenal (HPA) axis and autonomic nervous system (ANS) are independent physiological systems but likely interact and influence socioemotional behavior in ASD. The objective of the preliminary study was to explore differences in arousal response between a pilot sample of children with ASD or typical development (TD) across multiple physiological systems, as atypical arousal response may contribute to increased risk for physical and mental health conditions. Twenty-seven children ages 10-12 years with ASD (n=14; 4 females) or TD (n=13; 4 females) participated in a social interaction paradigm with a novel peer. Physiological measures included respiratory sinus arrhythmia (RSA) as an index of parasympathetic regulation, pre-ejection period (PEP) to index sympathetic arousal, and salivary cortisol to assess HPA reactivity. Cardiac measures (RSA and PEP) were collected continuously throughout the paradigm, while cortisol was sampled before and several times following the interaction. Repeated measures ANOVAs assessed group differences in RSA, PEP, or cortisol across time (baseline, social interaction, recovery). Pearson product correlations explored possible relationships between physiological variables within groups. There were no significant between-group differences for any of the three physiological variables ($p>0.05$). There were significant within-group effects of time for cortisol ($F(2.27,52.36)=12.35$, $p<0.001$, partial $eta^2=0.35$) and for RSA ($F(3,75)=6.62$, $p<0.001$, partial $eta^2=0.21$). A diagnosis*time interaction was shown for PEP ($F(3,66)=3.88$, $p=0.01$, partial $eta^2=0.15$), such that the two groups differed in change in PEP over time, suggesting a different pattern of sympathetic response to social interaction. In TD children, decreased cortisol reactivity to social interaction was associated with an increase in parasympathetic regulation (RSA) ($r=-0.63$, $p=0.03$), while no significant relationship was seen in the ASD group. Additionally, RSA increased from baseline to social interaction ($t(12)=2.80$,$p=0.02$, Cohen's $d=0.78$) in the TD

group. Decreased HPA reactivity along with greater parasympathetic regulation likely calms the body and primes one to socially engage. Children with ASD did not show this adaptive relationship, which could impact social behavior and warrants further investigation. Larger planned studies will elucidate whether substantial differences exist in arousal response across stress systems in ASD and to what extent any differences may be maladaptive.

Disclosures: R.A. Muscatello: None. **B.A. Corbett:** None.

Poster

121. Autism Spectrum Disorders: Neural Correlates in Humans

Location: SDCC Halls B-H

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Topic: A.07. Developmental Disorders

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Title: Mental imagery of emotions and observation of facial expressions requires recruitment of the precuneus in autism spectrum disorder

Authors: *M. CASTELO-BRANCO[1], M. SIMÕES[2], R. MONTEIRO[2], J. ANDRADE[2], S. MOUGA[2,3], G. OLIVEIRA[3,4], P. CARVALHO[5]
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Abstract: The neural correlates of recognition of facial expressions in autism spectrum disorder (ASD) have remained widely debated. Moreover, most studies have used static images, which lack the inherent dynamical nature of emotion recognition and representation, and do not address emotional imagery. We conducted an experiment directly combining for the first time both the observation and mental imagery of dynamic facial expressions and investigated both scalp and electroencephalography (EEG) source responses to pure face expression contrasts. Our results show a distinct recruitment pattern of the Precuneus in autism spectrum disorder (n = 17), as

compared to healthy controls (n=17), for both observation and mental imagery conditions. These task-related differences showed to yield signals that could be robustly classified as ASD biomarkers. Accordingly, we conducted a machine learning procedure to automatically identify the group that a participant belongs to, based on its EEG activity during the mental imagery of the facial expressions. We compared several classifiers and achieved the accuracy of approximately 93% using 25 features of the signal, from the theta, beta and gamma bands. This level of accuracy shows that both observation and imagery-related signals reflect differences that can be used as biomarkers of impaired processing of dynamic facial expressions.

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Poster

121. Autism Spectrum Disorders: Neural Correlates in Humans

Location: SDCC Halls B-H

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Topic: A.07. Developmental Disorders

Support: Department of Defense AR140105
Arizona Biomedical Research Commission (ADHS16-00005488)
Arizona Alzheimer's Consortium

Title: Hippocampal anatomy age-related differences in autism spectrum disorder: Correlates with episodic memory and executive function

Authors: *C. RIECKEN[1], B. BRADEN[1], J. ALVAR[1], M. WALSH[1], L. BAXTER[2], C. SMITH[3]
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Abstract: Over the last 30 years, a rise in autism spectrum disorder (ASD) diagnoses has led to a large group of aging individuals with ASD. Little is known about how aging will affect these individuals' neuroanatomy, compared to the neurotypical (NT) population. Recently, our group found that older adults with ASD have reduced executive functioning and smaller hippocampi compared to NT adults (Braden et al., 2017). This project expanded on those findings by using Freesurfer 6.0 to perform a cross-sectional analysis of 12 hippocampal subfield volumes in adults with ASD (n = 53) and NT (n =39). Using ANOVA with discrete age cohorts (young-adult: ages 18-25 years, n=42; middle-age: ages 40-70 years, n=50), we predicted a larger magnitude of age-related hippocampal subfield volume difference in adults with ASD, compared to NT adults. Correlations were examined between subfield volumes and measures of executive function (Wisconsin Card Sorting Task [WCST] and Tower of London [ToL]), as well as episodic memory (Auditory Verbal Learning Task [AVLT]) with a false discovery rate of p=0.05 to

correct for multiple comparisons. All participants with ASD had their diagnosis confirmed with the Autism Diagnostic Observation Schedule-2. There were significant interactions in several hippocampal subfield volumes (e.g. left and right hippocampal tail, subiculum, CA1, molecular layer, dentate gyrus, CA4, and fimbria, right presubiculum, and left hippocampal amygdala transition area) such that adults with ASD showed larger age-related differences compared to NT adults. Correlations with behavioral measures showed that AVLT performance was related to many subfield volumes across all participants. There were no significant relationships between hippocampal subfield volumes and WCST or ToL performance that survived correction for multiple comparisons. Correlations between behavioral measures and hippocampal subfield volumes were not significantly different between ASD and NT participants. Results indicate that adults with ASD may be at risk for accelerated age-related hippocampal volume loss; however, this warrants confirmation with a longitudinal sample (in progress). Behaviorally, our study suggests hippocampal size is more strongly related to episodic memory than executive functioning performance, and that this relationship does not differ between adults with ASD and NT adults of either cohort age.

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Poster

121. Autism Spectrum Disorders: Neural Correlates in Humans

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Program #/Poster #: 121.05/E24

Topic: A.07. Developmental Disorders

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Waterloo Foundation 1167-1684 (McPartland)
Patterson Trust 13-002909 (McPartland)

Title: Neural response to dynamic and static faces in adults with autism spectrum disorder and typical development

Authors: *K. A. MCNAUGHTON, A. NAPLES, T. MCALLISTER, D. STAHL, S. HASSELMO, T. DAY, T. WINKELMAN, L. CHAN, J. MCPARTLAND
McPartland Lab., Child Study Center, Yale Univ., New Haven, CT

Abstract: Individuals with autism spectrum disorder (ASD) process social information differently than individuals with typical development (TD). When viewing static faces, adults with ASD exhibit slower latency of the N170, a face-sensitive event-related potential (ERP), than adults with TD (McPartland et al., 2004). Because social processing differences are most evident in real-world situations, dynamic faces that respond to participant gaze offer an opportunity to probe neural activity in a more ecologically valid context. This study examined ERPs to static and gaze-responsive dynamic faces in participants with ASD and TD (n=16, 2 female; n=25, 11 female, respectively) matched for nonverbal IQ and age. The static face paradigm featured photographs of faces and control stimuli preceded by a crosshair to direct attention to the eyes, nose, or mouth. The dynamic face paradigm featured faces that moved their mouths and eyes in response to participant fixation on the mouth or eyes of the face. ERPs were segmented to appearance of the face for the static faces and fixation to the eyes/mouth for the dynamic faces. For the static faces, there was a significant main effect of diagnosis, such that individuals with ASD had a slower N170 response than individuals with TD (F(1, 39)=6.04, p<0.05), specifically in the eye and nose condition (ps<0.05). For the dynamic faces, 7 individuals (n=6 ASD, n=1 TD) did not contribute enough artifact-free trials and were excluded from further analysis. Among the 34 included individuals, there was no effect of diagnosis (p>0.05). In individuals who viewed both paradigms, a longer N170 latency to static faces was associated with fewer artifact-free trials in the dynamic face condition (r(39)=-0.36, p<0.05). Furthermore, 3 of 6 individuals with ASD who did not contribute sufficient artifact-free data exhibited the longest N170 latencies to static faces. Although dynamic gaze-contingent social neuroscience paradigms offer an opportunity to probe neural markers of social interaction, these results suggest that participants who display atypical neural response to static faces are more likely to be excluded from naturalistic paradigms. These findings highlight the potentially informative nature of "lost data" in clinical groups and the importance of developing inclusive paradigms and analysis strategies to effectively capture neural markers of interpersonal interactions in ecologically valid contexts.

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Poster

121. Autism Spectrum Disorders: Neural Correlates in Humans

Location: SDCC Halls B-H

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Program #/Poster #: 121.06/E25

Topic: A.07. Developmental Disorders

Support: Brain/MINDS, AMED

Title: Emotion-independent alteration of cortical response in the left middle temporal voice area in autism spectrum disorder

Authors: *R.-I. HASHIMOTO[1], T. ITAHASHI[2], H. OHTA[2], C. KANAI[1], J. FUJINO[2], Y. AOKI[2], M. NAKAMURA[2], N. KATO[2]
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Abstract: Individuals with autism spectrum disorder generally have difficulty with communication with others, particularly in the form of speech. This difficulty is thought to derive from the fact that human voice conveys a diverse range of prosodic information including the speaker's identity, attitudes, and emotions, and that individuals with ASD are reduced in sensitivity to such prosodic information. In the typically-developed brain, there exist specialized regions in the lateral temporal cortex called temporal voice area (TVA) for processing information carried by the human voice. Although previous functional imaging studies have reported that the TVA is functionally altered in the ASD brain, the TVA is a complex of multiple subregions extending over the bilateral superior temporal gyrus (STG) and/or sulcus (STS) and it remains unknown which subregions in the TVA complex show altered response to human voice in ASD. Furthermore, it remains unknown which prosodic factors, such as types of emotion and emotional intensity, modulate altered TVA response in ASD.
In order to identify functional alterations in TVA in the ASD brain, we recorded cortical response to multiple types of emotional voice using fMRI. 29 male adults with ASD and 37 sex- and age-matched typically-developed (TD) controls participated in this study. During the scan, the participant passively listened to 3 types of emotional voice of "angry", "sad", and "happy" voice, together with "neutral" voice. For each emotional type, intensity of emotion was graded as 35%, 75%, and 100%. In total, 20 different stimuli were presented as to each of the 10 stimulus conditions (3 emotions x 3 intensity levels, and 1 neutral condition). After conventional preprocessing using SPM12, condition-specific effects were compared between the ASD and TD groups using the general linear model with the voxel-based threshold of uncorrected $P < 0.001$. We identified a cluster in the middle part of the left STS that showed consistently reduced responses to all the 10 conditions of emotional voice, including the neutral voice. The center of the significant cluster of each contrast was highly invariant with a shift of no more than 2 mm in either of the direction. Furthermore, the magnitude of group difference was highly comparable across types of emotion and levels of emotion intensity ($P > 0.5$). These results suggest that functional alterations of TVA are localized in the left middle STS in ASD and that its alteration reflects reduced cortical response to human voice in general, regardless of emotion and its intensity.

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Poster

121. Autism Spectrum Disorders: Neural Correlates in Humans

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 121.07/E26

Topic: A.07. Developmental Disorders

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CTSA Grant Number UL1 RR024139 (McPartland)
NIMH K23 MH086785 (McPartland)
NIMH R21 MH091309 (McPartland)
Autism Speaks Translational Postdoctoral Fellowship (Naples)
Waterloo Foundation 1167-1684 (McPartland)
Patterson Trust 13-002909 (McPartland)

Title: Neural response to direct and averted gaze in children with autism spectrum disorder during a gaze contingent social simulation

Authors: *M. R. ALTSCHULER, A. NAPLES, T. C. DAY, K. A. MCNAUGHTON, T. WINKELMAN, D. STAHL, S. HASSELMO, T. HALLIGAN, B. LEWIS, J. WOLF, E. JARZABEK, K. ELLISON, J. MCPARTLAND
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Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by difficulties in social communication, such as maintaining reciprocal eye contact. Studies with static faces have demonstrated that direct and averted gaze elicit atypical neural responses in ASD, yet electrophysiological markers of gaze during reciprocal social interactions are not well understood. Moreover, though executive function (EF) impairment is common in ASD and associated with social difficulties, the influence of EF-related symptoms on neural response to gaze in ASD has not been studied. This study utilized a gaze-contingent paradigm to explore event-related potentials (ERPs) and EF-related symptoms in children with ASD and typical development (TD). During concurrent recording of electroencephalography (EEG) and eye-tracking (ET), children with ASD (n=53, age 5-17 years, 9 female) and TD controls (n=51, age 8-17 years, 23 female) matched on age viewed faces that responded to their gaze by looking back (direct) or looking away (averted). EEG was recorded using a 128-channel net, and ET was collected using an Eyelink-1000 remote camera system. The amplitude and latency of the N170, a neural marker of face processing, and of the P100, a neural marker of directed attention to a target, were analyzed. EF problems were measured with the Child and Adolescent Symptom Inventory Attention Deficit/Hyperactivity (ADHD) Subscale. Across groups, N170 amplitude was greater over right hemisphere (RH) compared to left hemisphere (LH), $F(1,102)=18.40$, $p<0.01$, and to direct gaze compared to averted gaze, $F(1,102)=5.54$, $p<0.05$. There was a

significant three-way interaction between hemisphere, condition, and diagnosis, $F(1,102)=4.41$, $p<0.05$; N170 amplitude to direct gaze was right lateralized in the ASD group, $F(1,52)=14.04$, $p<0.01$, and left lateralized in the TD group, $F(1,50)=5.63$, $p<0.05$. While the P100 did not differ across condition or hemisphere, children with ASD with greater EF impairment showed more positive LH P100 amplitudes to averted gaze, $r(51)=.46$, $p<0.01$. Results contribute to literature demonstrating modulation of face-related ERPs by direct versus averted gaze and reveal atypical lateralization of this neural response in ASD. In addition, a distinct neural marker of directed attention to a target was associated with EF-related symptoms in ASD but not TD. This pattern of findings suggests that EF difficulties may contribute to clinically observed difficulties with eye contact in ASD and may account, in part, for variability in neural response to faces in ASD. Future studies should account for EF as an influential factor in social neuroscience research in ASD.

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Poster

121. Autism Spectrum Disorders: Neural Correlates in Humans

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Topic: A.07. Developmental Disorders

Support: U.S. Department of Health and Human Services UA3 MC11054
Autism Speaks Autism Intervention Research Network on Physical Health
University of Missouri School of Medicine Summer Research Fellowship

Title: Associations between nutritional intake and gastrointestinal symptoms in autism spectrum disorder

Authors: *B. FERGUSON[1], S. FAMULINER[2], K. DOVGAN[3], D. SEVERNS[4], D. Q. BEVERSDORF[5]
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Abstract: Many individuals with autism spectrum disorder (ASD) have significant gastrointestinal (GI) symptoms, but the etiology is currently unknown. Studies have shown conflicting evidence on whether there are nutritional alterations in the various diets of individuals with ASD, which may be associated with GI functioning. However, little is known

about the relationship between nutritional intake—including gluten and casein—and GI symptomatology in ASD. The objective of this study is to assess relationships between GI symptoms and nutrient intake from diet in those with ASD. A total of 120 individuals with ASD aged 6-18 participated in the study. A food frequency questionnaire for nutritional intake over the past month was completed by the parents. The USDA Food Composition Database was utilized to provide nutritional data for the food items consumed by each participant. GI symptomatology was assessed using the Questionnaire on Pediatric Gastrointestinal Symptoms, Rome III. Upper GI tract symptoms were significantly associated with total dietary fiber intake and Vitamin B6 intake. However, this relationship could be bidirectional, and longitudinal studies are needed to determine causality. There were no significant associations between lower GI tract symptoms and dietary intake. Exploratory analyses will be discussed regarding associations between gluten, casein, and GI symptoms. This study supports the hypothesis that there may be other factors associated with the lower GI tract disorders in ASD, such as increased stress response. Further studies are needed to explore non-diet associations with GI disorders in ASD.

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Poster

121. Autism Spectrum Disorders: Neural Correlates in Humans

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Program #/Poster #: 121.09/E28

Topic: A.07. Developmental Disorders

Support: NIMH RO1MH100028

Title: Sex differences in atypical sensory processing and its relation to salience network functional connectivity in autism spectrum disorder

Authors: *K. K. CUMMINGS, S. A. GREEN, K. E. LAWRENCE, L. HERNANDEZ, M. DAPRETTO
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Abstract: Youth with autism spectrum disorder (ASD) show higher rates of sensory over-responsivity (SOR) and atypical sensory seeking than typically developing (TD) youth These atypical behaviors are related to abnormal connectivity in the Salience Network (SN), suggesting that sensory stimuli are overly salient for ASD youth. This research is mostly conducted in males, offering little understanding of sex differences in brain connectivity underlying sensory abnormalities, which can provide insight into the known gender bias in the diagnosis of ASD. Resting-state fMRI was conducted in 78 ASD (35 M/43 F) and 103 TD (48 M/55 F) youth, aged

8-17; the right anterior insula (AI) was used as the seed for the SN. In ASD females, SOR was related to increased connectivity between the SN and auditory language areas as compared to males, for whom SOR was instead related to increased SN connectivity with somatosensory cortex. In ASD females, abnormal sensory seeking behavior was related to increased SN connectivity with prefrontal regions, compared to increased SN connectivity with somatosensory/subcortical areas in ASD males. Results suggest that the mechanisms underlying sensory processing issues in ASD are sex-specific, despite similar behavioral symptoms. Abnormal sensory processing may be related to regulatory strategies in girls with ASD vs. more subcortically driven and less well-regulated processes in boys. Implications for sex differences in ASD diagnosis and presentation are discussed.

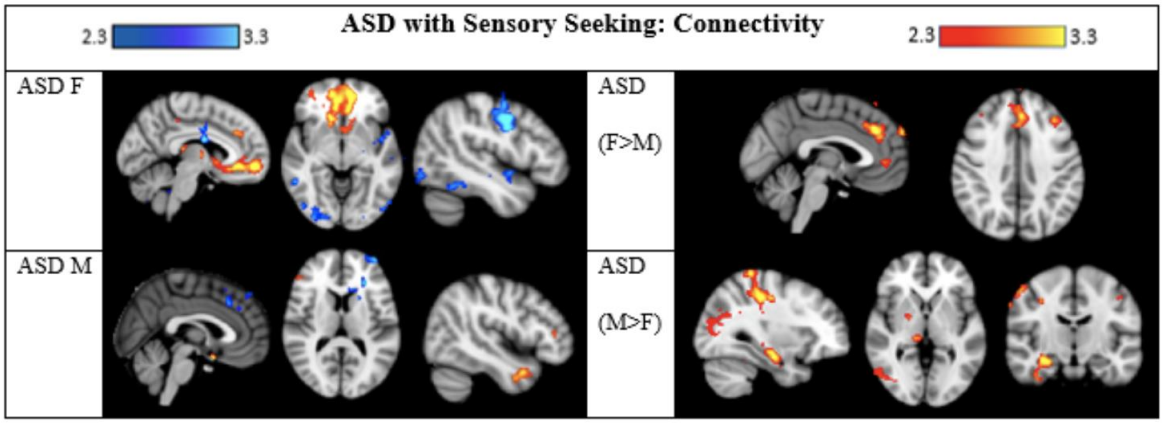

Figure 2. ASD with Sensory Seeking: Connectivity

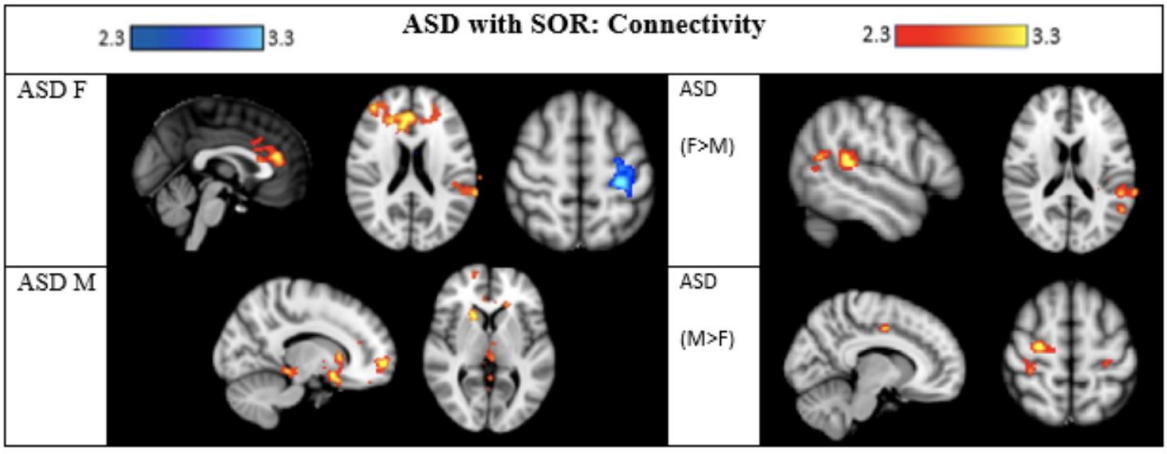

Figure 1. ASD with SOR: Connectivity

Disclosures: K.K. Cummings: None. **S.A. Green:** None. **K.E. Lawrence:** None. **L. Hernandez:** None. **M. Dapretto:** None.

Poster

121. Autism Spectrum Disorders: Neural Correlates in Humans

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Pfizer Inc. (no award number)
Shire Development LLC (no award number)

Title: Dissociating regional gray matter density and volume in children and adolescents with autism spectrum disorder

Authors: L. D. YANKOWITZ[1,2], B. E. YERYS[2,1], *J. D. HERRINGTON[2,1], J. PANDEY[2,1], R. T. SCHULTZ[2,1]
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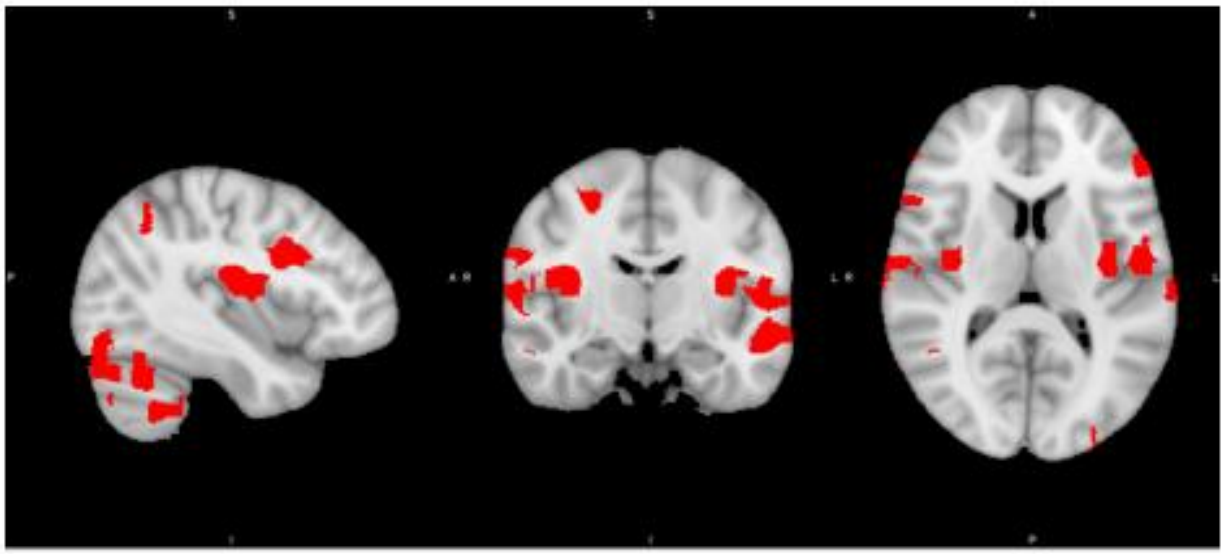
Abstract: Regional differences in gray matter structure have previously been explored in autism spectrum disorder (ASD) compared to typically developing controls (TDC) using voxel-based morphometry (VBM), often with regionally inconsistent results. VBM methods generally represent a combination of gray matter density (GMD) and gray matter volume (GMV). Combining these measures may obscure detection of underlying biological differences associated with ASD in either one of them, which may vary independently of one another.
In this study, we investigate whether regional differences in GMD or GMV are observed in 6-19 year-olds with ASD (n=211, 80.1% male, IQ 47-154) and TDC (n=179, 71.5% male, IQ 67-155). High-resolution T1-weighted anatomical images were collected on a single MRI scanner. A recently published GM parcellation of 1625 parcels was transformed into native space for each subject. GMD and GMV values were extracted from probabilistic segmentations per parcel. Analyses were conducted for each parcel using generalized additive models, corrected for false discovery rate ($q<0.05$).
Within the TDC sample, we replicate sex differences of greater GMD but smaller GMV in females compared to males, and age effects of GMD increasing and GMV decreasing across adolescence. However, no GMD differences were observed between groups, either in individual parcels or the whole brain mean GMD. Increased GMV was observed in ASD compared to TDC

when controlling for IQ, both at the level of the whole brain (total GMV) and in 94 parcels (6% of total parcels). Parcels with greater GMV included bilateral cerebellum, insula, superior and middle temporal cortex, anterior cingulate, paracingulate gyrus, orbital frontal cortex, occipital fusiform cortex, and inferior frontal gyrus (pars triangularis). These results show that regional volumetric differences between ASD and TDC groups may be more important than density differences, and underscore the importance of examining volume and density separately.



Parcels showing significant GMV effects, ASD>TDC controlling for age, sex, and IQ, corrected for multiple comparisons q<0.05.

Disclosures: L.D. Yankowitz: None. **B.E. Yerys:** None. **J.D. Herrington:** None. **J. Pandey:** None. **R.T. Schultz:** None.

Poster

121. Autism Spectrum Disorders: Neural Correlates in Humans

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Program #/Poster #: 121.11/E30

Topic: A.07. Developmental Disorders

Support: Grant-in-aid for scientific research,MEXT,No. 15H01581
Grant-in-aid for encouragement of young scientists,MEXT,No. 26870333

Title: Abnormal oscillatory patterns elicited by audio-visual movies in autism spectrum disorder with abnormal sensory sensitivity: A magnetoencephalographic study

Authors: *J. MATSUZAKI[1], K. KAGITANI-SHIMONO[1], S. AOKI[1], Y. KATO[1], R. HANAIE[1], M. NAKANISHI[1], A. TATSUMI[1], T. YAMAMOTO[1], K. TOMINAGA[1], Y. NAGAI[2], I. MOHRI[1], M. TANIIKE[1]
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Abstract: Aim: Abnormal sensory sensitivity is the common characteristics associated with autism spectrum disorder (ASD) and interrupts their adaptive behavior. We reported that abnormal auditory sensitivity in ASD was correlated with delayed and increased M50, M100 responses in the auditory cortex resulting from atypical myelination process and increased MMF responses which may resulting from the abnormal temporo-frontal network (Matsuzaki, Shimono-Kagitani et al., 2012, 2014, 2017). As we often experience audio-visual (AV) multisensory stimulus in our daily life, the present study has highlighted AV multisensory processing, and investigated the association between the oscillatory patterns and abnormal sensory sensitivity in children with ASD. **Methods:** We used magnetoencephalography (MEG) to measure cortical responses during AV multisensory movies, in twenty-three high-functioning boys with ASD (thirteen with abnormal AV sensory sensitivity: 10.00 ± 2.62 years, ten without it: 10.92 ± 1.84 years) and twenty-three typically developing boys (TD, 9.96 ± 2.67 years). Abnormal sensory sensitivity was assessed by the Japanese version of the Sensory Profile (SP). We determined the oscillatory patterns of each brain regions using brainstorm (http: // neuroimage. usc.edu / brainstorm). Written informed consent was obtained from the parents of all the participants. This study was approved by the Institutional Review Board of Osaka University Hospital. **Results:** The ASD with abnormal AV sensory sensitivity groups showed increased theta to low gamma ERS/D patterns compared with the ASD without it and TD group in the bilateral superior temporal sulcus (STS) and right temporal ($p < .05$). On the other hand, ASD group showed decreased theta to low gamma ERS/D patterns compared with TD groups in right ventrolateral prefrontal cortex (VLPFC, $p < .05$). **Discussion:** It has been known that gamma band oscillations were related with negative feedback inhibition of pyramidal cells by Gamma aminobutyric acid (GABA) ergic interneurons and glutamate receptor-feedforward excitatory inputs. In addition, theta to beta oscillatory patterns have been implicated in top-down sensory inhibitory processing associated with thalamic sensory gating system. We found abnormal oscillatory patterns in the bilateral STS, right temporal and right VLPFC which is engaged AV multisensory processing and motion perception including primary sensory cortex. Based on the results, we suggested neurophysiological mechanism underlying abnormal AV sensory abnormalities might be associated with GABAergic dysfunction and impairment of sensory gating system in children with ASD.

Disclosures: K. Kagitani-Shimono: None. **S. Aoki:** None. **Y. Kato:** None. **R. Hanaie:** None. **M. Nakanishi:** None. **A. Tatsumi:** None. **T. Yamamoto:** None. **K. Tominaga:** None. **Y. Nagai:** None. **I. Mohri:** None. **M. Taniike:** None.

Poster

121. Autism Spectrum Disorders: Neural Correlates in Humans

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Topic: A.07. Developmental Disorders

Support: NIMH R01 MH100173
UL1 RR024139
Autism Science Foundation Undergraduate Summer Research Grant

Title: Children with autism and sleep problems show abnormal regulation of resting EEG

Authors: *T. WINKELMAN, A. NAPLES, M. ROLISON, T. C. DAY, K. A. MCNAUGHTON, S. HASSELMO, T. MCALLISTER, K. ELLISON, E. JARZABEK, B. LEWIS, J. WOLF, J. MCPARTLAND
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Abstract: Sleep problems are a common feature of autism spectrum disorder (ASD). Forty to eighty percent of children with ASD experience sleep problems; however, little is known about its relationship with waking brain activity. Resting EEG provides a measure of baseline brain activity sensitive to age and sleepiness in children with typical development (TD). For example, theta power is especially sensitive to sleep deprivation. This research study examines relationships among sleep problems and resting EEG power in children with ASD and TD. In contrast to prior research focusing on pre-defined frequency bands, we also examined frequency as a continuous metric. Children with ASD (n=16) and TD (n=12) between the ages of 7 and 15 were clinically characterized and parents completed the Children's Sleep Habits Questionnaire (CSHQ), a 33-item measure of sleep problems, validated for children with ASD. Two minutes of eyes open resting EEG was collected from all participants. EEG data was filtered, re-referenced to a common average, segmented into 2-second epochs. Trials with movement artifact were rejected, and children with <20 seconds of data were excluded from analyses. EEG power was extracted from 3 to 12 Hz, using bin widths of 1 Hz (named below based on the lowest value in the range), along with conventional bands theta (4-8 Hz) and alpha (8-13 Hz). Participants were grouped by diagnosis (ASD or TD) and sleep group (sleep problems or normal sleep; defined by a whole sample median split of CSHQ score). A two-way MANOVA of all frequency ranges revealed a statistically significant difference in power between groups at 8 Hz [F(1,24), p=0.016]; post hoc analyses controlling for multiple comparisons with Tukey's HSD showed children with TD and sleep problems have greater 8 Hz power than children with TD and normal sleep (p=0.026) and children with ASD and sleep problems (p=0.036). No differences were observed in the theta or alpha range. The difference in power in the 8 Hz range between TD children with and without sleep problems is consistent with literature noting an endogenous

component of sleepiness in resting EEG. However, the absence of a significant difference between sleep groups in children with ASD suggests an atypical regulation of resting EEG power in response to decreased sleep. Results demonstrate the importance of exploring resting EEG data outside of conventional frequency bands when examining clinical populations.

Disclosures: T. Winkelman: None. **A. Naples:** None. **M. Rolison:** None. **T.C. Day:** None. **K.A. McNaughton:** None. **S. Hasselmo:** None. **T. McAllister:** None. **K. Ellison:** None. **E. Jarzabek:** None. **B. Lewis:** None. **J. Wolf:** None. **J. McPartland:** None.

Poster

121. Autism Spectrum Disorders: Neural Correlates in Humans

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Topic: A.07. Developmental Disorders

Support: 5T32MH019112-27
K01MH096091
K08 MH085100
R21MH098204
R01DC008871
U54 HD086984
P30HD026979

Title: Children with autism spectrum disorder demonstrate regionally altered resting-state alpha-to-gamma phase-amplitude coupling as well as resting-state band-passed power

Authors: *R. G. PORT[1], S. LIU[2], L. BLASKEY[3], J. C. EDGAR[2], T. P. ROBERTS[2], J. BERMAN[2]
[1]Neurosci. Grad. Group, Univ. Of Pennsylvania, Philadelphia, PA; [2]Radiology, [3]Ctr. for Autism Research/Neuropsychology Service, Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: Children with Autism Spectrum Disorder (ASD) exhibit alterations to resting-state (RS) neural synchrony as compared to typically developing (TD) controls, as indexed by changes to low-frequency power as well as alpha-to-gamma phase-amplitude coupling (PAC). The current study extends previous findings by examining brain neural activity in brain space in a large sample (~200) of children age 5.94 to 15.7 years old.
Data were collected using a 275-channel MEG system (VSM MedTech Inc). Children were scanned in a supine position and were instructed to lie still with their eyes gently closed during a at least 2-minute resting-state exam. MEG data were processed using BESA Research 6.1 (MEGIS Software GmbH), with a 15 regional source model applied to project each individual's raw MEG surface data into brain source space. Regional RS power spectra were calculated using

fast Fourier transformations, and subsequently parsed into canonical frequency bands. Linear Mixed-Effects Models examined RS power, testing for main effects of Diagnosis, Frequency-band and Region as well as their interactions. Age was used as a covariate and Holm's multiple comparisons correction was applied.
PAC was calculated using Tort and colleagues' method (2010), with additional variable bandwidth filtering applied. A PAC comodulogram was obtained via pairing low-frequency phase (3-15Hz, 1Hz steps) to high-frequency amplitude (20-100Hz, 5Hz steps). Clusters were identified and masked based on cluster size to provide a familywise corrected p=0.05.
To compute stable PAC estimates, any subject with less than 70 seconds of artifact free data was removed. Additionally, subjects that exhibited outlier (3 standard deviations (SD) from group mean) RS power or PAC were also rejected. 63 TD and 128 ASD participants remained for analysis. There was no group difference in either the amount of artifact-free data (TD=172.7±10.5s (mean ± SD); ASD156.3±6.6s; p>0.05) or age (TD=9.3±2.3 years old; ASD 10.0±2.12 years old; p>0.05).
ASD demonstrated increased low-frequency power at frontal regions as well as decreased occipital midline alpha than TD. Whereas at central midline locations higher alpha-gamma PAC was observed in ASD versus TD, at central as well as temporal parietal right-hemisphere locations higher alpha-to-gamma PAC was observed in TD versus ASD. Temporal parietal right-hemisphere PAC exhibited the largest effect size, and also maturational decreases (p<0.001) with differential rates of maturation between TD and ASD (p<0.02) As such, children with ASD demonstrate regionally specific and heterogeneous alterations to RS alpha-gamma PAC, as well as RS power.

Disclosures: R.G. Port: None. **S. Liu:** None. **L. Blaskey:** None. **J.C. Edgar:** None. **T.P. Roberts:** None. **J. Berman:** None.

Poster

121. Autism Spectrum Disorders: Neural Correlates in Humans

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Program #/Poster #: 121.14/E33

Topic: A.07. Developmental Disorders

Support: NIH Grant MH107802

Title: Atypical brain connectivity of pSTS in toddlers with autism spectrum disorders

Authors: S. REYNOLDS, A. C. LINKE, B. CHEN, C. FONG, L. OLSON, M. KINNEAR, *I. FISHMAN
San Diego State Univ., San Diego, CA

Abstract: Introduction Children with autism spectrum disorders (ASDs) display deficits in processing biological motion (BM), a fundamental aspect of perceiving and understanding others. There is evidence of atypical brain activation and connectivity associated with BM processing in ASDs, although it is not known how early in the first years of life the aberrant neural substrates of BM can be detected. The posterior superior temporal sulcus (pSTS) has been implicated in BM, as well as in language processing. Using resting state fMRI (rs-fMRI), we investigated pSTS to whole-brain functional connectivity (FC) in 16 to 33-month-old toddlers with and without ASDs. Methods Toddlers' motor, language and social development, and autism symptoms were assessed with the Mullen Scales of Early Learning, Vineland Adaptive Behavior Scales, and Autism Diagnostic Observation Schedule II. T1-weighted anatomical MRI and two rs-fMRI runs were acquired during natural sleep from 11 toddlers with ASDs (5F; 26±4m) and 14 typically developing (TD) toddlers (4F; 23±5m). Following standard preprocessing of the fMRI data, seed to region of interest (ROI) FC analyses were performed between left and right pSTS (ROIs derived from NeuroSynth) and 91 cortical ROIs from the Harvard-Oxford atlas. Group differences in FC between the pSTS seeds and cortical ROIs were tested with two-sample t-tests. Results Toddlers with ASDs displayed greater connectivity between the left pSTS and language regions (right and left inferior frontal gyrus (IFG); uncorr. $p<.01$), but weaker connectivity between the left pSTS and visual processing areas (right supracalcarine cortex; uncorr. $p<.01$), relative to TD toddlers. While left pSTS-left IFG connectivity was positively correlated with expressive language in TD toddlers ($r=0.67$, $p=.009$), it was positively correlated with fine motor scores in ASD toddlers ($r=.67$, $p=.041$). Conclusions The pattern of overconnectivity with language regions and underconnectivity with visual cortex observed in the ASD group may suggest that pSTS region is less differentiated or specialized for BM processing in toddlers with ASDs compared to TD toddlers. Paradoxical correlations with behavioral indices suggest that pSTS may have a different functional role in ASDs, with left pSTS contributing to the emerging language system in TD but not in ASD toddlers.

Disclosures: S. Reynolds: None. **A.C. Linke:** None. **B. Chen:** None. **C. Fong:** None. **L. Olson:** None. **M. Kinnear:** None. **I. Fishman:** None.

Poster

121. Autism Spectrum Disorders: Neural Correlates in Humans

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 121.15/E34

Topic: A.07. Developmental Disorders

Support: NIMH

Title: Atypical salience network connectivity underlying early emotion dysregulation in toddlers with ASD

Authors: *C. IBARRA[1], B. CHEN[1], S. REYNOLDS, 92115[1], L. OLSON[4], C. FONG[2], A. C. LINKE[3], M. KINNEAR[1], I. FISHMAN[1]
[1]San Diego State Univ., San Diego, CA; [2]San Diego State Univ., San Diego State University, CA; [3]Psychology, San Diego State Univ., San Diego, CA; [4]Joint Doctoral Program in Clin. Psychology, SDSU / UC San Diego, San Diego, CA

Abstract: Introduction:
There is substantial evidence demonstrating that individuals with autism spectrum disorders (ASDs) have increased prevalence of dysregulated emotions and affective psychopathology, including anxiety disorders. The anterior insula (AI) is a brain region involved in emotional awareness and is considered to be a part of the salience network (SN). The salience brain network plays a central role in detecting and orienting to relevant internal and external stimuli, an impaired ability in ASDs. However, little is known about functional network organization of the SN early in life in ASDs. Using resting state fMRI (rs-fMRI), we examined SN functional connectivity patterns in toddlers with and without ASDs.
Methods:
Toddlers' behavioral, social and emotional development was assessed using the Childhood Behavior Checklist (CBCL), Mullen Scales of Early Learning, and autism symptoms were quantified with the Autism Diagnostic Observation Schedule (ADOS-2). T1-weighted anatomical MRI and two rs-fMRI runs were acquired during natural sleep from 11 toddlers with ASDs (4 females; age 26±4 months) and 15 typically developing (TD) toddlers (5 females; age 23±5 months). Following standard preprocessing of fMRI data, seed to whole-brain functional connectivity (FC) analyses were performed with right anterior insula (rAI) as a seed.
Results:
Toddlers with ASDs displayed weaker connectivity between right AI and left primary auditory cortex (Heschl's gyrus), but greater connectivity with a language region (right planum temporale; all at uncorr. $p<.05$). An additional cluster of underconnectivity was identified in right cerebellum. Behavioral correlational analyses revealed negative correlation between rAI-left auditory cortex underconnectivity and CBCL Emotional Reactivity score in toddlers with ASDs ($r=-0.65$, $p=0.058$).
Conclusions:
The pattern of atypical connectivity was identified between the rAI and auditory and language regions in toddlers with ASDs. Further, behavioral correlations indicate that this atypical connectivity is associated with greater emotional reactivity in toddlers with ASDs, thus impacting their emotional regulation. Overall, these findings indicate that, in ASDs, atypical SN connectivity patterns may be an early indicator of emotion dysregulation.

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Poster

121. Autism Spectrum Disorders: Neural Correlates in Humans

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 121.16/E35

Topic: A.07. Developmental Disorders

Title: Transient improvement in autism symptoms following ketamine anesthesia

Authors: *E. T. CHOW[1], M. FAZAL[2], A. W. ZIMMERMAN[3], M. L. BAUMAN[4]
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Abstract: Background: Anecdotally, there have been cases of individuals with autism spectrum disorder (ASD) whose ASD symptoms improve temporarily after anesthesia administration. However, a MeSH search in Pubmed of "anesthesia" and "autism spectrum disorder" revealed no case reports and a Google Scholar search revealed two case reports. In particular, ketamine has recently been proposed as a potentially novel treatment for neuropsychiatric conditions, and efforts have been made to investigate its use as a treatment for ASD, including a current randomized clinical trial at Children's Hospital Medical Center in Cincinnati. We present here a patient with ASD who experienced transient improvement after each of three ketamine administrations. **Case report**: Patient is a 35-year-old non-verbal female with atypical ASD, diagnosed with a urea cycle disorder, who underwent general anesthesia with ketamine for three different procedures: at age 4 for a colonoscopy, at age 8 for a tooth abscess, and third at age 11 for a muscle biopsy and lumbar puncture. Upon emergence from anesthesia, the patient began to speak. A striking example of speech occurred after the lumbar puncture in which the patient responded to a verbal command not to sit up by responding "lie down." However, by the time the patient fully emerged from anesthesia and was out of the recovery room, she was non-verbal again. In all three cases, verbalizations were witnessed by recovery room staff as well as parents. **Conclusion**: This case is unique in that the transient improvement was reproducible and not limited to one instance. Ketamine has been shown to be both neurotoxic and neuroprotective. A Pubmed search of "ketamine" and "autism" reveals two studies showing that ketamine reduces inflammatory markers in patients and improves sensorimotor function in Rett syndrome mouse models, but also one study showing that ketamine worsens joint visual attention in common marmosets. The effect that ketamine has on ASD is complex and may be dose dependent, potentially indicating a narrow therapeutic index - a study in which ketamine was administered at very high doses in children with ASD revealed decreased cerebellar glucose utilization. The relationship between ketamine and autism is one that bears further investigation in order to better understand possible mechanisms by which ketamine may result in the temporary alleviation of ASD symptoms.

Disclosures: E.T. Chow: None. **M. Fazal:** None. **A.W. Zimmerman:** None. **M.L. Bauman:** None.

Poster

121. Autism Spectrum Disorders: Neural Correlates in Humans

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 121.17/E36

Topic: A.07. Developmental Disorders

Support: 5R01MH106520

Title: The regulation of neural responses in autism spectrum disorder

Authors: *T. KOLODNY[1], R. MILLIN[1], M.-P. SCHALLMO[2], A. M. KALE[1], R. A. BERNIER[1], S. O. MURRAY[1]
[1]Univ. of Washington, Seattle, WA; [2]Univ. of Minnesota, Minneapolis, MN

Abstract: A unifying theme of numerous proposals about the pathophysiology of autism spectrum disorder (ASD) is that a pervasive alteration in neural excitability underlies ASD development. However, experimental evidence for this has been inconsistent, with results from animal models of ASD demonstrating both increased and decreased excitability. Here we utilize the well-established hierarchical structure of human visual motion processing to test a novel hypothesis that is consistent with apparent contradictory evidence: *that neural excitability in ASD differ between adjacent stages of cortical processing.*
We used functional magnetic resonance imaging (fMRI) to measure neural responses to visual motion stimuli in early visual cortex (EVC) and in human MT complex (hMT+), a motion selective region in the lateral occipital lobe. Young adults with ASD and neurotypical controls (NT) matched for age, sex and IQ, were presented with foveally-located drifting sine-wave gratings, while their attention was diverted to a demanding central fixation task.
Neural response magnitudes of ASD subjects were lower than response magnitudes of NT subjects in EVC, but substantially higher than response magnitudes of NT subjects in MT. Moreover, examining individual differences in response amplitudes revealed that among ASD subjects the responses in the two regions were highly negatively correlated: individuals with low responses in EVC had high responses in MT. The magnitudes of attenuation in EVC and of amplification in MT were both correlated with ASD symptom severity.
A possible underlying mechanism for this striking pattern of responses in ASD could be that regulatory processes are amplifying the attenuated signal from EVC while it travels to MT, resulting in over-compensation in the subsequent response. Alternatively, it is possible that amplified responses in MT are suppressing the activation of EVC, resulting in abnormally low responses in these earlier stages of processing. Overall, our results indicate that neural excitability in ASD differs between cortical regions, reconciling seemingly contradictory

previous findings. Moreover, our results suggest that alterations in excitability lie in the inter-regional functional connections in the cortex. Whether this alteration is dependent on feedforward or feedback projections within the visual system remains an open question.

Disclosures: T. Kolodny: None. **R. Millin:** None. **M. Schallmo:** None. **A.M. Kale:** None. **R.A. Bernier:** None. **S.O. Murray:** None.

Poster

121. Autism Spectrum Disorders: Neural Correlates in Humans

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 121.18/E37

Topic: A.07. Developmental Disorders

Support: JSPS KAKENHI Grant Number 15K09843

Title: White matter microstructure of autism spectrum disorder and attention deficit hyperactivity disorder

Authors: *H. OHTA[1], T. ITAHASHI[2], Y. AOKI[4], M. NAKAMURA[3], J. FUJINO[3], C. KANAI[3], A. IWANAMI[3], N. KATO[3], R.-I. HASHIMOTO[3]
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Abstract: Background: Previous researches have shown high rate of comorbidity (30-50%) between attention deficit hyperactivity disorder (ADHD) and autism spectrum disorder (ASD) . Some researches reported that impaired structural brain connectivity was related to the core features of ASD and ADHD. However, only a few studies directly compared the white matter microstructure between ASD and ADHD. Objectives: The aim of the present study is to reveal the commonality and difference of white matter microstructure between adults with ASD and those with ADHD using Diffusion Tenor Imaging (DTI). Methods: A total of 196 adults participated in this study; 68 Normal Controls (NC), 83 ASD, 45 ADHD. Two medical specialists diagnosed ASD and ADHD according to DSM-5 criteria. ADOS-2 was conducted on 71 out of 83 ASDs. Individuals who have mental retardation were excluded from this study. MRI scans were conducted to all participants. DTI data were processed using programs in the FMRIB Software Library (FSL) version 5.0. TBSS (Tract-Based Spatial Statistics) was used for voxelwise statistical analysis. The statistical threshold was defined at $p < 0.05$ (corrected for multiple comparisons). Age and gender were included as covariates. Results: Lower FA of corpus callosum, bilateral superior and middle cerebellar peduncle, bilateral cerebral peduncle, right superior temporal cortex was found in ASD compared to HC. Lower FA of bilateral superior and middle cerebellar peduncle and right superior temporal cortex was found in ADHD

compared to HC. Although the differences were disappeared after controlling for multiple comparison, lower FA of genu of corpus callosum in ASD and right cingulum in ADHD comparing directly between the two disorders. Conclusions: The present study specified common white matter alternations between ASD and ADHD in cerebellum peduncle and white matter under superior temporal cortex. ASD specific alterations were observed in corpus callosum and cerebral peduncle.In the direct comparison of ASD and ADHD, lower FA values were found in genu of corpus callosum in ASD and in right cingulum in ADHD. To reveal more complete picture of association between ASD and ADHD, further studies are needed by adopting not only the categorical but also dimensional approach.

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Poster

121. Autism Spectrum Disorders: Neural Correlates in Humans

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 121.19/E38

Topic: A.07. Developmental Disorders

Title: The neurological motor deficits as an endophenotype of autism shared by affected and unaffected siblings

Authors: *M. FABBRI-DESTRO[1,2], A. NUARA[3], V. GIZZONIO[4], G. RIZZOLATTI[5], P. AVANZINI[5]
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Abstract: A large body of evidence reports that, beyond the core socio-communicative deficits, autism is accompanied by an impairment of motor functions, including abnormalities in coordination, gait, praxis, action preparation and imitation. However, at present it is still an open question whether motor abnormalities may be found also in unaffected siblings. The aim of our study was to profile the neuro-motor abilities of typically development children (TD), autistic children (ASD) and their unaffected siblings (SIB), thus, providing an overall picture of neurological impairment of ASD and, possibly, identifying motor endophenotypes typical of autism. Seventy children were enrolled: 24 TD, 27 ASD and 19 SIB. The neurological motor picture was obtained by administering the Physical and Neurological Examination of Subtle Signs (PANESS) test. The test is composed by two main subscales, namely Gaits-Station and Timed Movements, further composed by several trials.The statistical analysis was conducted according to a top-down approach following the hierarchical structure of PANESS test: starting from the top (total score), we moved backward in search of the domains distinguishing ASD

from all other groups and, most interestingly, of the domains in which SIB assumed an intermediate position in between TD and ASD. Statistical analysis on total score revealed a triple significant contrast, with ASD exhibiting the worst scores (27.9±11.5), TD the lowest (10.8±5.2) and SIB located in between (15.7±6.1). Also for the two subscales separately, we obtained significant main effect of Group. However, while post-hoc analysis on Gaits-Stations indicated only a difference between ASD and the other groups (both $p<0.001$), the analysis on Timed Movements scores revealed an additional difference between SIB and TD ($p<0.05$). Moving to single timed-trial contrasts SIB proved similarly to ASD, and worse than TD, in finger apposition speed ($p<0.01$) and in adiadochokinesia ($p<0.05$). As expected, our results confirmed the presence of a diffuse motor impairment in all domains for ASD children. Most interestingly, while TD and SIB performance is equivalent in Gaits and Station, when required to perform timed movements (where speed and accuracy have to be properly combined), SIB performed worse than TD, indicating an overall weakness in timed movements as a possible motor endophenotype of autism. If confirmed, this finding would represent a key step for the definition of biomarkers accessible in children at risk for autism at an early onset, by far preceding the speech and cognitive impairments onset, thus facilitating the early diagnosis of autism and a consequent early intervention.

Disclosures: M. Fabbri-Destro: None. **A. Nuara:** None. **V. Gizzonio:** None. **G. Rizzolatti:** None. **P. Avanzini:** None.

Poster

121. Autism Spectrum Disorders: Neural Correlates in Humans

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 121.20/E39

Topic: A.07. Developmental Disorders

Title: Intra- individual trial- to trial neural variability in autism spectrum disorders (asd) and attention deficit hyperactivity disorder (adhd): A biomarker of asd with high specificity

Authors: *A. GIANNADOU[1], E. MILNE[1], M. FREETH[1], A. SAMSON[2]
[1]Univ. of Sheffield, Sheffield, United Kingdom; [2]Univ. of Geneva, Geneva, Switzerland

Abstract: Abstract:
Intra- individual trial- to trial neural variability is suggested as a potential measurable indicator of the presence of ASD. High functioning individuals with ASD show greater neural variability compared to individuals without ASD. A similar pattern has been observed in ADHD, a disorder that share genetic risk factors with ASD. The aim of the present study was to test the specificity of the biomarker and explore overlapping or shared neural mechanisms in ASD and ADHD. EEG was used to measure neural variability, in the form of inter-trial phase coherence (ITPC), in an ASD (N=28), ADHD (N=32) and a neurotypical (N=34) sample. There was a significant

difference between groups as determined by a one-way ANOVA ($F(2,91)=4.475$, $p=0.014$). Post-hoc tests revealed that ITPC values were significantly lower in the ASD group than the ADHD ($p=0.022$) and the neurotypical group ($p=0.034$), suggesting that increased neural variability is specific to the ASD group. The information obtained will advance our understanding of the etiology of ASD and the identification of such biomarkers will contribute towards the development of tools to diagnose ASC at an early stage.

Disclosures: E. Milne: None. **M. Freeth:** None. **A. Samson:** None.

Poster

121. Autism Spectrum Disorders: Neural Correlates in Humans

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 121.21/E40

Topic: A.07. Developmental Disorders

Support: Brigham Young University Mentored Environment grant to MS.
SISSA award stipend to VP

Title: Neural characterization of olfaction in adults with autism spectrum disorder

Authors: *V. PARMA[1], M. FURLAN[1], K. STEPHENSON[2], D. N. TOP[2], N. HUNSAKER[2], A. HEDGES-MUNCY[2], N. MUNCY[2], J. BECK[2], N. RUSSELL[2], M. SOUTH[2]
[1]Cognitive Neurosci., Intl. Sch. For Advanced Studies, Trieste, Italy; [2]Brigham Young Univ., Provo, UT

Abstract: Individuals diagnosed with Autism spectrum disorder (ASD) exhibit abnormal sensory (including chemosensory) experiences. Even though these impairments extend into adulthood, the behavioral manifestations and neural mechanisms of mature common and body odor perception in ASD are still unclear. Here, we characterize the behavioral performance and the structural and functional underpinnings of olfactory perception in ASD. Thirty-three adults with ASD (13F) and 39 controls (19F), selected on a dimensional continuum for anxiety, participated in the study. Preliminary results indicate that: i) ASD are ~3 times more likely to show reduced odor identification skills than controls; ii) odor identification skills are underlined by significant changes in the tractography of the frontal part of the inferior fronto-occipital and the inferior longitudinal fasciculus across groups; iii) ASD and TD processed body odors in distinct networks from the nonsocial common odor. However, the ASD group activated more sensory areas than controls for both body odor conditions. In contrast, the control group activated insula and amygdala more than the ASD group for familiar but not unfamiliar body odors. Behavioral results are discussed in the context of piriform cortex, orbitofrontal, and limbic functional activations as well as ASD severity. Taken together, our findings describe for the first time explicit and implicit olfactory behaviors and their neural underpinnings in adults with ASD

and suggest that olfactory perception and neuroimaging can provide non-invasive tools for ASD diagnosis and subgroup characterization.
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Poster

121. Autism Spectrum Disorders: Neural Correlates in Humans

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 121.22/E41

Topic: A.07. Developmental Disorders

Support: NIMH R01MH080826

Title: Typicality of functional connectivity predicts healthy social function

Authors: *F. L. WEATHERSBY[1], J. B. KING[2], J. C. FOX[4], A. D. LORET[1], J. S. ANDERSON[3]
[2]Univ. of Utah Program in Neurosci., [3]Radiology and Imaging Neurosci., [1]Univ. of Utah, Salt Lake City, UT; [4]Brigham Young Univ., Provo, UT

Abstract: Introduction: Differences in functional connectivity MRI have been associated with both normal behavior and psychopathology, although limited brain imaging metrics are described for predicting healthy social function. Here, we compared the brain network synchrony of 1003 subjects from the Human Connectome Project (HCP) of individual principal components (PCs) to the population mean values.
Methods: The resting state PCs of each HCP subject were compared to PCs of group-averaged connectivity data. For each component in a given subject, the most similar group component was selected, and correlation across the brain was recorded between the subject and group component. Covariate data for 81 behavioral tests was compared across subjects for the first ten synchrony values, controlling for age, sex, and head motion. The resulting matrix was the correlation of synchrony to behavior.
Results: Similarity to the group mean is a predictor of behavior. Specifically, for the second, fifth, and ninth PCs, similarity to group mean is positively correlated to agreeableness and negatively to anger/aggression. The third principal component is positively correlated to predictors for friendship and life satisfaction and negatively to predictors for sadness and perceived rejection.
Conclusion: The similarity of a subject's functional connectivity as measured by principal

components to a group mean value predicts behavior related to social function and well-being. Thus, if a subject is more similar to a particular population PC, then the subject is more likely to exhibit healthy social behaviors. Conversely, the more an individual deviates from group-average brain connectivity, the more likely the individual is to exhibit maladaptive social behaviors. Alternately, conforming to and empathizing with "typical" patterns of social cognition may arise from similarity to other individuals within a social community, and normative forces in society may dictate how successfully an individual integrates with the community.

Disclosures: F.L. Weathersby: None. **J.B. King:** None. **J.C. Fox:** None. **A.D. Loret:** None. **J.S. Anderson:** None.

Poster

121. Autism Spectrum Disorders: Neural Correlates in Humans

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 121.23/E42

Topic: A.07. Developmental Disorders

Support: CTSA Grant Number UL1 RR024139 (McPartland)
NIMH R01 MH107426 (McPartland, Srihari)

Title: Relationship between resting state EEG in autism and comorbid depressive symptoms

Authors: *T. C. DAY, T. WINKELMAN, K. A. MCNAUGHTON, B. LEWIS, K. ELLISON, E. JARZABEK, J. WOLF, A. NAPLES, J. MCPARTLAND
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Abstract: Autism spectrum disorder (ASD) is characterized by social impairments and repetitive behaviors, and comorbid psychiatric conditions are common and exacerbate core symptoms. In ASD (Wang et al., 2013) and depression (Alhaj, Wisniewski, McAllister-Williams, 2011), abnormalities in neural activity have been observed during resting state EEG. Though resting EEG has been proposed as a biomarker to predict treatment response in depression (Alhaj, Wisniewski, McAllister-Williams, 2011), its utility in comorbid conditions to ASD is poorly understood. We examined resting state EEG in relation to depressive symptoms in adults with ASD compared to adults with typical development (TD).
Participants included adults with ASD (n=18) and TD (n=21) matched on age (M=23.9 years) and performance IQ (M=106). Medication status and clinician rating of depressive symptoms were collected. EEG was recorded at 500 Hz with a 128-channel net while participants watched abstract screensavers or closed their eyes. Absolute and relative power in the delta and theta frequency bands were examined between groups and in relation to depressive symptoms. Repeated measure ANOVAs with follow-up t-tests and Spearman's rho correlation were utilized to examine differences between groups.

Because participants with ASD on antidepressant medication *(n*=9) did not differ in relative or absolute delta and theta power from unmedicated ASD participants (*n*=9, *p*s>.10), unmedicated and medicated participants with ASD were analyzed together. The ASD group had higher absolute frontal delta power than the TD group [$F(1,32)=7.8$, $p<.01$]. Regarding absolute frontal theta power, an interaction effect between diagnosis, condition, and hemisphere emerged [$F(1,32)=4.9$, $p<.05$] revealing higher theta power in the ASD group in the right hemisphere while eyes were closed [$t(34)=-2.0$, $p=.05$]. In the ASD group only, more depressive symptoms were related to lower relative frontal theta power while eyes were open [$r(14)=-.58$, $p<.05$]. Enhanced absolute frontal delta and theta power was observed in adults with ASD. This finding is consistent with other studies showing excessive power in low-frequency bands in ASD (Wang et al., 2013). Interestingly, more depressive symptoms were related to lower relative theta power which differs from previous findings of increased theta power in individuals with depression (Knott, Mahoney, Kennedy, Evans, 2000). This highlights the need to consider comorbid conditions in the identification of biomarkers, such as resting state EEG, of treatment response since baseline neural activity in ASD and comorbid depression may be distinct from ASD or depression.

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Poster

121. Autism Spectrum Disorders: Neural Correlates in Humans

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 121.24/E43

Topic: A.07. Developmental Disorders

Support: NIH Grant MH109685
Hartwell Foundation

Title: Defining neurophysiological biotypes in autism spectrum disorder using human resting-state connectivity

Authors: *A. BUCH[1], C. LISTON[1,2]
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Abstract: Autism Spectrum Disorder (ASD) describes a diverse group of neurodevelopmental disorders associated with a wide range of often debilitating clinical impairments. Core symptoms that define ASD include impaired social communication and interaction as well as stereotyped and restricted repetitive behaviors. Many patients also have attention and learning disorders of

varying severity. How distinct neurobiological substrates give rise to differing clinical symptoms in subsets of ASD patients is unknown. In this study, we aim to address this question of heterogeneity in ASD by grouping ASD patients into distinct neurophysiological subtypes based on patterns of dysfunctional connectivity and clinical symptom profiles. Preliminary results demonstrate the feasibility and biological validity of this subtyping approach, although further optimization and investigation are required. My central hypothesis is that distinct patterns of abnormal corticocortical and corticostriatal functional connectivity will define discrete ASD subtypes and will predict subtype-specific deficits in social communication and stereotyped restricted and repetitive behaviors. The results of this study have the potential to reveal new insights into how distinct patterns of dysfunctional connectivity give rise to clinical heterogeneity in the diverse symptoms that define ASD. This advance would enhance our understanding of the disease risk factors and symptomology and may suggest new avenues for developing more targeted and personalized therapeutic interventions.

Disclosures: A. Buch: None. **C. Liston:** None.

Poster

121. Autism Spectrum Disorders: Neural Correlates in Humans

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 121.25/E44

Topic: A.07. Developmental Disorders

Title: Diffusion MRI of cortico-basal ganglia and cerebellar pathways in autism spectrum disorder: Relationships to repetitive behavior

Authors: *B. J. WILKES[1], H. E. KORAH[4], D. B. ARCHER[2], D. E. VAILLANCOURT[3], M. H. LEWIS[5]
[1]Psychology, [3]Applied Physiol. and Kinesiology, [2]Univ. of Florida, Gainesville, FL; [4]Univ. of Florid, Gainesville, FL; [5]Psychiatry, UF Col. of Med., Gainesville, FL

Abstract: Restricted, repetitive behavior is diagnostic for autism spectrum disorder (ASD) and a prominent feature of other neurodevelopmental disorders. Magnetic resonance imaging (MRI) has been widely used to study ASD, but with relatively little focus on the diagnostic domain of restricted, repetitive behavior. In this study we analyzed diffusion-weighted MRI data from a large dataset of children and adolescents with ASD (n=96) and typically developing (TD) controls (n=96), with nearly equal number of males and females, acquired from the National Database for Autism Research (NDAR). We performed targeted investigations of cortico-basal ganglia and cerebello-thalamic white matter (WM) pathways using customized analyses pipelines in order to determine whether these pathways differ in individuals with ASD compared to TD controls, covarying for age and IQ. Moreover, we investigated the relationship between these neuroimaging metrics and clinical assessments of repetitive behavior within individuals

with ASD. We found that individuals with ASD had lower fractional anisotropy (FA) in the WM pathway between dorsolateral prefrontal cortex and the caudate. Although no group differences were observed in the WM pathway between primary motor cortex and putamen, within the ASD group higher rates of repetitive behavior were correlated with higher FA in this pathway. These results support prior evidence of altered cortico-basal ganglia WM in ASD, extend evidence of altered WM to females with ASD, and provide novel evidence about the relationship between repetitive behavior WM alterations in ASD.

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Poster

121. Autism Spectrum Disorders: Neural Correlates in Humans

Location: SDCC Halls B-H

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Program #/Poster #: 121.26/E45

Topic: A.07. Developmental Disorders

Support: R01 MH081023
R01 MH101173
K01 MH097972

Title: Developmental trajectories of functional connectivity in resting state networks in Autism Spectrum Disorders

Authors: *M. OLSON[1], R.-A. MÜLLER[2]
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Abstract: *Background:* Atypical cortical connectivity is thought to underlie the complex cognitive and behavioral profile observed in individuals diagnosed with Autism Spectrum Disorders (ASDs). Previous literature has reported findings of both under and over-connectivity in individuals with ASDs. A recent meta-analysis spurred a developmental hypothesis, suggesting that these inconsistencies could be reconciled by accounting for atypical developmental trajectories (Uddin et al., 2013). The specific hypothesis predicts over-connectivity in pre-pubertal children, but under-connectivity in adolescents and young adults with ASDs, compared to matched typically developing (TD) individuals. However, relevant empirical findings remain mixed, warranting further study.*Methods:* We tested the developmental hypothesis in ASDs by examining within and between-network connectivity using in-house resting state-fMRI data from 76 participants (n=25 per Adolescent groups: ASD⁢13yrs, TD⁢13yrs, and n=13 per Childhood groups: ASD>13yrs, TD>13yrs), matched on motion, age, PIQ, and handedness. Independent component analysis (ICA) was run for all groups combined and dual regression was performed to examine the effects of participant

age on between and within-network whole-brain connectivity patterns in ASD and TD groups. *Results:* ICA produced 20 resting state networks. 12 were determined to represent non-artifactual networks. We found diverse network-specific effects of under and over-functional connectivity when comparing children (ages 9 -13 years) and adolescents (13-18 years) with ASDs to matched TD individuals. *Conclusion:* Overall, findings from cross-sectional comparisons do not support the hypothesis of broad overconnectivity in pre-pubertal children with ASDs contrasting with broad underconnectivity in post-pubertal adolescents with ASDs. Instead, group differences at both ages were mixed and network-specific, suggesting that maturational trajectories of abnormal connectivity in ASDs cannot be captured by a single principle. Supported by: R01 MH081023, R01 MH101173, K01 MH097972

Disclosures: M. Olson: None. **R. Müller:** None.

Poster

121. Autism Spectrum Disorders: Neural Correlates in Humans

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Program #/Poster #: 121.27/E46

Topic: A.07. Developmental Disorders

Support: NICHD 2P50HD055784-08
Autism Speaks
ARCS Foundation

Title: Hyperconnectivity during language processing in infants at risk for ASD

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Abstract: Autism spectrum disorder (ASD) is a disorder of connectivity, with ASD genes converging to disrupt neural development and cortical connectivity prenatally (Torre-Ubieta 2016). Familial risk (FR) infants, defined by having at least 1 older sibling with ASD, have increased risks for ASD and language delay (Messinger 2013, Charman 2017). Neuroimaging studies have identified abnormal structural and functional connectivity in FR infants at 6 months that relate to future ASD symptoms (Shen 2017). No prior study has examined connectivity during language processing in FR infants at 3 months. We asked if connectivity, as measured by phase coherence during language processing, differentiates infants based on risk status and ASD symptoms, and whether 3-month connectivity relates to 18-month language ability and ASD symptoms. We hypothesized that atypical connectivity during language processing is detectable at 3 months of age in FR infants. Participants included 40 familial-risk infants (FR) and 36 low-

risk (LR) infants, from an ongoing longitudinal study at the UCLA Autism Center of Excellence (NICHD 2P50HD055784-08). EEG was acquired at 3-month while subjects listened to a stream of concatenated syllables for 2 minutes. EEG data was cleaned using EEGlab independent component analysis and transformed to current source density (Dickinson 2018). Phase coherence was calculated in the theta (4-6 Hz), alpha (6-12 Hz) and gamma (30-50 Hz) bands between 22 electrode pairs in putative language networks (frontal-temporal, frontal-posterior, and interhemispheric connections). Language ability and ASD symptoms were assessed at 18-month using the Mullen Scales of Early Learning (MSEL) and Autism Diagnostic Observation Schedule-Toddler Module (ADOS-T), respectively. Independent samples t-tests were used to compute group differences, and Pearson's correlations were used to relate connectivity to behavioral outcomes. FR infants had greater left frontal-posterior connectivity than LR infants in both alpha ($p=0.003$) and gamma bands ($p=0.002$). In the theta band, ASD-concern infants had increased frontal-posterior connectivity compared to No-concern infants ($p=0.001$). Theta coherence negatively correlated with MSEL verbal t-score ($r=-0.37$, $p=0.003$), and positively correlated with ADOS-T clinical severity score ($r=0.33$, $p=0.009$) across risk groups. This study documents the earliest manifestation of altered connectivity during language processing in FR infants. Hyperconnectivity during language processing at 3 months of age could serve as an early marker of atypical neurodevelopmental trajectories.

Disclosures: X.A. Tran: None. **N.M. McDonald:** None. **A. Dickinson:** None. **M. Dapretto:** None. **S.S. Jeste:** None.

Poster

121. Autism Spectrum Disorders: Neural Correlates in Humans

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 121.28/E47

Topic: A.07. Developmental Disorders

Support: : Big Data in Medicine Project University of Louisville 21st Century Initiative

Title: An early-detection diagnostic framework for autism spectrum disorder using DTI and sMRI

Authors: Y. ELNAKIEB[1], O. DEKHIL[1], A. SHALABY[1], B. AYINDE[2], A. MAHMOUD[1], A. SWITALA[1], A. ELMAGHRABY[3], R. KEYNTON[1], M. GHAZAL[1,5], *E. GOZAL[6], A. EL-BAZ[1], G. BARNES[4]
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Abstract: Introduction: Autism is a complex neurological disorder which affects behavioral and communication skills. While conventional diagnosis is subjective and requires long time before confirmation, neuroimaging provides a promising alternative. In this study, a personalized autism diagnosis system is introduced using both structural MRI and diffusion weighted imaging to generate personalized maps showing the most affected areas with autism implications. The system analyzes both the shape features of the cerebral cortex (Cx) from sMRI, and the white matter connectivity patterns from the DTI, and applies a deep learning technique for subject classification and personalized maps generation. For structural MRI analysis, the brain cortex is segmented, then spherical harmonic decomposition is used to measure the surface complexity and curvatures. White matter integrity is examined by extracting the most prominent features representing the white matter connectivity, after segmenting white matter from diffusion weighted images. A multi-stage deep network, based on several autoencoders and soft-max classifier, is constructed to provide the final global diagnosis. The presented CAD system has been tested on 153 subjects who have available data for DTI or sMRI or both, with overall accuracy of 85%
Materials and Methods: For sMRI, the cortex complexity was calculated after using a fixed number of spherical harmonics. In addition, the two principle curvatures were calculated at each mesh vertex. Both features are parcellated onto local brain areas using Brodmann areas atlas. For DTI, the diffusion tensors are used to describe the white matter connectivity patterns. Four global features were calculated based on the 3 eigenvalues at each voxel: fractional anisotropy, axial and radial diffusivity, and mean diffusivity. These features are parcellated on John-Hopkins white matter atlas. Autoencoders trained for each area are used to reduce the dimensionality of the features. Local diagnosis decisions were then made using soft-max classifiers. Finally, the output of the two classifiers was averaged to decide if a subject was autistic.
Results and Discussion: Two approaches were used to ensure system robustness: Leave one subject out, and k-fold cross validation, with k = 4 and 10. Fusing features from each modality achieved a global diagnostic accuracy of 95.2%, sensitivity of 0.90, and specificity of 0.90 for sMRI; while the accuracy for DTI is 96.8%, the sensitivity 0.9, and the specificity 0.97. The system achieved per-subject overall accuracy of 85% (sensitivity = 0.82, specificity = 86, area under ROC curve = 0.84). This work provides early diagnosis accuracy.

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Poster

121. Autism Spectrum Disorders: Neural Correlates in Humans

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 121.29/E48

Topic: A.07. Developmental Disorders

Support: University of Louisville 21st Century Initiative

Title: A deep learning based generative model for functional mri analysis

Authors: *O. DEKHIL[1], M. ALI[2], A. SHALABY[2], R. KEYNTON[2], M. GHAZAL[2], A. ELMAGHRABY[2], A. EL BAZ[2], G. N. BARNES[3]
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Abstract: Introduction: Resting state fMRI is a trending imaging modality. It is frequently used in identifying abnormalities in brain activation either at rest or while performing a task. In resting fMRI (RfMRI), the most common analysis technique is Independent Component Analysis (ICA), however, the basic limitation in the ICA analysis technique is the constraining the components to be non-Gaussian. To overcome this constraint, we propose a technique that utilizes semi-restricted Boltzmann machines (SRBMs) to analyze RfMRI. SRBMs are graphical generative models that are used to learn an unknown input distribution using latent variable representation. In this study, we use SRBMs to analyze the data from 100 subjects (50 autistic and 50 typically developed) and extract components of statistically significant differences between the two groups. **Materials and Methods:** The results shows the architecture of the SRBM used, where the input layer nodes represent the voxels of a single RfMRI volume, the hidden nodes represent the number of latent components used and the weights between each hidden node and set of visible units represent a spatial component. To obtain the time courses that are not given explicitly by SRBMs, the learned weights are used as a de-mixing matrix of a linear model and obtain the time courses using: Where S are the time courses, W are the learned weights and x is the input RfMRI volume. The main advantage of using SRBMs instead of RBMs is having connections between visible units; these connections account for spatial connections between voxels which is informative than RBMs. After extracting both spatial and temporal components from the RfMRI data, and to check for significance, non-parametric permutation testing is used. **Experimental Results:** Using the proposed approach on a dataset of 100 subjects obtained from the National Database for Autism Research (NDAR), 3 significant components were extracted. In this experiment, a number of hidden nodes are used. The functional correspondence between the significant components and behavioral aspects was checked. **Conclusions:**The major impaired functionalities were fully related to autistic behaviors such as restricted and repetitive behaviors, attention, language, social interaction and executive functions.

Disclosures: O. Dekhil: None. **M. Ali:** None. **A. Shalaby:** None. **R. Keynton:** None. **M. Ghazal:** None. **A. Elmaghraby:** None. **A. El Baz:** None. **G.N. Barnes:** None.

Poster

122. Metabotropic Glutamate and GABA B Receptors

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 122.01/E49

Topic: B.03. G-Protein Coupled Receptors

Support: This study was financed by The Polish National Centre for Research and Development. Project MAESTRO no. 2012/6/06/A/NZ7/00014

Title: 5ht1a and mglur4 interactions, possible link to schizophrenia?

Authors: G. BURNAT[1], P. BRANSKI[1], J. SOLICH[2], M. KOLASA[2], B. CHRUSCICKA[1], M. DZIEDZICKA-WASYLEWSKA[2], *A. PILC[2]
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Abstract: Introduction: In previous reports our group showed beneficial effect of antipsychotic action of mGluR4 and 5HT1a activators (Wierońska et al., 2015, 2017). In present study we examined the heteromerization of members two different class of GPCR. The first one, the metabotropic glutamate receptor 4 (mGluR4) is a representative of class C. Like other members of group III mGluRs its activation inhibits activity adenylate cyclase thus leading to a decrease of cAMP concentration in cells. In CNS mGluR4 is predominantly expressed pre-synaptically. Its highest expression is observed in the cerebellum as well as in the cerebral cortex and in the hippocampus. The second receptor is 5HT1a receptor which belongs to A class of GPCR. Similarly to mGlur4 it is preferentially coupled with Gi protein to inhibit cAMP formation. 5HT1a in most brain structures is located post-synapticaly. The highest densities are observed in the hippocampus, amygdala, and cortical limbic areas. Moderate expression is observed in the raphe nuclei (pre-synaptic localization as an autoreceptor).
Due to high expression of mGluR4 and 5HT1a in the hippocampus and cortex both receptors seem to be a promising pharmacological targets for treatment of anxiety and schizophrenia.
Methods: To analyze distribution of both receptors in the mouse brain, a double immunofluorescence with DAPI on cortical section was performed. The occurrence of mGluR4-5HT1a hetero complex was studied in the brain regions where co-localization was observed. PLA red dots indicates close proximity <17 nm. Similar researches ware conducted on mouse primary neuron and astrocyte cells culture. Also both receptors were co-expressed in HEK293 cells. To study possible interaction between mGluR4 and 5-HT1A receptors SNAP or HALO - tag wer used. Potential oligomerization was measured by HTR-FRET assay in presence agonists L-Glu , 8-OH DPAT or both. Additionally, we evaluated pharmacological response of mGlu4R and 5-HT1a by measuring cAMP level upon stimulation with reference compounds.
Results: We measured the distribution of 5HT1a and mGluR4 through the mouse brain structures. The hippocampus could be most interesting structure where strong co-expression and

PLA signal were seen. Also in *in-vitro* experiments at heterologous cell system indicate the possibility of interaction of both receptors on molecular level. We also looked on receptors distribution and potential oligomerization in *in-vivo* and *in-vitro* experiments. Up to date there is no data concerning oligomerization or interaction at molecular level between 5HT1a and mGluR4.

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Poster

122. Metabotropic Glutamate and GABA B Receptors

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 122.02/E50

Topic: B.03. G-Protein Coupled Receptors

Title: Functional independence of IP_3- and Ry-sensitive Ca^{2+} stores in hippocampal pyramidal neurons

Authors: *J. HARTMANN[1], H.-J. CHEN[2], R. M. KARL[2], S. FESKE[3], A. KONNERTH[2]
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Abstract: Endoplasmic reticulum (ER) Ca^{2+} stores are a major source and sink for cytosolic Ca^{2+} and thus crucial for neuronal function. Two types of channels release Ca^{2+} ions from the ER into the cytosol: inositoltrisphosphate receptors (IP_3Rs) and ryanodine receptors (RyRs). A high Ca^{2+} concentration in the ER is maintained by store-operated Ca^{2+} entry (SOCE) through the plasma membrane. In non-excitable cells the protein Orai1 has been identified as the pore-forming subunit of SOCE channels. In neurons ER Ca^{2+} homeostasis and SOCE are poorly understood. Here, we investigated the specific functional properties of IP_3- and RyR-stores in hippocampal CA1 pyramidal neurons (CA1 PNs). With a quantitative RT-PCR approach we found that Orai2 is the predominant Orai homolog in CA1 PNs. Using a combination of confocal Ca^{2+} imaging and the patch-clamp technique in acute hippocampal slices, we investigated the contribution of Orai1 and Orai2 to RyR- and IP_3R-mediated Ca^{2+} signaling in wild type, CA1 PN-specific Orai1 knockout ($Orai1^{CA1ko}$) and Orai2-deficient knockout (Orai2-/-) mice. IP_3R-dependent Ca^{2+} release in somata of CA1 PNs was stimulated by local application of dihydrophenylglycine (DHPG), an agonist of metabotropic glutamate receptors 1 and 5 (mGluR1/5) that are highly expressed in CA1 PNs. Analogously, Ca^{2+} release through RyRs was evoked using their agonist caffeine. Neither IP_3R nor RyR-mediated Ca^{2+} transients were affected by the absence of Orai1 in $Orai1^{CA1ko}$ mice. In contrast to that, IP_3R-dependent Ca^{2+} release in CA1 PNs was largely abolished in Orai2-/- mice, irrespective whether it was evoked by local DHPG application or intracellular photolytic uncaging of IP_3. Most remarkably,

however, responses to caffeine remain unaltered in Orai2-/- mice. The discovery of an almost complete independence of IP_3R- and RyR-dependent Ca^{2+} release in CA1 PNs was substantiated by additional experiments in which IP_3R-mediated Ca^{2+} responses persisted when Ry-sensitive stores were depleted and *vice versa*. In contrast to DHPG-evoked Ca^{2+} transients, the spontaneous recovery of caffeine-induced Ca^{2+} responses after store depletion is sensitive to the antagonist of voltage-gated Ca^{2+} channels (VGCCs) D600. Moreover, activation of VGCCs by depolarization increases Ca^{2+} responses to caffeine but fails to restore DHPG responses in Orai2-/- mice. Together, our results demonstrate that IP_3-sensitive and Ry-sensitive Ca^{2+} stores in CA1 PNs represent distinct Ca^{2+} pools, with different molecular mechanisms of store refilling. Furthermore, our data reveal for the first time the critical role of Orai2 for mGluR1/5-dependent Ca^{2+} signaling in central mammalian neurons.

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Poster

122. Metabotropic Glutamate and GABA B Receptors

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 122.03/E51

Topic: B.03. G-Protein Coupled Receptors

Title: Unraveling the mechanism underlying the anti-absence activity of mGlu5 receptor activation

Authors: R. CELLI[1], I. SANTOLINI[1], M. VERGASSOLA[2], M. J. WALL[3], R. GINERETE[1], G. MASCIO[1], V. D'AMORE[1], *R. GRADINI[4,1], G. VAN LUIJTELAAR[5], G. BATTAGLIA[1], V. BRUNO[1,4], R. T. NGOMBA[6], A. PITTALUGA[2], F. NICOLETTI[1,4]
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Abstract: We found that systemic or intrathalamic treatment with the selective mGlu5 receptor positive allosteric modulator (PAM), VU0360172, reduced spike-and-wave discharges (SWDs) in the WAG/Rij rat model of absence epilepsy. No tolerance developed to the anti-absence activity of VU0360172, suggesting that the mGlu5 receptor is a valuable candidate drug target for the treatment of absence epilepsy. Searching for the mechanism(s) linking mGlu5 receptors to the control of SWDs, we moved from the evidence that the GABA transporter inhibitor, tiagabine, could reverse the anti-absence activity of VU0360172 when locally infused in the thalamus at doses that did not affect SWD frequency on their own. This finding gave us impetus to examine whether mGlu5 receptors could affect SWDs by influencing synaptic clearance of

GABA in the ventrobasal thalamus. In a first series of experiments, we measured protein levels of the high affinity GABA transporter, GAT-1, after systemic administration of VU0360172 (3 mg/kg, s.c.) in 5-month old symptomatic WAG/Rij rats. We were surprised to find that a single administration of the mGlu5 receptor PAM was sufficient to up-regulate GAT-1 expression in the thalamus after 1 hour. No significant changes in GAT-1 were seen in the somatosensory cortex. The effect of VU0360172 on thalamic GAT-1 expression was not disease-dependent because could also be observed in non-epileptic control rats at different ages. We also performed functional studies measuring [3H]GABA uptake in synaptosomal preparations prepared from the thalamus and somatosensory cortex of symptomatic WAG/Rij rats. An increased [3H]GABA uptake was measured in thalamic synaptosomes prepared from rats that had been acutely injected with VU0360172 (rats were killed 1 hour after injection). Opposite findings were obtained in synaptosomes prepared from the somatosensory cortex. We are now exploring the molecular mechanism underlying the fast up-regulation of GAT-1 and the ensuing GABA uptake in the thalamus in response to mGlu5 receptor activation using primary cultures of thalamic astrocytes and mixed thalamic cultures containing astrocytes and neurons incubated with selective inhibitors of enzymes directly involved in mGlu5 receptor signaling. In addition, electrophysiological experiments are under way to examine whether activation of mGlu5 receptors influences tonic GABAergic inhibition as a result of an increased GABA uptake in astrocytes or neurons.

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Poster

122. Metabotropic Glutamate and GABA B Receptors

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 122.04/F1

Topic: B.03. G-Protein Coupled Receptors

Support: Dept of Veterans Affairs BLR&D, Merit Review Award 1I01BX0027450IA1

Title: Modulation of extrasynaptic $GABA_A$ receptor function in dentate gyrus granule cells by $GABA_B$ receptors and severe TBI

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Abstract: Tonic inhibition mediated by extrasynaptic $GABA_A$ receptors importantly affects many physiological and pathophysiological processes, including epileptic seizures. Clinical experience and experimental data indicate that seizures can be exacerbated by both decreases or increases in tonic inhibition, highlighting the importance of regulating tonic inhibition to appropriate levels for normal brain function. Previous work has identified postsynaptic $GABA_B$ receptors as mechanism that enhances extrasynaptic $GABA_A$ receptor function in dentate gyrus granule cells (DGGCs) (Tao et al., J Neurosci 2013). Using a combination of electrophysiology, biochemical techniques, and analytical approaches (biotinylated western blots, nonstationary noise analysis) we report here that $GABA_B$ receptor activation with baclofen (10 µM) increased tonic GABA currents and plasma membrane expression of both delta and gamma subunits of $GABA_A$ receptors. Estimates of single channel conductance and mean open time (noise analysis) were unaffected by baclofen. Thus, modulation of tonic inhibition by $GABA_B$ receptors is primarily due to increased receptor number in plasma membrane. Traumatic brain injury (TBI) affects many cellular and molecular processes, including $GABA_A$ receptor function. Literature describing effects of TBI on tonic inhibition are highly variable, warranting further investigation. We used the controlled cortical impact model (CCI) to investigate changes in GABAergic signaling of DGGCs following severe TBI. CCI reduced endogenous tonic GABA currents of DGGCs in ipsilateral hippocampus by 86% compared to control/sham ($p<0.01$). Tonic GABA currents induced by the δ subunit-selective agonist THIP (10 µM) were also reduced ipsilateral to CCI. Spontaneous IPSC frequency, but not amplitude or kinetics, was reduced by CCI in ipsilateral DGGC. Because TBI produces chronic reductions in cAMP levels and PKA activity (Atkins et al., Exp. Neurol. 2007), we hypothesized that the effects of $GABA_B$ receptor activation ($G_{i/o}$) on tonic GABA currents would be attenuated following CCI. However, baclofen significantly potentiated tonic GABA currents in both control/sham DGGCs and DGGCs ipsilateral to CCI. These results indicate that extrasynaptic $GABA_{A[[Unsupported Character - Codename]]}$ receptors are affected by CCI but their modulation by $GABA_B$ receptors remains intact.

Disclosures: A. Parga: None. **C.B. Ransom:** None.

Poster

122. Metabotropic Glutamate and GABA B Receptors

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 122.05/F2

Topic: B.03. G-Protein Coupled Receptors

Title: Genetic deletion of mGlu3 receptors causes developmental abnormalities in GABAergic neurons and cortical neural synchronization in mice: A multi-modal and -scale approach

Authors: *G. BATTAGLIA[1], T. IMBRIGLIO[2], R. VERHAEGHE[2,1], K. MARTINELLO[1], C. DEL PERCIO[2], M. T. PASCARELLI[2], S. LOPEZ[2], G. CHECE[2], D. BUCCI[1], S.

NOTARTOMASO[1], G. MASCIO[1], M. QUATTROMANI[3], F. SCALABRI[1], A. SIMEONE[4], S. MACCARI[5,1], T. WIELOCH[3], S. FUCILE[2,1], C. BABILONI[2], C. LIMATOLA[2,1], F. NICOLETTI[2,1], M. CANNELLA[1]
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Abstract: A large body of evidence suggests that genetic variants of mGlu3 receptors are associated with schizophrenia, and mice lacking mGlu3 receptors show a psychotic-like phenotype (Fujioka et al, 2014; Lainiola et al, 2014). Here we tested the hypothesis that the lack of mGlu3 receptors may have a significant impact on 1. biochemical and functional parameters related to the development of GABAergic interneurons and GABAergic transmission in the prefrontal cortex and hippocampus; and, 2. cortical neural synchronization in wakefulness as revealed by on-going electroencephalographic (EEG) rhythms. To this aim, we used wild-type, mGlu3$^{-/-}$ and mGlu2$^{-/-}$ mice. Compared with wild-type mice, mGlu3$^{-/-}$ and mGlu2$^{-/-}$ mice showed no changes in the expression of genes involved in specification and/or migration of GABAergic interneurons, such as Msh1, Otx2, Pax6, and Nkx2.1 at embryonic day 12.5. In contrast, mGlu3$^{-/-}$ mice showed alterations in the transcripts of genes encoding for biochemical markers of cortical and hippocampal interneurons at postnatal days (PNDs) 1 and 9, with a large reduction in parvalbumin (PV) mRNA levels at PND9. Furthermore, the analysis of $GABA_A$ receptor-mediated responses in layer V pyramidal neurons at PND9 showed a more depolarized somatic inversion potential of Cl^-, associated with a reduced expression of the neuronal K^+/Cl^- symport, KCC2, in the prefrontal cortex. In addition, the number of perineuronal nets, which are formed by chondroitin sulphate containing proteoglycan (mainly aggrecan) supers structures, that enwrap PV^+ GABAergic interneurons, was significantly increased in the cortex of mGlu3$^{-/-}$ mice at PND21. mGlu3$^{-/-}$ mice also showed reduced PV mRNA and protein levels at PND30. As compared to mGlu3$^{-/-}$ mice, mGlu2$^{-/-}$ mice had a lower impact on the expression of interneuron-relate genes, and did not induce a reduction in PV mRNA. Finally, cortical neural synchronization was tested in both wild-type and mGlu3$^{-/-}$ mice at PND75. Compared to wild-type mice, mGlu3$^{-/-}$ mice showed a significant background reduction in the amplitude of on-going EEG rhythms in wakefulness in all frequency bands from δ to γ (1-100 Hz), with sporadic short-bursts (300-400 ms) of wide-band EEG oscillations at high amplitude. These findings suggest that the lack of mGlu3 receptors may alter the development of GABAergic transmission in the prefrontal cortex and hippocampus as well as cortical neural synchronization in wakefulness. Future studies may test the correlation of those biomarkers with behavioral and cognitive symptoms in mouse models of schizophrenia before and after antipsychotic drugs.

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Poster

122. Metabotropic Glutamate and GABA B Receptors

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 122.06/F3

Topic: B.03. G-Protein Coupled Receptors

Title: *In vivo* measurement of receptor-activated polyphosphoinositide hydrolysis: A valuable tool for the assessment of mGlu5 receptor function in physiology and pathology

Authors: *V. BRUNO[1,2], A. R. ZUENA[1], L. IACOVELLI[1], R. ORLANDO[1], L. DI MENNA[2], S. ALEMÀ[1], P. CASOLINI[1], S. NOTARTOMASO[2], G. MASCIO[2], G. DI CICCO[1], G. BATTAGLIA[2], F. NICOLETTI[1,2]
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Abstract: Measurement of inositolmonophosphate (InsP) levels by ELISA (IP-One, Cisbio, Codolet, France) allows the *in vivo* assessment of receptor-activated polyphosphoinositide (PI) hydrolysis in the CNS. This method limits the biases inherent to the conventional measurement of receptor-activated PI hydrolysis in brain slices incubated with radioactive inositol. Systemic treatment with the selective positive allosteric modulator (PAM) of mGlu5 receptors, VU0360172 (30 mg/kg, i.p.), enhanced InsP formation in different brain regions of mice pretreated with lithium ions (LiCl, 105-420 mg/kg, i.p.). VU0360172-stimulated PI hydrolysis was greater in the hippocampus, followed by the striatum, cerebral cortex, hypothalamus, olfactory bulb, and cerebellum. This correlated with mGlu5 receptor expression, as detected by immunoblot analysis. The action of VU0360172 was abrogated by the mGlu5 receptor negative allosteric modulator, MTEP (10 mg/kg, i.p.), and was absent in mGlu5 receptor knockout mice. Comparison between wild-type and mGlu5 receptor knockout mice also indicated that a large component of the basal PI hydrolysis was mediated by endogenous activation of mGlu5 receptors. We used this new method to examine how mGlu5 receptor signaling changes in mice performing a spatial learning task in the water maze, and in transgenic mice harboring a mutated form of ataxin-1 (a model of type-1 spinocerebellar ataxia - SCA1). We found that mGlu5 receptor-mediated PI hydrolysis was blunted in the dorsal and ventral hippocampus of mice that learned to find the hidden platform in the water maze, as compared to non-learner or naïve mice. In SCA1 mice, we also found a blunted mGlu5 receptor-mediated PI hydrolysis in the hippocampus as compared to their wild-type counterparts. No stimulation of PI hydrolysis was found in the cerebellum of both strains of mice. These data indicate that mGlu5 receptor-mediated PI hydrolysis can be measured *in vivo* in animals systemically treated with a selective receptor PAM, and that this method can be applied to the assessment of mGlu5 receptor function in physiological and pathological conditions.

Disclosures: V. Bruno: None. **A.R. Zuena:** None. **L. Iacovelli:** None. **R. Orlando:** None. **L. Di Menna:** None. **S. Alemà:** None. **P. Casolini:** None. **S. Notartomaso:** None. **G. Mascio:** None. **G. Di Cicco:** None. **G. Battaglia:** None. **F. Nicoletti:** None.

Poster

122. Metabotropic Glutamate and GABA B Receptors

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 122.07/F4

Topic: B.03. G-Protein Coupled Receptors

Title: Antipsychotic-like activity of the kynurenine metabolite, cinnabarinic acid, in mice

Authors: F. FAZIO[1], M. ULIVIERI[2], J. M. WIERONSKA[3], P. CIEśLIK[3], G. MASCIO[1], A. TRAFICANTE[1], F. LIBERATORE[2], N. ANTENUCCI[1], G. GIANNINO[1], V. BRUNO[2,1], G. BATTAGLIA[1], *F. NICOLETTI[2,1], A. PILC[3]
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Abstract: The kynurenine pathway of tryptophan metabolism generates a series of neuroactive compounds, of which kynureninc and quinolinc acids have been extensively studied for their ability to interact with NMDA receptors. It has been proposed that schizophrenia is associated with an imbalance between kynurenic and quinolinic acids formation, contributing to NMDA receptor hypoactivity in the CNS (reviewed by Schwarcz et al., Nat. Rev. Neurosci., 2012). More recently, another kynurenine metabolite, xanthurenic acid, has been associated with schizophrenia (Fazio et al., Sci. Rep., 2015). The kynurenine pathway also generates cinnabarinic acid (CA), a condensation product of 3-hydroxyanthranylic acid, which has been shown to activate mGlu4 metabotropic glutamate receptors (Fazio et al., Mol. Pharmacol., 2012). The evidence that mGlu4 receptor agonists display antipsychotic-like activity in rodents (Wozniak et al., Neuropharmacol., 2016) gave us impetus to investigate the effect of CA in experimental animal models of psychosis. We first determined that i.p. injected CA could enter the brain, as assessed by immunohistochemistry with an anti-CA antibody. We were surprised to find that very low doses of CA (0.125-5 mg/kg, i.p.) were able to reduce MK-801-induced hyperactivity in C57Bl/J mice. Higher doses of CA were inactive. Inhibition of MK-801-induced hyperactivity by CA (0.5 mg/kg) was blunted in mice lacking mGlu4 receptors. CA (0.5 mg/kg) was also able to reduced MK-801-stimulated glutamate release in the frontal cortex, as assessed by microdialysis in freely moving mice. In addition, CA could also reverse the reduced expression of the K^+/Cl^- symporter, KCC2, induced by MK-801 in the frontal cortex. We extended the study to another model of psychosis, i.e., head twitches induced by systemic administration of the 5-HT_{2A} serotonin receptor agonist, DOI, in mice. Interestingly, very low doses (0.125 mg/kg, i.p.) of CA significantly reduced DOI-induced head twitches, whereas doses

of 0.5 and 5 mg/kg were inactive. Taken together these findings indicate that CA displays an unusually high potency in experimental animal models that are predictive of antipsychotic activity. This supports the view that mGlu4 receptors are potential drug targets for the treatment of psychosis, and encourages the study of CA in biological fluids or autoptic brain samples of patients affected by schizophrenia.

Disclosures: F. Fazio: None. **M. Ulivieri:** None. **J.M. Wieronska:** None. **P. Cieślik:** None. **G. Mascio:** None. **A. Traficante:** None. **F. Liberatore:** None. **N. Antenucci:** None. **G. Giannino:** None. **V. Bruno:** None. **G. Battaglia:** None. **F. Nicoletti:** None. **A. Pilc:** None.

Poster

122. Metabotropic Glutamate and GABA B Receptors

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 122.08/F5

Topic: B.03. G-Protein Coupled Receptors

Title: mGlu1 receptors drive the developmental decline of mGlu5 receptors in cerebellar Purkinje cells

Authors: *S. NOTARTOMASO[1], H. NAKAO[2], G. MASCIO[1], P. SCARSELLI[1], M. CANNELLA[1], C. ZAPPULLA[1], M. MADONNA[1], M. MOTOLESE[1], R. GRADINI[1,3], F. LIBERATORE[3], M. ZONTA[4], G. CARMIGNOTO[4], G. BATTAGLIA[1], V. BRUNO[1,3], M. WATANABE[5], A. AIBA[2], F. NICOLETTI[1,3]
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Abstract: mGlu1 receptors are highly expressed in mature cerebellar Purkinje cells (PCs) and play a key role in motor learning and motor coordination. In contrast, mGlu5 receptors are nearly absent in mature PCs, and their expression is low in the whole cerebellar cortex. Here we show that mGlu5 receptors are expressed by PCs and functionally active in the first two weeks of postnatal life, as assessed by immunohistochemistry and measurements of polyphosphoinositide (PI) hydrolysis and intracellular Ca^{2+} responses. The developmental decline of mGlu5 receptors in PCs coincides with the appearance of mGlu1 receptors. Interestingly, there was a cause-to-effect relationship between endogenous activation of mGlu1 receptors and the reduced expression of mGlu5 receptors. Accordingly, mGlu5 receptors were still present at postnatal day (PND) 16 after conditional knockdown of mGlu1 receptors in PCs in the first two weeks of postnatal life. Similar findings were obtained after repeated administrations of the mGlu1 receptor negative allosteric modulator, JNJ16259685 (2.5 mg/kg, i.p.), from PND9 to PND14. In contrast, a 3- or 5-days treatment with the mGlu1 receptor positive allosteric modulator, Ro0711401 (10 mg/kg, s.c.), starting from PND7, accelerated the developmental decline of mGlu5 receptors. Finally, the mGlu1 receptor maintaines the suppressing activity on mGlu5

receptors in mature PCs because conditional mGlu1 knockdown or JNJ16259685 treatment in the adult life caused the reappearance of mGlu5 receptors in PCs. These findings raise the intriguing possibility that mGlu5 receptor activation plays a role in the early maturation of PCs.

Disclosures: S. Notartomaso: None. **H. Nakao:** None. **G. Mascio:** None. **P. Scarselli:** None. **M. Cannella:** None. **C. Zappulla:** None. **M. Madonna:** None. **M. Motolese:** None. **R. Gradini:** None. **F. Liberatore:** None. **M. Zonta:** None. **G. Carmignoto:** None. **G. Battaglia:** None. **V. Bruno:** None. **M. Watanabe:** None. **A. Aiba:** None. **F. Nicoletti:** None.

Poster

122. Metabotropic Glutamate and GABA B Receptors

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 122.09/F6

Topic: B.03. G-Protein Coupled Receptors

Support: NIAAA DICBR
NIH Grant K99 AA025403

Title: Operant self-stimulation of thalamic terminals in the dorsomedial striatum is modulated by group II metabotropic glutamate receptors

Authors: *K. A. JOHNSON[1], D. M. LOVINGER[2]
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Abstract: The striatum plays a central role in the learning and performance of motivated behaviors. Cortical and thalamic glutamatergic inputs to the striatum are critical determinants of striatal neuron activity. In addition, both corticostriatal and thalamostriatal activation have been shown to elicit dopamine release, and thalamostriatal stimulation has recently been shown to support reinforcement of operant behavior. However, surprisingly little is known about how thalamostriatal transmission is regulated. Presynaptic G protein-coupled receptors such as group II metabotropic glutamate ($mGlu_{2/3}$) receptors can robustly modulate the strength of transmission at many synapses. We previously demonstrated that activation of $mGlu_2$ dramatically decreases the strength of thalamically-driven glutamate and dopamine release in the dorsal striatum. Because both glutamate and dopamine could play roles in the reinforcing properties of thalamostriatal stimulation, we predicted that $mGlu_2$ manipulation would modulate this form of reinforcement. To test this, we established a thalamostriatal intracranial self-stimulation paradigm. We expressed channelrhodopsin-2 (ChR2) in striatum-projecting thalamic neurons in the intralaminar nuclei of the thalamus and bilaterally implanted mice with optical fibers in the dorsomedial striatum. We trained the mice to press a lever for a brief (1 second, 20 Hz) train of optical stimulation on an FR1 reinforcement schedule. Mice responded vigorously to this form of

ICSS, averaging 400 presses in a 30-minute training session. We then evaluated the effects of pharmacological manipulation of $mGlu_{2/3}$ receptors. Consistent with its ability to dampen thalamically-driven glutamate and dopamine release, the $mGlu_{2/3}$ agonist LY379268 (3 mg/kg, i.p.) robustly reduced the rate of pressing for thalamostriatal ICSS. Conversely, the $mGlu_{2/3}$-preferring antagonist LY341495 (3 mg/kg, i.p.) did not significantly alter response rates. To determine the specificity of these effects, we trained a separate cohort of mice to lever press for a palatable food reinforcer. In contrast to the effects on thalamostriatal ICSS, LY379268 caused a very modest decrease in response rates, whereas LY341495 robustly decreased rates of responding for food. Our findings suggest that group II mGlu receptors differentially influence operant behavior depending on the type of reinforcement. Ongoing studies aim to dissect the circuit-specific roles of $mGlu_2$ in reinforcement of operant behaviors.

Disclosures: K.A. Johnson: None. **D.M. Lovinger:** None.

Poster

122. Metabotropic Glutamate and GABA B Receptors

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 122.10/F7

Topic: B.03. G-Protein Coupled Receptors

Support: NIH Pharmacology Training Grant T32-GM07628
Rettsyndrome.org

Title: Modulating the metabotropic glutamate receptor 3 as a potential therapeutic intervention for MECP2-associated disorders

Authors: *S. D. VERMUDEZ[1,2], R. G. GOGLIOTTI[1,2], B. J. STANSLEY[1,2], N. M. FISHER[1,2], J. L. ENGERS[1,2], C. W. LINDSLEY[1,2,3], P. J. CONN[1,2,4], C. M. NISWENDER[1,2,4]
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Abstract: Both loss-of-function mutations and duplication of the *methyl-CpG-binding protein 2* (*MECP2*) gene are associated with the neurodevelopmental disorders, Rett syndrome (RTT) and *MECP2* Duplication syndrome (MDS), respectively. Consistent with their opposing molecular origins, many phenotypes of RTT and MDS are anti-parallel, spanning symptom domains of cognition, anxiety and sociability. These phenotypes are recapitulated in mouse models of each disorder, and, importantly, almost all phenotypes can be rescued through genetic normalization of MeCP2, even at advanced symptomatic time points. This indicates that the neurodevelopmental components of RTT and MDS are not static, but rather responsive to interventions that target the pathophysiology of the disease.
Our therapeutic approach focuses on identifying potentially druggable candidates that are both

involved in neurotransmission and sensitive to changes in MeCP2 dosage. One broad class of receptors that match these criteria is the metabotropic glutamate receptors. Specifically, we are evaluating the Group II mGlu receptor, $mGlu_3$, which our preliminary data indicate is decreased in the brains of RTT patients and has previously been linked to cognitive deficits in other neurological disorders. **We hypothesize that modulating $mGlu_3$ function using allosteric modulators will validate $mGlu_3$ as a potential therapeutic target for cognitive phenotypes in *MECP2*-associated disorders.**
Using mouse models of RTT and MDS, we are evaluating the efficacy of a Group II mGlu receptor agonist and/or an $mGlu_3$ negative allosteric modulator (NAM, VU0650786) in improving deficits in synaptic plasticity and cognitive phenotypes. Here, we present our current findings that, despite the opposing molecular origins of these disorders, negatively modulating $mGlu_3$ does not correct behavioral phenotypes of MDS mouse model, but may enhance a form of long-term synaptic plasticity that is impaired in MDS mice. This implies a more complex role of $mGlu_3$ in MDS that needs to be further explored. Conversely, we have data suggesting that decreased $mGlu_3$ expression is a conserved aspect of RTT in patients and mouse models, which may contribute to cognitive impairments that we predict to be sensitive to $mGlu_3$ positive modulation.

Disclosures: S.D. Vermudez: None. **R.G. Gogliotti:** None. **B.J. Stansley:** None. **N.M. Fisher:** None. **J.L. Engers:** None. **C.W. Lindsley:** None. **P.J. Conn:** None. **C.M. Niswender:** None.

Poster

122. Metabotropic Glutamate and GABA B Receptors

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 122.11/F8

Topic: B.03. G-Protein Coupled Receptors

Support: NIH Grant RO1MH062646
NIH Grant R37NS031373
NIH Grant T32MH093366

Title: Efficacy of $mGlu_2$and $mGlu_3$negative allosteric modulators in preclinical models of major depressive disorder

Authors: *C. I. SANTIAGO[1], M. E. JOFFE[2], J. L. ENGERS[3], C. W. LINDSLEY[4], P. J. CONN[2]
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Abstract: Introduction: Major depressive disorder (MDD) is one of the most prevalent psychiatric illnesses, affecting more than 6% of the U.S. population. Primary symptoms include

persistently depressed mood and a loss of motivation to engage in pleasurable activities. While currently available medications are generally safe and well-tolerated, they exhibit unsatisfactory remission rates and onset latency to reduce depressive symptoms, necessitating the development of new treatments that will increase remission rates and alleviate symptoms faster. Nonselective antagonists for metabotropic glutamate (mGlu) receptor subtypes 2 and 3 exhibit rapid anti-depressant-like effects in rodent models, however the relative contribution of the individual mGlu subtypes remains unclear. Therefore, we are investigating whether selective negative allosteric modulators (NAMs) of $mGlu_2$ and $mGlu_3$ will reverse MDD-like behavioral disruptions in preclinical models of chronic stress.
Methods: To model MDD symptomology, we treated adult C57Bl/6J mice with the stress hormone corticosterone over a 4-week period. Following a week period, we conducted a behavioral battery consisting of the sucrose preference test, tail suspension test, and forced swim test. In addition, we implemented a chronic variable stress (CVS) model and performed similar behavioral assays. The $mGlu_2$ NAM VU6001966 and $mGlu_3$ NAM VU0650786 were administered systemically.
Results: A single dose with either VU6001966 or VU0650786 reversed anhedonic-like behaviors in the sucrose preference test, 24-hours after drug treatment. Both mechanisms decreased immobility in the forced swim test, while only VU0650786 retained efficacy in the tail suspension test. Similar findings were observed using the CVS model.
Conclusion: Taken together, these data posit that both $mGlu_2$ and $mGlu_3$ NAMs exert antidepressant-like effects and that both targets warrant further scrutiny in preclinical models of MDD and other affective disorders. Continued investigation into the differential antidepressant effects of $mGlu_2$ and $mGlu_3$ NAMs will allow us to parse apart the diverging mechanisms through which these receptors operate and will aid in translational efforts to develop these approaches into efficacious treatments.

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Poster

123. Sodium Channels

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 123.01/F9

Topic: B.04. Ion Channels

Support: R01 MH111107 (FL & JZ)
R01 MH095995 (FL)
John Sealy Memorial Endowment Funds (FL)
T32 AG051131 (PW)

Title: Protein:protein interaction-based peptidomimetics targeting the Nav channel complex

Authors: *A. K. SINGH[1], P. WADSWORTH[2], Z. LIU[3], P. WANG[3], S. R. ALI[3], H. CHEN[3], J. ZHOU[3], F. LAEZZA[3]
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Abstract: The voltage-gated Na+ (Nav) channel Nav1.6 is regulated by accessory proteins such as intracellular fibroblast growth factor 14 (FGF14). Studies have shown that FGF14 binds directly to the Nav1.6 C-tail leading to modulation of the channel's biophysical properties. Thus, short peptides or small molecules interfering with the FGF14:Nav1.6 channel interface might serve as specific inhibitors of Nav1.6 and modulators of neuronal excitability. Recently, we have applied homology modeling to define the FGF14:Nav1.6 interface and designed putative interfering peptides of the FGF14:Nav1.6 channel complex formation. Here, we applied *in silico* docking and a combination of split-luciferase complementation assay (LCA) and patch-clamp electrophysiology to reconstitute the FGF14:Nav1.6 channel complex and test each peptide efficacy in disrupting the complex formation. *In silico* peptide docking predicted FLPK to interact with the previously identified "hot-spots", $FGF14^{Y158}$ and $FGF14^{V160}$, at the FGF14:Nav1.6 channel complex interface, finding that was confirmed by surface plasmon resonance (SPR) studies. In cell LCA demonstrated that FLPK disrupts the FGF14:Nav1.6 channel complex formation and that the effect is abolished by $FGF14^{Y158N/V160N}$ mutations. Whole-cell patch clamp electrophysiology studies showed that FLPK prevents FGF14-dependent regulation of Nav1.6 currents, reversing previously reported changes in peak current density and voltage sensitivity of Nav1.6 that occur in the presence of FGF14. On the basis of these results, we designed a series of peptidomimetics derived from FLPK which are currently under investigation for functional activities toward Nav1.6. In conclusion, our findings identify the FGF14:Nav1.6 interface as an attractive target for future therapeutics against Nav channelopathies.
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Poster

123. Sodium Channels

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Program #/Poster #: 123.02/F10

Topic: B.04. Ion Channels

Support: R01 MH095995-A1
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5T32AG051131-02
T32 GM089657-04
John Sealy Memorial Endowment Funds/UTMB
MD-PhD Training Program in Health Disparities and Aging T32
Jeane B. Kempner Post-doctoral Fellowship

Title: Functional modulation of FGF13-1A or -1B mediated voltage-gated sodium channel (Nav 1.6) activity by novel peptidomimetics

Authors: *O. FOLORUNSO[1], P. WANG[1], P. WADSWORTH[2], Z. LIU[1], P. SCADUTO[3], L. BOURNER[1], S. R. ALI[1], H. CHEN[4], J. ZHOU[1], F. LAEZZA[1]
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Abstract: Voltage-gated sodium (Nav) channels interact with auxiliary proteins, including intracellular fibroblast growth factor 13 (FGF13), which modulate their biophysical properties in different regions within the CNS and PNS. These protein-protein interactions (PPI) are necessary to maintain neuronal excitability, and FGF13 dysfunction is associated with epilepsy and neuropathic pain. The physiologically relevant Nav1.6 C-terminal tail binds to FGF13, and the functional specificity of this interaction provides an opportunity for the development of novel Nav isoform-specific probes. Here, we combined the split-luciferase complementation assay (LCA) with molecular modeling to screen various peptidomimetics to target FGF:Nav channel interfaces. We identified two peptidomimetics (ZL192 and PW164) as potential regulators of FGF13-1A /-1B isoforms interaction with Nav1.6. We used whole-cell patch clamp electrophysiology to validate the effect these peptidomimetics on Nav1.6 channel activity in a heterologous system stably expressing human Nav1.6, and transiently transfected with GFP (Nav1.6) or FGF13-1A-GFP (FGF13-1A-Nav1.6) or FGF13- 1B- GFP (FGF13-1B-Nav1.6). There was no effect on the peak current density derived from transient Na^+ current of Nav1.6-ZL192 compared to Nav1.6-DMSO or FGF13-1A-Nav1.6-ZL192 compared to FGF13-1A-Nav1.6-DMSO. At voltage step 10mV, there was an increase in peak current density in Nav1.6-PW164 (-50.16 ± 11.28 pA/pF, n=8) compared to Nav1.6-DMSO (-12.95 ± 3.243 pA/pF, n=8), which mimicked FGF13-1A-Nav1.6-DMSO (-83.07 ± 19.7 pA/pF, n=4) (one-way ANOVA,

Homl-Sidak multiple comparison test). There was a ~6mV hyperpolarizing shift of $V_{1/2}$ of steady-state inactivation which indicates channel availability of Nav1.6-ZL192 compared to Nav1.6-DMSO, which mimicked FGF13-1B-Nav1.6-DMSO. FGF13-1A-Nav1.6-ZL192 masks the effect seen in Nav1.6-ZL192. Futhermore, there was no change in steady state inactivation Nav1.6-PW164 compared to Nav1.6-DMSO. However, there was a ~18mV hyperpolarizing shift of $V_{1/2}$ steady-state inactivation of FGF13-1B-Nav1.6-PW164 compared to FGF13-1B-Nav1.6-DMSO. Our results show that ZL192 mimics aspects of FGF13-1B-Nav1.6 channel activity, while PW164 mimics aspects of FGF13-1A-Nav1.6. The significance of this study is to generate novel specific modulators of excitability in epilepsy or pain, which we plan to investigate using animal models.

Disclosures: O. Folorunso: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); The compounds used for this work are covered under a "patent application' filed by the University of Texas Medical Branch, Galveston TX. This patent application has not been licensed to any company. **P. Wang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); The compounds used for this work are covered under a "patent application' filed by the University of Texas Medical Branch, Galveston TX. This patent application has not been licensed to any company.. **P. Wadsworth:** None. **Z. Liu:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); The compounds used for this work are covered under a "patent application' filed by the University of Texas Medical Branch, Galveston TX. This patent application has not been licensed to any company.. **P. Scaduto:** None. **L. Bourner:** None. **S.R. Ali:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); The compounds used for this work are covered under a "patent application' filed by the University of Texas Medical Branch, Galveston TX. This patent application has not been licensed to any company.. **H. Chen:** None. **J. Zhou:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); The compounds used for this work are covered under a "patent application' filed by the University of Texas Medical Branch, Galveston TX. This patent application has not been licensed to any company. **F. Laezza:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); The compounds used for this work are covered under a "patent application' filed by the University of Texas Medical Branch, Galveston TX. This patent application has not been licensed to any company..

Poster

123. Sodium Channels

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 123.03/F11

Topic: B.04. Ion Channels

Support: NIH Grant R37-NS39395

Title: Regulation of resurgent Na current of cerebellar Purkinje neurons by FGF14

Authors: *H. V. WHITE[1,2,3], T. C. BOZZA[2], J. M. NERBONNE[3], I. M. RAMAN[2]
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Abstract: Resurgent sodium current is a component of TTX-sensitive voltage gated Na current, in which channels that were blocked by an intracellular particle upon depolarization become unblocked upon repolarization.
The blocking particle(s) in Purkinje cells is likely to be a protein with amino acid sequence homology to the intracellular tail of Na channel β4 (Scn4b). To investigate the identity of the Purkinje cell blocker, we generated mice in which β4 was deleted throughout development and recorded voltage-clamped TTX-sensitive transient, persistent, and resurgent currents from dissociated Purkinje cells (P12-P20). Resurgent current was still present, with kinetics indistinguishable from wildtype cells, suggesting that other blocking proteins are present, either normally or owing to compensation. To identify such proteins, we searched RNAseq databases for proteins expressed by Purkinje cells containing an amino acid sequence with similarity to the proposed β4 blocking sequence. Nine candidate proteins resulted. We then tested the ability of synthetic peptides that mimicked each protein's putative blocking sequence to produce resurgent current in dissociated CA3 hippocampal neurons of P8-P15 mice, which normally lack an open-channel blocker. The most effective peptide came from fibroblast growth factor 14 isoform 1a (FGF14-1a). Mutation of FGF14, which is implicated in SCA27, has been previously shown to disrupt Purkinje cell firing and reduce resurgent current, although many of these effects are ascribed to FGF14-1b. To test whether FGF14 might directly block Na channels in Purkinje neurons, we recorded Na currents in dissociated Purkinje neurons of FGF14 -/- mice. In these cells, transient current decayed more rapidly, relative resurgent current was reduced across voltages, and persistent current was decreased. To test whether the FGF14 deletion reduced resurgent current simply by promoting fast inactivation owing to loss of FGF14-1b, we slowed fast inactivation with ATX-II, reasoning that this might remove the difference between wildtype and FGF14 -/- Na currents. In CA3 neurons, which express FGF14, ATX-II-modified Na current was indistinguishable in deletion and wildtype neurons. However, in ATX-II-modified Purkinje neurons, FGF14 -/- cells still had faster transient current, reduced persistent current, and smaller resurgent current than wildtype. The data suggest that multiple blockers may be present in Purkinje cells and that FGF14 may directly participate in generating resurgent current.

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Poster

123. Sodium Channels

Location: SDCC Halls B-H

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Program #/Poster #: 123.04/F12

Topic: B.04. Ion Channels

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Title: Identification of JAK2 and Src tyrosine kinases as regulators of the Nav1.6 channel complex by high-throughput drug screening

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Abstract: Neuronal firing is a highly regulated process that depends on the integrity of ion channel macromolecular complexes, which in turn rely on the stability of cellular signaling networks. In neurodegenerative disease states, the cellular milieu of neurons is skewed away from growth and toward pro-inflammatory mediators. These changes have drastic consequences on neuronal firing, which could be a consequence of changes in phosphorylation events at the voltage-gated Na^+ (Nav) channel macromolecular complex. This complex is made up of multiple Nav channel regulators including fibroblast growth factor 14 (FGF14), which we have previously demonstrated affects the biophysical properties of the Nav channel through phosphorylation mediated by glycogen synthase kinase 3 (GSK3). However, the specific upstream signaling events that control this complex have not been well characterized. Here, we posited that the cytokine tumor necrosis factor-α (TNF-α) pathway, which has been previously shown to modulate Nav1.6 firing, could play a role in regulating the FGF14 interaction with Nav1.6 through the cascade of Ser/Thr and/or Tyr kinases downstream of the TNF receptor. To test this hypothesis, we applied the split-luciferase complementation assay (LCA) to reconstitute the FGF14:Nav1.6 channel complex in the cellular milieu and examined the sensitivity of the complex to variable concentrations of TNF-α. We showed that human recombinant TNF-α concentrations as low as 50 pg/mL produce a significant increase in the FGF14:Nav1.6 complex assembly (156% vs no treatment). Based on these findings, we predict that formation of this

protein complex might be dysfunctional in neuroinflammatory states due to aberrant cellular signaling. Thus, we have adapted the LCA for a high-throughput drug screening to search for kinases activated by TNF-α signaling that may control the formation of this complex. We screened > 3,000 compounds against known mediators of cellular signaling, and hits were selected based on counter-screening against full-length luciferase, cellular toxicity, potency, and dose response relationships. Bioinformatic analysis revealed that TNF-α regulates the Nav1.6 complex through activation of signaling cascades skewed toward tyrosine kinase phosphorylation events that converge on GSK3 and FGF14. Specifically, we have identified the JAK2 and Src tyrosine kinases as primary regulators of the Nav complex, in addition to other mediators of TNF-α signaling. Altogether, these studies identify new determinants of Nav complex regulation that might serve as a base for therapeutic development.

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Poster

123. Sodium Channels

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Topic: B.04. Ion Channels

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NS078171

Title: CaMKII modulation of aberrant Nav1.6 activity

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Abstract: Altered sodium currents (I_{Na}) produced by neuronal voltage-gated sodium channel 1.6 (Nav1.6) are critical drivers of hyperexcitability and a hallmark characteristic associated with epilepsy. To this end, an important component of I_{Na} is the persistent sodium current (I_{NaP}). Produced by Nav1.6, I_{NaP} can amplify neuronal synaptic inputs and mediate repetitive action potential firing. Therefore, aberrant alterations in these currents can significantly affect neuronal excitability by contributing to the pro-excitatory changes observed in epilepsy. The Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) is also a critical driver of excitability and is known to modulate transient and persistent I_{Na} in other Nav isoforms. For these reasons, we sought to investigate the ability of CaMKII to modulate Nav1.6 sodium currents. We first

determined the biophysical consequences of CaMKII modulation on Nav1.6 by obtaining whole-cell voltage clamp recordings of ND7/23 cells transiently expressing a TTX-resistant Nav1.6 with >1μM Ca^{2+}/calmodulin in the pipette solution to activate endogenous CaMKII. To assess CaMKII-specific effects, we compared Nav1.6 sodium currents and biophysical properties with and without the CaMKII peptide inhibitor CN21 (1 μM) or its inactive analogue, CN21Ala, in the pipette. We found that inhibition of CaMKII with CN21 resulted in significant decreases in peak transient and persistent current densities in addition to alterations in the voltage-dependence of activation and channel availability. To further identify if CaMKII-induced alterations in Nav1.6 activity are occurring via direct phosphorylation of the channel, we analyzed αCaMKII-dependent phosphorylation of the intracellularly accessible regions of Nav1.6 using immobilized peptide arrays. We identified five putative CaMKII phosphorylation sites within the first intracellular loop that links domains I and II of the channel. These findings suggest that Nav1.6 is a substrate for αCaMKII phosphorylation and mediates enhanced Nav1.6 channel activity that could contribute to the hyperexcitability associated with inherited and acquired epilepsies.

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Poster

123. Sodium Channels

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Title: Sodium channel $Na_V1.3$ regulation of human cerebral cortical folding and oral motor development

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Abstract: Channelopathies are disorders caused by abnormal ion channel function in differentiated, excitable tissues. We discovered a unique neurodevelopmental channelopathy resulting from pathogenic variants in *SCN3A*, a gene encoding the voltage-gated sodium channel $Na_V1.3$. Pathogenic $Na_V1.3$ channels showed altered biophysical properties including increased persistent current. Remarkably, affected individuals showed disrupted folding of the perisylvian

cortex of the brain (polymicrogyria) but did not typically exhibit epilepsy; they presented with prominent speech and oral motor dysfunction, implicating *SCN3A* in prenatal development of human cortical language areas. The development of this embryonic disorder parallels *SCN3A* expression, which was highest early in fetal cortical development in progenitor cells of the outer subventricular zone and cortical plate neurons, and decreased postnatally when *SCN1A* ($Na_V1.1$) expression increased. Disrupted cerebral cortical folding and neuronal migration were recapitulated in ferrets expressing the mutant channel, underscoring the unexpected role of *SCN3A* in progenitors and migrating neurons.

Disclosures: R.S. Smith: None. **C. Kenny:** None. **R. Borges-Monroy:** None. **A. Jang:** None. **J. Partlow:** None. **M.K. Lehtinen:** None. **C.A. Walsh:** None.

Poster

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Topic: B.04. Ion Channels

Support: NRF-2016R1A2B4011333

Title: Norquetiapine blocks the human cardiac sodium channel, $Na_v1.5$, in a state-dependent manner

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Abstract: Quetiapine, an atypical antipsychotic, has been used for the treatment of schizophrenia and acute mania. Although a previous report showed that quetiapine blocked hERG potassium current, it has not been reported serious cardiac toxicity and whether the drug affects on other channels such as sodium channels. In the present study, we investigated whether quetiapine and its a major metabolite, norquetiapine, blocked the human cardiac sodium channel, Nav1.5 (hNav1.5), using the whole-cell patch-clamp technique. Quetiapine and norquetiapine inhibited hNav1.5 currents with more potency at more depolarized membrane potentials. The half-maximal inhibitory concentration of quetiapine and norquetiapine at -90 mV of holding potential near the resting potentials of cardiomyocyte were 30 and 6 μM, respectively. While the voltage dependency of activation of hNav1.5 was not changed by norquetiapine, the steady-state inactivation curve was hyperpolarized in the presence of norquetiapine. The recovery from inactivation in the presence of norquetiapine was slower than in the absence of norquetiapine. Norquetiapine also showed strong use-dependent inhibition of hNav1.5 current. Our results indicate that norquetiapine blocks hNav1.5 current in concentration-, state- and use-dependent manners. The blocking mechanism of norquetiapine is similar to the class IB antiarrythmic

sodium channel blockers, suggesting that the drug can shorten the cardiac action potential duration and may reduce the risk of QT interval prolongation induced by the inhibition of hERG potassium current.

Disclosures: S. Kim: None. **D. Kim:** None. **J. Choi:** None.

Poster

123. Sodium Channels

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Topic: B.04. Ion Channels

Support: NIH grant MH065339
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Title: Expression of sodium channels in the developing grasshopper brain and *Drosophila*

Authors: H. WANG[1], T. A. RAVENSCROFT[2], B. FOQUET[3], H. SONG[3], H. BELLEN[4], M. N. RASBAND[5], H. DIERICK[4], *F. GABBIANI[6]
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Abstract: Sodium channels play a critical role in the initiation of action potentials in most neurons. We investigated the expression pattern of sodium channels in the brains of developing grasshoppers (*Schistocerca americana*) and *Drosophila melanogaster* using a mouse monoclonal pan-Nav antibody. In *Drosophila*, sodium channels are encoded by the paralytic gene. We used its sequence to locate *S. americana* orthologs in a transcriptome database and found transcripts with a high degree of homology. Further, we found high homology of both the *Drosophila* and *S. americana* amino acid sequence with that used to generate pan-Nav. Immunohistochemistry showed extensive staining in the optic lobe and the mushroom body of juvenile grasshoppers. Similarly, in *Drosophila*, pan-Nav was found to be highly expressed in the optic lobe and the brain. Its staining pattern largely coincided with a GFP-tagged endogenous Para protein that was generated through RMCE conversion of a MiMIC insertion in the *para* locus. These results show that sodium channels are highly conserved in insects and suggest they may be highly clustered to initiate spikes in grasshopper neurons.

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Poster

123. Sodium Channels

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Topic: B.04. Ion Channels

Title: Kinetic properties and pharmacology of voltage-gated Na channels involved in pain pathways

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Abstract: Voltage-gated Na (NaV) channels expressed in dorsal root ganglion neurons (DRG) such as $Na_V1.8$ and $Na_V1.9$ have been proposed to play important roles in nociception and pain signalling. $Na_V1.8$ and $Na_V1.9$ are exclusively expressed in dorsal root ganglion (DRG) neurons, with $Na_V1.9$ being selectively expressed in small diameter C fibres. The $Na_V1.8$ channel is the predominant channel driving TTX-resistant action potentials (AP) in DRG neurons and plays a major role in shaping the AP waveform due to its slow rate of inactivation. Given its relatively depolarized voltage dependence of inactivation, $Na_V1.8$ can contribute to action potential generation even at depolarized membrane potentials which may occur during nerve injury or pain signalling. This property, coupled with its location in DRG neurons and the modification of expression patterns in animal models of pain and human pain states, has meant that $Na_V1.8$ has received attention as a novel target for pain therapeutics for chronic, inflammatory and neuropathic pain. Although $Na_V1.9$ probably does not contribute to action potential amplitude, it most likely acts as a threshold channel, contributing to resting membrane potential and lowering the threshold for action potentials thereby increasing repetitive firing. Gain-of function mutations in human pain disorders points to a role of $Na_V1.9$ in pain sensation and transmission in humans. Until recently the expression of $Na_V1.8$ or 1.9 proved problematic in heterologous expression systems. We have utilized two cell lines for $Na_V1.8$, either in $hNa_V1.8$ expressed in CHO cells or rNaV1.8 expressed in ND7-23 cells, and one cell line for $hNa_V1.9$ expressed in HEK293 cells. We have used automated patch clamp to investigate the activation, inactivation and pharmacological properties of $hNa_V1.8$, $rNa_V1.8$ and $hNa_V1.9$. For $Na_V1.8$, the Vhalf of activation and inactivation was approximately -9 mV and -24 mV, respectively, in good agreement with the literature. For $Na_V1.9$, the Vhalf of activation and inactivation was approximately -40 mV and -36 mV, respectively. Concentration dependence of known blockers on $Na_V1.8$ and $Na_V1.9$ such as lidocaine and tetracaine will be shown. The potency of tetracaine showed state dependence with a higher affinity for the inactivated state of $Na_V1.8$.

Disclosures: **T. Strassmaier:** A. Employment/Salary (full or part-time):; Nanion Technologies. **J.L. Costantin:** A. Employment/Salary (full or part-time):; Nanion Technologies. **N. Brinkwirth:** A. Employment/Salary (full or part-time):; Nanion Technologies. **A. Obergrussberger:** A. Employment/Salary (full or part-time):; Nanion Technologies. **S. Stölzle-Feix:** A. Employment/Salary (full or part-time):; Nanion Technologies. **N. Becker:** A. Employment/Salary (full or part-time):; Nanion Technologies. **C. Haarmann:** A. Employment/Salary (full or part-time):; Nanion Technologies. **M. Rapedius:** A. Employment/Salary (full or part-time):; Nanion Technologies. **T.A. Goetze:** A. Employment/Salary (full or part-time):; Nanion Technologies. **I. Rinke-Weiß:** A. Employment/Salary (full or part-time):; Nanion Technologies. **C.T. Bot:** A. Employment/Salary (full or part-time):; Nanion Technologies. **R. Haedo:** A. Employment/Salary (full or part-time):; Nanion Technologies. **M. George:** A. Employment/Salary (full or part-time):; Nanion Technologies. **A. Brüggemann:** A. Employment/Salary (full or part-time):; Nanion Technologies. **N. Fertig:** A. Employment/Salary (full or part-time):; Nanion Technologies.

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123. Sodium Channels

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Title: Loss of NF-186 alters the distal accumulation of sodium channels within the axon initial segment

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Abstract: Voltage gated sodium channels (Na_vs) are recruited to the axon initial segment (AIS) following the enrichment of AnkyrinG (AnkG), a scaffolding protein commonly used to define the AIS. Previous studies have proposed that an AnkyrinG-binding motif within an intracellular loop of the Na_v is sufficient for targeting Na_vs to the AIS. However, the role of other AIS proteins in the targeting of Na_vs to this region remain undetermined. Also uniquely localized at the AIS are cellular adhesion molecules (CAMs) such as Neuronal Cellular Adhesion Molecule (NrCAM) and Neurofascin-186 (NF-186). While these molecules are known for their functions in neurite extension, axon formation, and neuron-glial interactions, the effect that they may have on AIS maintenance, organization and function is less clearly defined. Recent literature has demonstrated a role for NF-186 on Na_v stability, yet the specific mechanism in which this occurs remains elusive. Our aim is to identify a potential role of NF-186 in stabilizing critical AIS

components, thereby influencing structural parameters of the AIS, as well as to elucidate the functional consequences of NF-186 loss on action potential (AP) firing. Here, using primary rat hippocampal cultures, we combined localization measurements or various AIS proteins with genetic ablation of NF-186 using CRISPR. These localization experiments were combined with electrophysiology to elucidate any changes in AP firing or waveform shape. The loss of NF-186 selectively impaired AnkG accumulation relative to Na_Vs. Additionally, ablating NF-186 altered the distribution of Na_Vs relative to the remaining AnkG within the AIS, enriching them more proximally than in controls. These results challenge the dogma that the distribution of Na_Vs is solely influenced by the distribution of AnkG within the AIS. Moreover, no changes in AP firing or waveform shape were observed when NF-186 was depleted, suggesting compensatory mechanisms in action potential initiation. NF-186 may play a direct role in the distal stabilization of Na_Vs within the AIS, yet compensatory mechanisms may render this phenomenon unimportant for AP firing.

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Poster

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Support: NIH Grant CA196263
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Title: Excitability changes in trigeminal nerve induced by entrapment neuropathy: Role of voltage gated sodium channels

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Abstract: Chronic facial pain as a consequence of injury to one or more branches of the trigeminal nerve can be excruciating. Evidence suggests that increased excitability of injured nerves and local accumulation of certain ion channels have played an important role in the development and maintenance of chronic pain symptoms. Voltage gated sodium (NaV) channels are critical for the transmission of afferent signals as they contribute to majority of the inward current during action potential upstroke and abnormal accumulation of select NaV subtypes in injured nerves has been shown in animal models and human studies. However, the role of NaV in trigeminal nerve excitability still requires further investigation. To address this issue, we developed an *ex vivo* method to record compound action potentials (CAP) from rat infraorbital

nerves (IoN) using suction electrodes and compared the excitability changes of sham operated and neuropathic rats, before and after cumulative application of tetrodotoxin (TTX) and the NaV1.8 selective blocker, A-803467. Trigeminal neuropathy in rats was induced by placing two Tygon cuffs around the IoN. Pain behaviors were evaluated with von Frey stimulation of vibrissal pad region and video recordings of facial grooming. We also examined the expression of NaV1.8 in the infraorbital nerve with Western blot analysis and mRNA changes of NaV1.3, NaV1.5, NaV1.6, NaV1.7, NaV1.8 & NaV1.9 after IoN constriction. Results showed significant decreases in von Frey withdrawal thresholds ipsilateral to the side of nerve entrapment at 1 week and 2 weeks post-surgery. Neuropathic rats also displayed increased asymmetric grooming of facewash strokes directed to the area of IoN innervation. CAP recordings revealed increased excitability of A and C fibers in neuropathic rats compared to the sham group. Application of TTX (300 nM) and A-803467 (5 µM) significantly decreased A-fiber CAP amplitude in neuropathic rats with a trend of decreased C-CAP amplitude, compared to sham rats. Western blot analysis showed a trend towards increased expression NaV1.8 in the injured IoN. In addition, our qPCR data also showed a significant increase in the expression of NaV1.6, NaV1.8 and NaV1.9 mRNAs in the IoN of neuropathic rats. In conclusion, our data suggests that increased accumulation of select NaVs plays an important role in increased peripheral nerve excitability after trigeminal nerve injury and resultant chronic pain behaviors.

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Poster

123. Sodium Channels

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Title: cAMP signaling-independent acidity-sensing of olfactory sensory neurons is mediated by acid-sensing ion channels

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Abstract: Olfactory sensory neurons (OSNs) in the main olfactory epithelia (MOE) rely on odorant receptors and type 3 adenylyl cyclase 3 (AC3) in olfactory cilia to transmit olfactory

signal into the neurons. It is unknown how OSNs of mammals detect volatile acid (H+), the simplest odor ligand in the air and how volatile acidic odorants influence our normal olfaction. Here we show that OSNs can detect acidity in the presence and absence of AC3. Forskolin/IBMX treatment (interfering with cAMP signaling) blocked odor-mediated electro-olfactogram (EOG) responses, but not acid-induced EOG responses. In AC3 knockout mice, acid-evoked EOG responses in MOE were very pronounced compared to odorant-induced signal. Moreover, the acid-evoked EOG currents were completely blocked by diminazine, an acid-sensing ion channels (ASICs) inhibitor, but not affected by forskolin/IBMX. Behaviorally, AC3 knockout mice lose the sensitivity to detect regular odorants, but retain the ability to detect volatile acids in an olfactory habituation-dishabituation test. Immunofluorescence staining and fluorescence *in situ* hybridization results show that ASIC1a and 2a are highly expressed in OSNs, and enriched in the cell body and dendrites, but not in olfactory cilia of OSNs. We conclude that cAMP signaling-independent acidity-sensing of olfactory sensory neurons is directly mediated by ASICs, and that olfactory sensory neurons utilize a non-canonical signal pathway to sense acidity in the air.

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Poster

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Title: Enhancement of Multi-Electrode Array "Pain-in-a-dish" assays by adding optogenetic stimulation

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Abstract: An increasing number of ion channel mutations have been shown to cause disorders of excitability. As additional mutations have been identified, the need for assays with higher throughput than manual patch-clamping is growing. Multi-electrode array (MEA) instruments provide one such pathway to higher throughput. Multi-electrode array technology allows for the

simultaneous monitoring of large numbers of neurons for spontaneous spiking activity and has the additional advantage that the monitoring is non-invasive and the same preparation can be repeatedly monitored over several days, and neurons could be stimulated using physiologically relevant stimuli including physiological temperatures of skin (33ºC) and core body (37ºC). We have previously reported MEA data showing the enhancement of DRG excitability when disease-associated variants of Nav1.7 are transiently expressed compared to wild-type Nav1.7 (Yang et.al. 2017 - I234T, Geha et.al. 2016 - S241T, Yang et.al. 2016 - A1632G). Transient transfection efficiency, however, is not high enough to ensure that every spiking neuron observed with the MEA instrument is expressing the desired channel construct. Here we describe improvements to the resolution and scope of our MEA "Pain-in-a-dish" assay by utilizing an optogenetic construct as the transfection marker. Optogenetic responses should positively identify neurons that have been transfected and analysis of this subset of neurons may result in higher signal to noise when comparing populations of neurons transfected with mutant constructs to those transfected with wild-type constructs. In addition, reproducible control of optogenetic light power and duration allows for the programming of light stimulus protocols that mimic threshold and firing frequency responses. Quantifying stimulated responses in addition to spontaneous spiking should reveal additional metrics to characterize enhanced excitability caused by ion channel variants compared to their wild-type construct.

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Poster

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Title: Search for molecular determinants of Nav1.9 functional expression

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Abstract: Over the last decade sodium channel Nav1.9 gradually earned increased attention of the neuroscience community. Soon after its discovery in 1998, this channel was shown to be

involved in regulating inflammatory and neuropathic pain in rodents, while more recent studies of human pain disorders have demonstrated its important role in both regulating sensory neuron excitability and in pain signaling. These discoveries have made Nav1.9 a promising therapeutic target for pain. However, research involving Nav1.9 has been slowed down by lack of robust and reliable functional expression of this channel in a heterologous system. To address this issue, we are implementing an approach to identify molecular determinants that are required for functional expression of Nav1.9 in neurons and heterologous expression systems.
The small amplitude Nav1.9 currents in heterologous cells could be caused by low density of channels at the plasma membrane or by the absence of channel partners that regulate gating. Voltage-clamp recordings cannot discriminate between these two alternative explanations. To test the hypothesis that Nav1.9 channels are only present at low density at the plasma membrane in HEK293 cells, we made DNA constructs carrying a Biotin Acceptor Domain (BAD) inserted into one of the extracellular loops of domain IV in Nav1.9 and Nav1.7, as well as a fluorescent protein at the termini. We transiently transfected HEK293 cells with these constructs and compared the surface expression of Nav1.9 and Nav1.7 using Total Internal Reflection Fluorescence (TIRF) microscopy. We found that surface expression of Nav1.9 is dramatically lower compared to Nav1.7 in most transfected cells.
It has also been recently shown that the C-terminus of Nav1.9 makes a major contribution to the low functional expression of this channel in heterologous systems. To identify Nav1.9 specific protein partners that bind that region we made DNA constructs carrying C-terminal parts of either Nav1.9 or Nav1.7 linked to a Strep-Flag tag for easier identification of channel expression and for possible subsequent tandem affinity purification (TAP) of protein complex involved in Nav1.9 functional expression. In transiently transfected HEK cells, the C-terminal part of Nav1.9 showed dramatically lower steady-state levels of the protein compared to Nav1.7. We are using C- terminal Nav1.7-Nav1.9 chimeric constructs to narrow down the molecular determinants that regulate protein stability and will proceed to search for protein(s) that interact with these Nav1.9 sequences and which may regulate Nav1.9 functional levels.

Disclosures: D. Sizova: None. **L. Akin:** None. **S.D. Dib-Hajj:** None. **S.G. Waxman:** None.

Poster

123. Sodium Channels

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 123.15/DP02/F23

Topic: B.04. Ion Channels

Support: Center Grant B9253-C from the U.S. Department of Veterans Affairs Rehabilitation Research and Development Service
Paralyzed Veterans of America

Title: High-resolution imaging of $Na_v1.7$ cell-surface delivery and membrane dynamics in developing DRG neurons

Authors: *E. J. AKIN[1,2,3], S. LIU[1,2,3], F. B. DIB-HAJJ[1,2,3], S. D. DIB-HAJJ[1,2,3], S. G. WAXMAN[1,2,3]
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Abstract: Voltage-gated sodium (Na_v) channels are responsible for the rising phase of the action potential in excitable cells, including dorsal root ganglion (DRG) neurons. These peripheral sensory neurons primarily express a mixture of $Na_v1.6$, $Na_v1.7$, $Na_v1.8$, and $Na_v1.9$. The trafficking and insertion of Na_v channels in plasma membrane is precisely regulated since alterations in the number, type, or distribution of Na_v channels can be pathogenic. For example, increased expression levels of $Na_v1.3$ and accumulation of other Na_v channels within the plasma membrane of axonal neuromas after nerve injury contribute to neuropathic pain. Here we investigate how DRG neurons process $Na_v1.7$ proteins in real-time as they are building excitable membranes. $Na_v1.7$ is of major interest due to its key role in pain signaling. Gain-of-function mutations of $Na_v1.7$ can cause severe pain syndromes such as inherited erythromelagia (IEM), and loss-of-function mutations can cause an absence of pain phenotype. We describe the use of a modified $Na_v1.7$ construct suitable for live-cell imaging with a fluorescent protein tag (Venus) fused to the N-terminus and a biotin acceptor domain (BAD) tag inserted in an extracellular loop to allow specific labeling of surface channels. The BAD tag is a sequence of amino acids recognized by a bacterial biotin ligase (BirA) that biotinylates the lysine residue in the middle of the sequence. Thus, channels correctly inserted in the plasma membrane can be labeled with membrane impermeable streptavidin-conjugated fluorophores. We used this Venus-$Na_v1.7$-BAD construct to visualize $Na_v1.7$ trafficking and cell-surface dynamics in living DRG neurons. Neurons transiently transfected with Venus-$Na_v1.7$-BAD were imaged on a spinning disk confocal microscope 36-60 hrs after plating and transfection. The biotin tags for channels located on the cell surface at the start of the experiment were blocked with NeutrAvidin. A streptavidin-conjugated fluorophore, CF640R, was added to the bath during imaging where it selectively labeled channels that were newly inserted in the neuronal membrane. This high-resolution assay allows analysis of the location and number of channels inserted in the membrane in real-time with single-molecule accuracy, as well as the ability to observe cell-surface dynamics of $Na_v1.7$ molecules after membrane insertion. The development of this in vitro assay will allow us to better understand the regulation of trafficking and dynamic regulation of $Na_v1.7$ channels in real time and under different culture conditions, which could mimic normal and pathological conditions.

Disclosures: E.J. Akin: None. **S. Liu:** None. **F.B. Dib-Hajj:** None. **S.D. Dib-Hajj:** None. **S.G. Waxman:** None.

Poster

123. Sodium Channels

Location: SDCC Halls B-H

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Program #/Poster #: 123.16/F24

Topic: B.04. Ion Channels

Support: This work was supported in part by Center Award # B9253-C from the U.S. Department of Veterans Affairs Rehabilitation Research and Development Service

Title: Cell- and temperature-dependent effects of the Nav1.7-L858F inherited erythromelalgia mutation on the excitability of dorsal root ganglia neurons

Authors: *M. A. MIS, E. J. AKIN, F. DIB-HAJJ, P. ZHAO, S. DIB-HAJJ, S. G. WAXMAN
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Abstract: Nav1.7 sodium channels are widely expressed in small and large dorsal root ganglion (DRG) neurons, and contribute most of the TTX-sensitive current in small DRG neurons. Patients carrying inherited erythromelalgia (IEM) mutations in the Nav1.7 sodium channel experience pain attacks triggered by warm temperatures, but they have not reported mechanical allodynia. However, the cellular basis of the thermal sensitivity characteristic of this channelopathy is not well understood. IB4+ and IB4- DRG neurons have been shown to have different neurochemical makeups and be involved in different types of pain. Specifically, IB4- neurons, which have large heat-evoked currents, were implicated in thermal allodynia and inflammatory pain, while IB4+ neurons were linked to mechanical allodynia and chronic pain. We hypothesized that there is a cell-type specific effect of IEM mutations which may underlie thermal sensitivity in IEM.
Here, we report the properties of the Nav1.7-L858F IEM channel at the physiologically-relevant skin temperature of 33°C in small-diameter IB4+ and IB4- mouse DRG neurons. To determine the properties of the Nav1.7-L858F mutation at room temperature (RT, ~22°C) and 33°C, we expressed Nav1.7-Wild-Type (WT) and Nav1.7-L858F (L858F) channels in DRG neurons from 4-8 week-old C57Bl/6 mice. IB4+ neurons were identified using the vital label fluorescein isothiocyante-conjugated isolectin B4 (IB4-FITC). Our voltage-clamp recordings demonstrate significant difference in current density between WT and L858F channels at 33°C in IB4-, but not IB4+ DRG neurons. Current-clamp recordings demonstrate significant increase in firing frequency in IB4- neurons expressing L858F channels, but not IB4+ DRG neurons at 33°C. Since differences in current density may be the result of differential cell surface expression, constructs for WT and L858F channels containing a fluorescent protein tag (Venus) and an extracellular surface tag (biotin acceptor domain) (Venus-Nav1.7-BAD) were used to selectively label surface channels with a streptavidin-conjugated fluorophore, CF640R. IB4+ and IB4- neurons expressing either WT or L858F channels were imaged on a spinning disk

microscope at RT or 33°C. IB4- neurons expressing L858F-Venus-Nav1.7-BAD imaged at 33°C showed significantly more surface labeling than all other conditions. These data suggest that the increased current density is due to more surface expression of L858F channels in IB4- neurons at 33°C. Taken together, these results point to novel and cell-specific L858F mutation effects, and advance our current mechanistic understanding of the thermal sensitivity of the IEM phenotype.

Disclosures: M.A. Mis: None. **E.J. Akin:** None. **F. Dib-Hajj:** None. **P. Zhao:** None. **S. Dib-Hajj:** None. **S.G. Waxman:** None.

Poster

123. Sodium Channels

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 123.17/F25

Topic: B.04. Ion Channels

Support: Center Grant B9253-C from the U.S. Department of Veterans Affairs Rehabilitation Research and Development Service
Paralyzed Veterans of America

Title: Fibroblast growth factor homologous factor 2 (FGF13) differentially modulates the current properties of Nav1.7 in DRG neurons

Authors: *P. EFFRAIM[1,2,3,4], J. HUANG[2,3,4], A. LAMPERT[5], S. G. WAXMAN[2,3,4], S. D. DIB-HAJJ[2,3,4]
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Abstract: Voltage-gated sodium channel $Na_v1.7$ is a threshold channel preferentially expressed in peripheral sensory neurons that is known to play a key role in human pain signaling. Gain-of-function mutations to $Na_v1.7$ have been shown to increase excitability in dorsal root ganglion (DRG) neurons and have been linked to inherited pain disorders. Fibroblast Growth Factor Homologous Factors (FHFs) are a subfamily of intracellular FGF proteins with four members (FHF1-4, also known as FGF11-14). FHFs have been shown to bind directly to the membrane-proximal segment of sodium channel's C-terminus, and regulate current density, availability, and frequency-dependent inhibition of sodium currents. FHF2 is expressed in DRG neurons and is down-regulated following axotomy. FHF2 has two main isoforms, FHF2A and FHF2B, which differ in the length and sequence of the N-termini, and differentially regulate some voltage-gated sodium channels. Using biochemical assays, we have found that FHF2 interacts with $Na_v1.7$ in HEK293 cells. Voltage-clamp recordings show that the expression of FHF2A or FHF2B in

HEK293 cell line stably expressing $Na_v1.7$ channels causes a large depolarizing shift in availability of $Na_v1.7$, but no effect on current density. FHF2A causes an accumulation of inactivated channels at all frequencies tested due to a slowing of recovery from inactivation, whereas FHF2B has little effect on these properties of $Na_v1.7$. Thus, FHF2A/B factors modulate gating properties of $Na_v1.7$ in the stably-transfected HEK293 cell line so that they more closely match these properties in native DRG neurons. To evaluate the effects of FHF2 on $Na_v1.7$ in native dorsal route ganglion (DRG) neurons, we implemented a knockdown strategy using miRNAs against FHF2A and core FHF2 isoforms. Voltage-clamp analysis of DRG neurons from mice with $Na_v1.6$ and $Na_v1.8$ double knockout transfected with plasmids encoding miRNAs against either FHF2A, or core FHF2 (which knocks down all FHF2 isoforms), have revealed that FHF2 has a role in modifying the properties of $Na_v1.7$ in native DRG neurons. We have observed changes in the kinetics of activation, fast inactivation, and repriming. We did not observe a change in current density, however. These observations support the idea that FHFs modulate $Na_v1.7$ in vivo and may contribute to changes to the properties of $Na_v1.7$ following axotomy, including enhanced repriming.

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Poster

123. Sodium Channels

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 123.18/F26

Topic: B.04. Ion Channels

Support: Medical Research Service and Rehabilitation Research Service, Department of Veterans Affairs (SGW and SDH)
Paralyzed Veterans of America
VA Medical Center, West Haven, CT
Yale University

Title: A novel gain-of-function Nav1.9 mutation in an individual with episodic pain

Authors: *J. HUANG[1], M. ESTACION[1], P. ZHAO[1], B. SCHULMAN[1], A. ABICHT[2], K. BROCKMANN[3], S. WAXMAN[1], S. DIB-HAJJ[1]
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Abstract: Sodium channel Nav1.9, encoded by the *SCN11A* gene, is one of the nine isoforms in voltage-gated sodium channel (VGSC) family that plays an essential role in conducting nerve

impulses through action potential generation and propagation. Nav1.9 is preferentially expressed in intrinsic myenteric neurons, trigeminal ganglion neurons, and small-diameter dorsal root ganglion (DRG) neurons (<30μm) including nociceptors. Nav1.9 manifests unique biophysical properties that distinguish it from other VGSC isoforms, including hyperpolarized activation threshold (i.e. the voltage at which the channel opens) at ~ -70 mV and a large window current that occurs at a voltage domain overlapping the resting membrane potential (RMP) of DRG neurons. Recent studies demonstrate a monogenic Mendelian link of Nav1.9 to human pain disorders including the paradoxical connection of gain-of-function mutations in Nav1.9 channel and insensitivity to musculoskeletal pain in some patients, due to massive depolarization that inactivates VGSCs within DRG neurons. Gain-of-function variants in Nav1.9 have also been identified in patients with the more common pain disorder small fiber neuropathy. To shed more light on the phenotypic spectrum of Nav1.9 channelopathy, here we report a new mutation, N816K, in Nav1.9 found in a child with early-onset episodic pain in both legs, episodic abdominal pain, and chronic constipation. The clinical features fit very well with the descriptions of familial episodic pain associated with *SCN11A* variants. Sanger sequencing of *ATP1A3, GCH1, SCN9A, SCN10A, TRPA1* revealed normal results. The substitution occurs at a residue at the N-terminus of the cytoplasmic loop 2 which joins domains II and III, proximal to end of transmembrane segment 6 in domain II (DII/S6). Voltage-clamp recordings demonstrate that this mutation significantly increases current density of Nav1.9 channel by 39%, compared to wild-type channel in small DRG neurons. The mutant channel also hyperpolarizes the voltage-dependence of activation by 10 mV, enabling a larger window current that could impact RMP of DRG neurons. Functional assessment of the mutant channel in DRG neurons via current-clamp recordings shows that mutant channel depolarizes RMP of small DRG neurons by 7 mV. Additionally, the mutant channel reduces current threshold of firing an all-or-none action potential and renders hyperexcitability in DRG neurons. Taken together these data demonstrate the gain-of-function attributes of this mutation at the channel and cellular levels, and are consistent with pain phenotype in the carrier of this mutation, and expands the spectrum of Nav1.9-related pain mutations.

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Poster

123. Sodium Channels

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 123.19/G1

Topic: B.04. Ion Channels

Support: Medical Research Service and Rehabilitation Research Service, Dept. Veterans Affairs
Paralyzed Veterans of America

Yale University

Title: A novel gain-of-function $Na_v1.7$ mutation in a carbamazepine-responsive patient with adult-onset non-length-dependent small fiber neuropathy

Authors: *T. ADI[1,2,3], M. ESTACION[1,2,3], S. VERNINO[4], S. D. DIB-HAJJ[1,2,3], S. G. WAXMAN[1,2,3]
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Abstract: Voltage-gated sodium channel $Na_v1.7$ is a threshold channel preferentially expressed in peripheral sensory neurons that is known to play a key role in human pain signaling. Dominant gain-of-function mutations to $Na_v1.7$ have been shown to increase excitability in dorsal root ganglion (DRG) neurons and have been linked to several rare Mendelian pain disorders. Using genomic screening in subjects with a family history of adult-onset pain symptoms, we identify a novel gain-of-function mutation in $Na_v1.7$, valine 810 substitution to methionine (V810M), which results in atypical neuropathic pain and non-length-dependent small fiber neuropathy. Voltage-clamp analysis of HEK293 cells transiently expressing V810M channels revealed a small (~3 mV) hyperpolarized shift in activation, increased peak current density, and slowed deactivation of the mutant as compared to wild-type $Na_v1.7$ (WT), all of which are gain-of-function attributes. Use of multi-electrode array to assess excitability of DRG neurons transiently expressing the channels of interest revealed hyperexcitability of V810M-expressing neurons, consistent with a pain phenotype. Furthermore, because the proband is known to respond to treatment with carbamazepine (CBZ), we went on to assess the effects of CBZ on voltage-dependent properties of V810M channels. These findings suggest that CBZ is acting on V810M channels via the classical use-dependent mechanism as opposed to via the novel effect of depolarizing activation we have previously described with other $Na_v1.7$ mutations.

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Poster

124. Control of Neuronal Firing in Development and Disease

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 124.01/G2

Topic: B.08. Intrinsic Membrane Properties

Support: German National Academic Foundation

Promotionsstipendium der Medizinischen Fakultät Mannheim
Paracelsus Medical University PMU FFF: R-16/04/084-BEN
DFG SFB 1134

Title: Structural and functional remodeling of the axon initial segment in rat primary motor cortex principal neurons after spinal cord lesion

Authors: *D. DANNEHL[1,2,3], B. BENEDETTI[3,2], L. S. BIELER[3,2], J. M. JANSSEN[1], C. CORCELLI[1], S. SOLEYMANI[1], M. EWERTZ[1], C. SCHULTZ[1], S. COUILLARD-DESPRÉS[3,2], M. ENGELHARDT[1]
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Abstract: The axon initial segment (AIS) is an electrogenic microdomain usually located at the proximal axon in most cortical neurons. Its molecular architecture, length, and position as well as synaptic innervation pattern make it a key player for modulating neuronal excitability. Previous studies have shown that alterations of synaptic input parameters such as sensory deprivation can drive AIS plasticity in vivo. To which extent the modification of axonal output parameters, e.g. the ability to propagate action potentials to postsynaptic targets can affect AIS structure and function remains unclear. Thus our aim is to understand if M1 pyramidal neurons retain the ability to regulate their excitability when axonal output is altered. Using immunofluorescence, confocal microscopy, patch-clamp recordings, and surface reconstruction of synaptic complexes, we first investigated normal AIS development in layers II/III and V of primary motor cortex (M1) at various developmental stages. Data show that AIS gradually increase in length over time, but contrary to sensory cortices, AIS do not undergo activity-dependent length remodeling. To test potential AIS plasticity after distal axotomy, adult rats underwent laminectomy of C4 vertebra and wire-knife lesion of the dorsal corticospinal tract (CST). Lesioned CST neurons were visualized via retrograde tracer injection using hydroxystilbamidine (FluoroGold). Axotomized layer V pyramidal neurons in M1 have significantly longer AIS than non-lesioned controls. Furthermore, the number of axo-axonic GABAergic synapses at the AIS of axotomized neurons is significantly reduced. Strikingly, layer II/III pyramidal neurons show significant length reduction 5 days post-surgery with complete recovery to control length 2 days later. In summary, these results suggest that after distal lesion, M1 infragranular and supragranular pyramidal neurons dynamically adapt their excitability, possibly indicating putative cellular compensation mechanisms reminiscent of homeostatic plasticity due to CST lesion.

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Poster

124. Control of Neuronal Firing in Development and Disease

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 124.02/G3

Topic: B.08. Intrinsic Membrane Properties

Support: Institutional Support

Title: Development and optimization of an *in vitro* assay for neuronal sensitization by inflammatory mediators

Authors: *D. M. DUBREUIL[1], Y. SAPIR[2], B. WAINGER[2]
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Abstract: Inflammation is a significant contributor to short-term pain hypersensitivity, as well as long-term pain. Current *in vivo* animal models of inflammatory pain utilize peripheral injection of irritants (e.g. Formalin, Carrageenan) or heat-killed bacteria (e.g. complete Freund's adjuvant) to initiate an inflammatory response leading to spontaneous pain, hyperalgesia, and allodynia. These classic models have been critical for understanding the role of genetics in inflammatory signaling and for discovering many components of the inflammatory hyperalgesia signaling pathway; however, they are relatively low-throughput and poorly suited to drug screens and development of novel therapies aimed at disrupting inflammatory signaling. Furthermore, these assays are primarily restricted to rodent subjects, which may not adequately reflect the response of human nociceptors to inflammation. For these reasons, we developed a high-throughput *in vitro* assay to assess sensitization of primary mouse nociceptors as well as human iPSC-derived nociceptors by application of inflammatory mediators. Treatment of mouse or human nociceptors with a mixture of inflammatory mediators *in vitro* lowers the threshold for neuronal activation by Trpv1 channels. Using our high-throughput assay, we extend this finding to determine if this hyperexcitability encompasses activation by other sensory transduction channels, such as purinergic receptors and other Trp channels, or by optogenetic stimulation. Furthermore, we demonstrate how this method can be applied to understand differences between dorsal root and trigeminal ganglion nociceptors, with a direct relation to disease mechanism in migraine. Migraine results, in part, from pathological inflammatory sensitization of trigeminal nociceptors, with much smaller effects on nociceptors in dorsal root ganglia. We use our high-throughput assay to investigate functional differences between mouse primary trigeminal and dorsal root ganglia nociceptors, and how differences are reflected in human iPSC-derived nociceptors. The functional overlap of human iPSC-derived nociceptors with DRG or TG nociceptors is unclear and functional phenotyping may be able to provide new insight.

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Poster

124. Control of Neuronal Firing in Development and Disease

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 124.03/G4

Topic: B.08. Intrinsic Membrane Properties

Title: Dicer ablation in postmitotic VIP interneurons leads to circuit dysfunction and neuronal death

Authors: *F. QIU[1], L. GONG[2], M. HE[3]
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Abstract: Excitation & inhibition balance is crucial for normal brain development and function. Neocortex contains many inhibitory interneuron subtypes which are developmentally and functionally distinct. Neocortex interneurons are mainly generated from medial and caudal ganglionic eminence (MGE and CGE). Recent study has revealed that microRNAs (miRNAs) are essential for the survival and maturation of cortical interneurons derived from medial ganglionic eminence, but whether they play crucial roles in CGE derived interneurons remains elusive. Vasoactive intestinal peptide expressing interneurons are one of the major CGE derived interneuron subtypes. We conditionally ablated Dicer, an essential enzyme for canonical miRNA biogenesis, in postmitotic VIP interneurons using VIP-cre. Through in vitro and in vivo assays, we have demonstrated that miRNAs are critical for the survival of VIP interneurons and their normal function in the cortical circuit.

Disclosures: F. Qiu: None. **L. Gong:** None. **M. He:** None.

Poster

124. Control of Neuronal Firing in Development and Disease

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 124.04/G5

Topic: B.08. Intrinsic Membrane Properties

Support: NIH-RO1-NS036692
NIH-RO1-NS082851
NIH-RO1-NS052634

Title: Perineuronal nets regulate the excitability of GABAergic interneurons: Lessons from glioma-associated epilepsy

Authors: *B. P. TEWARI[1,2], L. CHAUNSALI[3], S. L. CAMPBELL[4], D. PATEL[3], A. GOODE[5], H. SONTHEIMER[6,3]
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Abstract: Imbalance of excitation and inhibition in the brain is the key determinant of neuronal hyperexcitability in epilepsy. In different acquired forms of epilepsies, this imbalance has been associated with elevated glutamatergic neurotransmission, diminished or disinhibition of GABAergic neurotransmissions, neuronal loss, astroglial dysfunctions etc. Recurrent and unprovoked epileptic seizures are often a presenting symptoms of high-grade glioma patients, and we have shown previously that upregulation of the glutamate-cysteine antiporter SXC in glioma cells causes excessive glutamate release. This in turn increases excitatory drive and causes GABAergic disinhibition and excitotoxic neuronal death that drive the seizure activity in a mouse model of human glioma-associated epilepsy. In the present study, we examined the epileptogenic consequences of glioma, which operate independent but in parallel to the elevated extracellular glutamate. Perineuronal nets (PNNs) are specialized extracellular matrix assemblies of highly negatively charged proteoglycans and proteins that surround fast spiking GABAergic interneurons. We show that glioma degrades perineuronal nets by releasing matrix-degrading enzymes thereby directly exposing these inhibitory interneurons to excitotoxic glutamate and causing preferential death of inhibitory interneurons. We also show that disintegration of PNN curtails the fast spiking property of GABAergic interneurons by increasing the membrane capacitance without altering other intrinsic properties. Simulation of capacitance alterations on PNN degradation using Hodgkin-Huxley modeling further confirmed the impact of increased capacitance on slowing the spike frequency. Hence our study provides evidence of a novel mechanism, whereby perineuronal nets regulate the excitability of GABAergic interneurons and implicated a role of PNNs in acquired epilepsies.

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Poster

124. Control of Neuronal Firing in Development and Disease

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 124.05/G6

Topic: B.08. Intrinsic Membrane Properties

Title: Subchronic phencyclidine (pcp) treatment differentially affects neuronal excitability in mPFC subdivisions

Authors: *H. R. KIM[1], L. RAJAGOPAL[2], H. Y. MELTZER[2], M. MARTINA[1]
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Abstract: The medial prefrontal cortex (mPFC) is critical for cognitive functions such as working memory and decision making. Previous studies suggested that alterations in mPFC activity have a critical role in the cognitive deficits of schizophrenia. This observation originally obtained in human patients is successfully mimicked in subchronic-phencyclidine (scPCP) treated mice, an animal model of schizophrenia. The mPFC is a complex area, where the dorsal (prelimbic cortex; PLC) and the ventral (infralimbic cortex; ILC) subdivisions have different anatomical connectivity and function. Yet, little is known of how these mPFC subdivisions are affected in the scPCP model, although this is important to develop effective treatments as several reports show that activity of the PLC and ILC can produce very different behavioral outcomes. We investigated whether neuronal excitability in the PLC and ILC is differentially affected by scPCP treatment. Adult male mice were injected with either saline or PCP for 7 consecutive days. After waiting for at least another 7 days, the animals were sacrificed and brain slices containing both PLC and ILC were prepared to perform whole-cell patch clamp recordings of layer 5 pyramidal neurons. We found that in saline-injected mice the excitability of PLC (n=13) and ILC (n=21) neurons is similar. However, in scPCP mice the excitability of ILC neurons (n=16) is significantly increased compared to PLC neurons (n=14). Indeed, in ILC neurons, 1s-long depolarizing pulses of 300 and 500 pA evoked 21.5±1.5 and 37.4±1.7 spikes in cells from control vs. 24.6±2.7 and 46.7±5.1 in cells from scPCP mice. We hypothesized that such difference may be caused by differential GABAergic inhibition; however, we found that the frequency of spontaneous inhibitory inputs in scPCP mice compared to controls was unaffected in PLC, while it was increased in ILC. Thus, the altered pyramidal cell excitability is likely due to intrinsic changes, rather than modified inhibitory control.

Disclosures: H.R. Kim: None. **L. Rajagopal:** None. **H.Y. Meltzer:** None. **M. Martina:** None.

Poster

124. Control of Neuronal Firing in Development and Disease

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 124.06/G7

Topic: B.08. Intrinsic Membrane Properties

Title: Two subpopulations of somatostatin-expressing interneurons with distinct postnatal developmental patterns in cortex of transgenic mice

Authors: *C. WANG
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Abstract: The GIN transgenic mice express the enhanced green fluorescent protein (eGFP) in a subset of somatostatin(SST)-expressing interneurons in the brain and have been widely used in a variety of studies. However, the early postnatal development of eGFP neurons in the cortex has not been well addressed. In this study, we observed two groups of eGFP neurons in the cortex: one was located in the white matter as clusters (cluster eGFP neurons), which was noticeable at birth, but disappeared after the second postnatal month; another was dispersed in mostly layer II to IV (cortical eGFP neurons), which appeared after postnatal day 2 or 3 and gradually increased in the density in the subsequent weeks, and existed throughout the life. All cortical eGFP neurons were immunoreactive for SST, but cluster eGFP neurons were not for either SST or Calcium-Calmodulin Kinase II. Cortical eGFP neurons showed dramatic increases in firing rate, the amplitude of afterhyperpolarization, and synaptic activity during the first postnatal month. These properties in cluster eGFP neurons, however, remained largely unaltered. Short-term plasticity of excitatory synapses evoked by train stimulations showed a robust facilitation in cortical eGFP neurons but a depression in most cluster eGFP neurons. These results indicate that eGFP neurons in GIN mice are heterogeneous in their properties and developmental patterns.

Disclosures: C. Wang: None.

Poster

124. Control of Neuronal Firing in Development and Disease

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 124.07/G8

Topic: B.08. Intrinsic Membrane Properties

Support: NIH (R01AA025784)
NIH (P50AA017823)
NIH (T32AA025606)
NARSAD Young Investigator Grant (#24989) from the Brain & Behavior Research Foundation

Title: Layer-specific effects of adolescent vs. adult ethanol exposure on the intrinsic excitability of prelimbic cortex

Authors: *E. J. GALAJ, Y.-Y. MA
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Abstract: Adolescence is a vulnerable developmental time period during which binge drinking, characterized by cycles of repeated drinking of alcohol followed by abstinence, can have long-

lasting effects on brain function. However, little is known about the effects of binge drinking on the excitability of pyramidal cells in the prelimbic cortex during acute and protracted withdrawal periods. In the present study, we performed whole-cell patch-clamp recordings to examine the intrinsic excitability of pyramidal cells in layer 2/3 and layer 5/6 of the prelimbic cortex in rats exposed to chronic intermittent ethanol (CIE) during adolescence or adulthood. Our data show differential effects of CIE during adolescence vs. adulthood on prelimbic neurons. After protracted withdrawal periods (21 days) adolescent CIE exposure increases the excitability of prelimbic neurons in layer 5/6 as compared to water-treated adolescent rats. However, compared to the water treated group, the excitability of these neurons appears to be suppressed in CIE rats at the prolonged withdrawal stage. Neither of the above effects was observed during the acute withdrawal period (2 days), indicating that both the adolescent CIE-increased excitability and the adult-CIE-decreased excitability in deep layers in the prelimbic cortex is incubated rather than the result of acute effects after CIE exposure. Our results reveal CIE exposure leads to age-specific and prelimbic cortex layer-specific effects, which is gradually accumulated during CIE withdrawal periods. This may constitute a predictive neuroadaptation of individual vulnerability to mental disorders involving dysfunctional prelimbic cortex after CIE at different developmental stages.

Disclosures: E.J. Galaj: None. **Y. Ma:** None.

Poster

124. Control of Neuronal Firing in Development and Disease

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 124.08/G9

Topic: B.08. Intrinsic Membrane Properties

Support: NEI 1R01EY027380

Title: Resurgent sodium current in neurons of the xenopus laevis optic tectum as a modulator of intrinsicexcitability during retinotectal circuit development

Authors: *C. D. AIZENMAN[1], A. C. THOMPSON[2], K. M. KEARY, III[2]
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Abstract: The retinotectal circuit of *Xenopus laevis* tadpoles undergoes significant activity-dependent refinement and strengthening during development, while maintaining the ability to stably respond to visual stimulation. To maintain a stable relationship between synaptic input and spike output, the intrinsic excitability of tectal neurons decreases as excitatory synaptic input increases with maturation. However, the mechanisms by which tectal neurons regulate their intrinsic excitability has remained largely unknown. To identify mediators of intrinsic excitability in tectal neurons, we investigated changes in gene expression as the retinotectal

circuit matures. This analysis revealed that scn4b, which encodes for the beta subunit of the voltage-gated sodium channel that regulates the resurgent component of voltage-gated sodium currents, is upregulated in tectal neurons in conditions where increased intrinsic excitability is observed. To explore the role of the resurgent current in mediating intrinsic excitability in tectal neurons, we used whole cell voltage-clamp electrophysiology. Here we describe the presence of a resurgent current in tectal neurons that is mediated by a TTX-resistant channel and is insensitive to voltage gated calcium channel blockade. Isolated whole-soma recordings indicate that this current is localized to the soma, not the axon initial segment. We also describe how resurgent current is regulated during retinotectal circuit development. We further explore the hypothesis that regulation of this resurgent current is responsible for regulating changes in tectal cell intrinsic excitability.

Disclosures: C.D. Aizenman: None. **A.C. Thompson:** None. **K.M. Keary:** None.

Poster

124. Control of Neuronal Firing in Development and Disease

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 124.09/G10

Topic: B.08. Intrinsic Membrane Properties

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Simons Foundation Pilot Award

Title: Neural activity triggers ectopic axonal spiking in parvalbumin-expressing inhibitory interneurons of the neocortex

Authors: *B. B. THEYEL[1], R. STEVENSON[2], B. CONNORS[2]
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Abstract: Action potentials can sometimes originate from ectopic sites in distal axon branches and terminals, far from the axon initial segment, and propagate antidromically to the soma (*Brain Res Rev.* 21:42, 1995). Ectopic spikes are often induced by intense neural activity; for example, they have frequently been observed in cortical and thalamic neurons whose axons terminate in seizure-generating foci. Recently, ectopic spikes have been induced in inhibitory interneurons in “normal” brain tissue when several hundred orthodromic spikes were triggered from the soma. In the hippocampus, the cells most predisposed to ectopic spiking were the NPY-expressing interneurons (*Nat Neurosci*. 14:200, 2011), whereas parvalbumin (PV)-expressing interneurons (PV+ cells) seldom spiked ectopically. Little is known about the origin, functional effects, and

mechanisms of this ectopic spiking in different interneuron populations and cortical areas. In acutely prepared slices of the mouse orbitofrontal cortex (OFC), we observed ectopic spikes in a great majority of interneurons, with particularly high prevalence (~90%) in PV+ cells. Ectopic spikes were induced in PV+ cells after an average of several hundred orthodromic spikes were triggered with somatic current injection or channelrhodopsin activation. Persistent trains of ectopic spikes in PV+ cells could last up to several seconds. In contrast, somatostatin-expressing interneurons in the OFC rarely exhibited more than a few scattered ectopic spikes after comparable stimulation. The ectopic, antidromic origins of the spikes were inferred from spike shapes, sizes, and thresholds. Persistent ectopic spiking was not limited to PV+ cells of the OFC; it was also robustly induced in PV+ cells in layer 4 of the primary somatosensory cortex. Given PV+ cells' strong inhibition of pyramidal cells and their role in generating network gamma rhythms, and the importance of OFC for cognitive flexibility, ectopic spiking may have significant implications for network activity, cognitive processing, and seizure susceptibility.

Disclosures: B.B. Theyel: None. **R. Stevenson:** None. **B. Connors:** None.

Poster

124. Control of Neuronal Firing in Development and Disease

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 124.10/G11

Topic: B.08. Intrinsic Membrane Properties

Support: TUBITAK, Project No: 214S673
EBILTEM, Project No: 2015BIL042

Title: Lipid composition of membranes could be an important variable regarding excitotoxicity of hippocampal neurons

Authors: *V. EVREN, A. YURT KILCAR, E. DERVIS, B. KARATAY, D. TASKIRAN
Ege Univ., Izmir, Turkey

Abstract: Objective: Chronic exitotoxic stress on neurons is blamed for certain neuropathological conditions such as epilepsy. Statistically, a large percentage of partial epilepsies is temporal lobe epilepsy. This brings the hippocampus into the focus of epileptogenic mechanisms. Many studies explored the different aspects of these mechanisms including ion channels, receptors and gene expressions but membrane lipid structure seems to be overlooked. The main objective of this study is to determine whether the lipid membrane reacts to excitotoxic stress and if so, how...
Methods: Primary neuronal cells were prepared from the hippocampus region of newborn (P0) rat brains. In order to determine the effective dose of glutamic acid, we performed cell viability tests (MTT) for different concentrations. Cells were incubated in culture medium including 100

μM glutamic acid for seven days. We also tested the effects of two antiepileptics (phenytoin and levetiracetam) on glutamic acid exitotoxicity. After the culture period, cells were homogenized, and membrane lipids were extracted by using modified Bligh Dyer and Folch methods. Lipid composition was determined by high performance liquid chromatography (HPLC). All data were evaluated by ANOVA and post-hoc Dunnet T3 test. A p value <0.05 was accepted statistically significant.
Results: High glutamic acid concentrations reduced viability of the cells. At effective concentration of glutamic acid (100μM), hippocampal neuronal membrane lipid composition changed significantly. Most prominent change was the increase of cholesterol ($p<0.001$). We found that phenytoin alone has an opposite effect on cholesterol and also reversed the change caused by glutamic acid when they were applied together.
Conclusion: The hippocampus, which is involved in the formation of the memory, is a site for continuous neurogenesis. This turnover seems to make it more susceptible to various stresses. This may be the cause of lipid composition changes. We know that an increase in membrane cholesterol thickens the hidrophobic (internal) segment of the membrane. This has implications regarding the cable properties of the neurons by decreasing the capacitance of the membranes which makes the neuron easier to stimulate. Our study indicates that the membrane lipid plasticity is a worthwhile candidate for epileptogenesis and should be investigated thoroughly.

Disclosures: V. Evren: None. **A. Yurt Kilcar:** None. **E. Dervis:** None. **B. Karatay:** None. **D. Taskiran:** None.

Poster

124. Control of Neuronal Firing in Development and Disease

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 124.11/G12

Topic: B.08. Intrinsic Membrane Properties

Title: The functional impact of sphingosine-1-phosphate signaling on neuronal excitability in the central amygdala is sex-specific

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Abstract: Sphingosine-1-phosphate (S1P) is a bioactive lipid that sensitizes peripheral nociceptors via activation of G-protein coupled receptors. S1P receptors (S1PRs) are expressed in the amygdala, which is a temporal lobe structure essential to the affective component of pain. S1P and S1P receptor 1 (S1PR1) agonist SEW2871 stimulate G-protein activity in the amygdala, however the functional effects of this signaling on defined amygdalar neurons is completely unknown. Using anatomical labeling techniques in mice, we identified neurons in the central nucleus of the amygdala (CeA) that send projections to the periaqueductal gray (PAG), a

midbrain structure essential to endogenous analgesia and affective behaviors. Using whole-cell electrophysiology in acute brain slices, we measured the effects of S1P and SEW2871 on the subthreshold and excitable properties of retrogradely-labeled CeA-PAG neurons. We find that exogenous S1P and SEW2871 significantly depolarize resting membrane potential in CeA-PAG neurons. Surprisingly, we find that S1P depolarizes CeA-PAG neurons only in female mice. However, our data show that SEW2871 depolarizes CeA-PAG neurons in both male and female mice. Mechanisms regarding this disparity remain unclear. Evidence shows that somatostatin (SOM+) neurons are primarily in the central lateral amygdala (CeL) and directly inhibit PAG neurons. Using offspring from SOM-Cre mice crossed with the Ai9 td-Tomato reporter mouse, we were able to identify SOM+ neurons in CeL via expression of red fluorescence. Using injection of green fluorescent tracer into the PAG of SOM-tdTomato mice, we confirmed that PAG projecting neurons in CeL are SOM+. Surprisingly, we find that the action potential half-width of CeL-SOM+ neurons is significantly narrower in males than females. Exogenous S1P does not depolarize CeL-SOM+ neurons in male mice. Further, preliminary data show that SEW2871 significantly reduces firing frequency of CeL-SOM+ neurons in female mice while having minimal effect in male mice. Overall, we demonstrate that the functional consequences of S1P signaling on CeA neurons is dependent on sex. We are currently testing S1P signaling effects on CeL-SOM- neurons as this may provide insight into whether S1P differentially alters the excitability of molecularly distinct neurons in the amygdala. Results from this work will potentially identify sex-specific mechanisms through which S1P modulates distinct pathways (i.e. CeA-PAG and CeL-SOM+) implicated in pain modulation and associated coping mechanisms. This could lead to novel approaches exploiting S1P pathways for treating chronic pain and anxiety disorders.

Disclosures: B. Mork: None. **J. Li:** None. **P.L. Sheets:** None.

Poster

125. Oscillations and Synchrony in the Human Brain

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 125.01/H1

Topic: B.09. Network Interactions

Support: NRF Grant 2015R1D1A1A02061486

Title: Decreased hippocampal theta activity in general anesthesia

Authors: *M. CHOE[1], S.-H. JIN[2,3], S. JUN[1], J. KIM[1], C. CHUNG[1,2,4]
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Abstract: Amnesia (loss of memory) is a major component of general anesthesia (GA) along with loss of consciousness and analgesia. Some patients unexpectedly become aware during surgery and form a traumatic memory. However, the mechanism underlying amnesia of GA has not been adequately studied. Theta rhythm of hippocampus plays a crucial role in memory formation. Hence, the disruption of theta oscillation during GA may be at least one of mechanisms of postoperative amnesia. In previous rodent studies, the hippocampal theta rhythm slowed during GA and correlated with anesthetic-induced amnesia. However, in humans, theta oscillation in hippocampus during GA has not been investigated. In this study, we investigated hippocampal theta activity during propofol-induced GA. Power spectrum of hippocampal theta frequency (4-7 Hz) was calculated from the intrahippocampal depth electrode in 9 epilepsy patients (age: 24-55 years) during propofol-induced GA, resting state (awake), and long-term memory task. Kruskal-Wallis one way ANOVA was performed for intergroup comparisons of theta power in hippocampus. Theta powers during propofol-induced GA (5, 4, and 3 ug/ml concentration) showed significant difference compared to those in resting state, and memory task state (p value < 0.01). Theta power in hippocampus was attenuated during propofol-induced GA. Suppression of the hippocampal theta activity may be a mechanism of amnesia during GA.

Disclosures: M. Choe: None. **S. Jin:** None. **S. Jun:** None. **J. Kim:** None. **C. Chung:** None.

Poster

125. Oscillations and Synchrony in the Human Brain

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 125.02/H2

Topic: B.09. Network Interactions

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Institute for Medical Engineering and Science, Massachusetts Institute of Technology
The Department of Anesthesia, Critical Care and Pain Medicine at Massachusetts General Hospital

Title: What's the difference between sleep and anesthesia? Evidence that posterior and anterior phase-amplitude coupling distinguish unconsciousness from unarousability

Authors: *E. P. STEPHEN[1], M. J. PRERAU[2], O. JOHNSON-AKEJU[3], G. C. HOTAN[1], S. KHAN[5], M. HÄMÄLÄINEN[5], E. N. BROWN[1], P. L. PURDON[4]
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Abstract: Recent theories regarding the neural correlates of consciousness and unconsciousness disagree about the involvement of frontal and posterior areas. For example, in sleep, the EEG slow wave is stronger over a "posterior hot zone" in non-dreaming sleep than dreaming sleep (Siclari and Tononi 2017). In contrast, research on propofol anesthesia has shown a frontal effect during unconsciousness where the amplitude of the frontal alpha rhythm is coupled to the trough or the peak of the slow wave, depending on the depth of anesthesia: so-called "troughmax" and "peakmax" phase-amplitude coupling (Purdon et al 2013). Both of these studies suggest that the EEG slow wave is fundamental to unconscious states, but they differ in which brain areas they prioritize.

Here we focus on the spatiotemporal properties of high frequency coupling to the slow wave in EEG recordings of sleep, propofol anesthesia, and dexmedetomidine sedation. We describe three progressively deeper states of unconsciousness based on the presence of troughmax or peakmax dynamics over frontal and posterior regions: (1) at the lightest level, frontal areas display troughmax dynamics (light NREM and dexmedetomidine sedation); (2) with deeper unconsciousness, posterior areas begin to show peakmax dynamics (deep NREM and light propofol); (3) at even deeper levels, both frontal and posterior areas display peakmax dynamics (deep propofol before burst suppression).

Based on the frequency bands that couple to the slow wave, we propose that troughmax dynamics reflect a preferred phase for sleep spindles and mechanistically related processes. In contrast, peakmax dynamics indicate a local state in which all cortical activity is disrupted by spatially incoherent up and down states. Dexmedetomidine sedation mimics light sleep, where spindling occurs but peakmax dynamics are not yet present. In sleep and propofol, peakmax first occurs over posterior areas, disabling regions involved in sensory perception, spatial awareness, and self-awareness from their waking functional networks. The spread of peakmax dynamics to frontal areas in deep propofol anesthesia, which has been linked to a state in which the subject is unarousable even in the presence of noxious stimuli (Gaskell et al 2017, Brown et al in press), could reflect disrupted high-order cognitive functions and global cortical communication. Hence we propose that troughmax and peakmax dynamics over posterior and frontal cortices can be used to distinguish different states of unconsciousness and arousability in general anesthesia and sleep.

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Poster

125. Oscillations and Synchrony in the Human Brain

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 125.03/H3

Topic: B.09. Network Interactions

Title: Triphasic wave-like generalized periodic discharges with a high negative component (Tri-HNC) on EEG as an indicator of cefepime-induced encephalopathy (CIE): Three case reports and neural mass modeling in silico

Authors: *H. TAMUNE[1,2,3], Y. HAMAMOTO[1,2], N. ASO[4], N. YAMAMOTO[1]
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Abstract: Cefepime, a fourth-generation cephalosporin (antibiotics), acts as a $GABA_A$ receptor antagonist. Cefepime-induced encephalopathy (CIE) is potentially diagnosed in 15% of patients treated with cefepime in ICU, but frequently overlooked. We suspected that the EEGs in patients with CIE were quite unique and homogenous; therefore, we aimed to clarify clinical features and mechanisms of CIE. We retrospectively reviewed CIE documented by a single-center consultation-liaison team between April 2015 and March 2017, and identified three patients with CIE. They refused medication/examination and showed overt pain, palilalia, and much greater deterioration of eye and verbal response than of the motor response; which were potentially related to GABAergic function. As expected, triphasic wave-like generalized periodic discharges with a high negative component (Tri-HNC, we coined and pronounced it as “Try high neck”) were identified on EEG in all three cases. For further investigation, neural mass modeling was done *in silico*. The computational simulation demonstrated the characteristic feature of 2-2.5 Hz Tri-HNC and recovery course on EEG, and a possible involvement of individual differences in pharmacological intervention. It also suggested that auto-inhibition (synaptic inputs from interneuron to interneuron) was the underlying mechanism of CIE. Since CIE is iatrogenic and continues unless cefepime is stopped, early recognition is crucial. Physicians should be vigilant about altered mental status, pain, and verbal changes in patients taking cefepime. In conclusion, Tri-HNC can expedite the diagnosis of CIE and the association between Tri-HNC and CIE suggested that an imbalance between excitation and inhibition due to the dysfunction of GABAergic interneurons is the underlying mechanism of CIE. Moreover, this modeling may offer a new method for investigating disorders related to GABAergic function. This study was approved by the IRB and there are no COIs to disclose.

Disclosures: H. Tamune: None. **Y. Hamamoto:** None. **N. Aso:** None. **N. Yamamoto:** None.

Poster

125. Oscillations and Synchrony in the Human Brain

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 125.04/H4

Topic: B.09. Network Interactions

Support: CIHR

Title: Sex-related changes in EEG during isoflurane-induced surgical anesthesia

Authors: *P. J. SOJA[1], T. MARIAM[2], S. MALEKI[2], R. TADAVARTY[2]
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Abstract: Sex-related differences are well known to occur in a variety of brain functions associated with cognition, emotion, social behavior, pain, etc. Isoflurane is a commonly used inhalational anesthetic to assess brain function in rodents. However, few data exist that describe in detail whether sex-related changes occur in cortical EEG activity during protracted periods of surgical isoflurane anesthesia. In the present study, the cortical EEG in age-matched (adult, 4-6 months) male (n=12) and female (n=8) Sprague Dawley rats, was continuously monitored during the surgical plane of isoflurane anesthesia (ISO). Each rat was initially anesthetized with ISO using an anesthetic chamber. The animal's trachea was intubated and its head mounted in a stereotaxic frame. Cortical EEG (S1) was recorded bilaterally using stainless steel electrodes. The concentration of ISO (1.0-1.25 %) was adjusted to keep the rats at a surgical plane of anesthesia characterized by delta (δ) wave EEG activity. ISO was maintained for 4 hr while recording the EEG using CED Spike 2 software. EEG records compressed over the entire 4 hr recording period revealed clear oscillatory behavior characterized by high-power "up"-state and low-power "down"-states. A tagging procedure was developed that marked the onset and offset of each "up" and "down" state. The tagged EEG states were then re-constructed as continuous up and down state EEG records. Sex-differences were apparent in the number and spectral features of the up-states. Male rats displayed on ~11 up-state oscillations/hr that lasted ~6.25 min whereas female rats revealed ~ 3 up state oscillations/hr that on average lasted ~15 min. The difference in relative power between the up-states vs. down-states in δ (1-4Hz), θ (5-8Hz), (9-12Hz), and (13-25Hz) bandwidths were analyzed with a customized script. Male rats displayed a ~30% decrease and ~35% increase in relative power values for δ and θ bands, respectively when comparing the up state vs. down state EEG traces. In contrast, in female rats, there was a 15% decrease in relative power in the δ band only when comparing upstate vs. downstate EEG activity. These findings demonstrate distinct differences in EEG activities between male and female rats during prolonged periods of surgical anesthesia. The functional consequence of these sex-related differences in EEG oscillations requires further study.

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Poster

125. Oscillations and Synchrony in the Human Brain

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Program #/Poster #: 125.05/H5

Topic: B.09. Network Interactions

Support: DFG EXC 307
ERC StG 335880

Title: Spectral fingerprints of cortical neuromodulation

Authors: *A. RADETZ, M. SIEGEL
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Abstract: Pupil dilation has been shown to be a reliable measure of neuromodulatory processes in the brain, originating from both, spontaneous or stimulus-driven activity of neuromodulatory brainstem-nuclei such as e.g. the Locus Coeruleus. Yet, little is known about cortical sources and effects of neuromodulation in the human brain. To address this, we recorded cortical activity during rest with magnetoencephalography (MEG) and simultaneously measured pupil dilation in 41 participants. We found a network of several brain regions, in which power significantly covaried with pupil dilation in a frequency- and latency-specific manner. These effects persisted after controlling for luminance-related changes in pupil size. Our results identify several potential cortical sources and targets of noradrenergic and cholinergic neuromodulation in the human brain.

Disclosures: A. Radetz: None. **M. Siegel:** None.

Poster

125. Oscillations and Synchrony in the Human Brain

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Topic: B.09. Network Interactions

Support: NIH Grant R01 GM111293

Title: Cortical dynamics during psychedelic and anesthetized states induced by ketamine

Authors: *D. LI, G. A. MASHOUR
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Abstract: Ketamine is a unique drug that has psychedelic and anesthetic properties in a dose-dependent manner. Recent studies have shown that ketamine anesthesia appears to maintain the spatiotemporal repertoire of cortical activation evoked by transcranial magnetic stimulation, while a psychedelic dose of ketamine is associated with increased spontaneous magnetoencephalography signal diversity. Here we investigated the spatiotemporal complexity of spontaneous high-density scalp electroencephalography (EEG) signals in healthy volunteers during alterations of consciousness induced by both subanesthetic and anesthetic doses of ketamine. Given the fast transient spectral dynamics, especially during the gamma-burst pattern after loss of consciousness, we employed a method based on Hidden Markov modeling to classify the EEG signals into a discrete set of brain states associated with different behavioral states. We characterized the spatiotemporal complexity specific for each brain state as measured through the Lempel-Ziv complexity (LZC) algorithm. After controlling for signal diversity due to spectral changes, we found that the subanesthetic dose of ketamine is associated with an elevated complexity level relative to baseline, while the brain activity following an anesthetic dose of ketamine is characterized by alternating low and high complexity levels until stabilizing at a high level comparable to that during baseline. These results demonstrate that the spatial complexity associated with ketamine-induced state transitions has features of general anesthesia, normal consciousness, and heightened states of consciousness, suggesting that ketamine may serve as a unique tool to probe different states of consciousness.

Disclosures: D. Li: None. **G.A. Mashour:** None.

Poster

125. Oscillations and Synchrony in the Human Brain

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Topic: B.09. Network Interactions

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MH067533
MH103222

Title: Tms evoked n100 reflects local gaba and glutamate balance

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Abstract: Background: Animal studies suggest that synchronized electrical activities in the brain are regulated by the primary inhibitory and excitatory neurotransmitters gamma-aminobutyric acid (GABA) and glutamate, respectively. Identifying direct evidence and measure that this same basic chemical-electrical neuroscience principle operates in the human brains is critical for translation of neuroscience to pathological research. **Objective/Hypothesis**: We hypothesize that the background neurochemical concentrations may affect the cortical excitability probed by transcranial magnetic stimulation (TMS). **Methods**: We used TMS with simultaneous Electroencephalography (EEG) recording to probe the cortical excitability and determined how background frontal cortical GABA and glutamate levels measured using magnetic resonance spectroscopy (MRS) modulate frontal electrical activities. **Results**: We found that TMS-evoked N100 component reflects a balance between GABA-inhibitory and glutamate-excitatory levels. About 46% of individual variances in frontal N100 can be explained by their glutamate/GABA ratio (r=-0.68, p=0.001). Both glutamate (r=-0.51, p=0.019) and GABA (r=0.55, p=0.01) significantly contributed to this relationship but in opposite directions. **Conclusion**: The current finding encourages additional mechanistic studies to develop TMS evoked N100 as a potential electrophysiological biomarker for translating the known inhibitory GABAergic vs. excitatory glutamatergic chemical-electrical principle from animal brain studies to human brain studies.

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Poster

125. Oscillations and Synchrony in the Human Brain

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 125.08/H8

Topic: B.09. Network Interactions

Support: University of Texas Brain Initiative, Direct Probes and Genetic Underpinnings of Neural Oscillations

Title: Effect of COMT and DAT1 genetic polymorphisms on resting EEG activity in healthy individuals

Authors: *N. RAMAKRISHNAN[1], C. P. WALKER[1], C. J. RODRIGUEZ[1], A. VENKATA SUBRAMANIAN[2], M. RAFFERTY[1], D. FRAHER[1], G. R. FRIES[3], N. R. POLIZZOTTO[3], N. MURPHY[1], C. WALSS-BASS[3], R. Y. CHO[1]
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Abstract: Background: Dopaminergic circuits and cortical gamma oscillations are associated with multiple sensory and cognitive processes, and are disturbed in many neuropsychiatric disorders such as schizophrenia and bipolar disorder. However, the interactions between dopamine (DA) and gamma activity in normal and pathological conditions have not been completely understood. (Furth, K. E., et. al, 2013). In this study, we studied the effects of functional polymorphisms of catechol-O-methyltransferase (COMT) gene which encodes the enzyme to degrade DA in prefrontal cortical areas (Gogos, J. A., et. al, 1998, Qinghua He., et. al, 2012) and the DA transporter gene (DAT1) and its association with variations in brain oscillations. **Methods:** 59 healthy individuals (17 M, 42 F) were recruited, restricted to African Americans to maintain relative homogeneity for genotype-related analyses. Genotyping was conducted on all subjects. The resting state electroencephalography (EEG) data for eyes open and close conditions (90 seconds each) was de-trended and lowpass filtered at 150 Hz. Continuous EEG data was divided into 2-second segments. Artifact removal was performed using Independent Component Analysis. Mean power estimates were calculated for each of the frequency bands, delta (0 to 3 Hz), theta (4 to 7 Hz), alpha (8 to 13 Hz), and beta (14 to 30 Hz) and gamma (31 to 54 Hz). In order to assess associations between each individual single nucleotide polymorphism (SNP) and EEG parameters, data was entered into a General Linear Model analysis. **Results:** One-way ANOVA for DAT1 and mean gamma power at fronto-central electrodes (F3C3, F4C4) yielded significant differences across the genotypes ($p < 0.05$) for both eyes open and closed conditions. One-way ANOVA for COMT and mean gamma power at parietal-occipital electrodes (P3O1, P4O2) for eyes open condition, yielded significant differences between the genotypes ($p < 0.05$). Met/Met Individuals exhibited higher mean gamma power in the eyes open condition compared to Val/Val or Val/Met Individuals. **Conclusion**: These results provide some preliminary evidence for genotypic variations in COMT and DAT1 and associated variations in DA signaling, modulating gamma oscillations in healthy individuals. Future studies will investigate the impact of other critical components of dopamine signaling (e.g. dopamine receptors) on neural oscillations, including the epistatic interactions between them as well as with other genes critical to oscillations such as glutamate and GABA neurotransmission.

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Poster

125. Oscillations and Synchrony in the Human Brain

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Topic: B.09. Network Interactions

Support: NSERC Research Chair in Neurovascular Coupling

Title: Frequency specific retinotopic and orientation tuning and relationship to hemodynamic responses in healthy human V1

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Abstract: The EEG spectral response to visual stimulation in healthy humans is characterized by decrease in low frequency (alpha/beta, 8-25Hz) amplitude and increase in high frequency (gamma, 40-80Hz) amplitude. The alpha/beta and gamma responses are thought to be functionally distinct, representative of feedback and feedforward neural activity respectively, but to date almost no work has been done examining functional differences in alpha/beta and gamma frequency response to low-level visual manipulations such as retinotopic location and orientation. To address this, we performed orientation and retinotopic tuning experiments in healthy human subjects (n=10), and examined the tuning preferences of alpha/beta and gamma rhythms, then compared these tuning preferences to results from the same stimuli using FMRI. We find that alpha/beta and gamma respond similarly to retinotopic location, with stronger responses to lower visual field than higher visual field. This lower field preference can be explained by a higher number of FMRI voxels activated in response to lower visual field, as well as these voxels being closer to the scalp. For orientation tuning, we find stronger gamma responses to oblique over cardinal orientations, and vertical over horizontal orientations. Alpha showed no orientation tuning, and beta preference was opposite to gamma, preferring cardinal over oblique orientations. FMRI orientation tuning more closely matched gamma band tuning, with a strong preference for vertical over horizontal orientations, and weaker preference for oblique over cardinal, both in terms of overall magnitude and number of activated voxels. The fact that low (beta) and high (gamma) EEG frequencies have opposite orientation tuning preferences, but similar retinotopic tuning preferences shows that while both bands are spatially specific, they convey different functional information within a given region of cortex.

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Poster

125. Oscillations and Synchrony in the Human Brain

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Topic: B.09. Network Interactions

Support: ISF grant 961/14

Title: Neural variability quenching is strongly associated with reductions in neural oscillations

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Abstract: Neural activity varies dramatically across repeated trials of the same behavioral task, for neural signals recorded at various different spatial scales. This trial-by-trial variability is reduced (quenched) approximately 200 ms after presentation of a sensory stimulus, for different experimental protocols, sensory stimuli, and species (animal models and humans). Other work has shown that sensory stimulation modulates the amplitude of neural oscillations that are not phase-locked to stimulus onset (i.e., "induced" oscillations). In extra-cranial EEG and MEG recordings these modulations are dominated by a suppression of alpha band (8-13Hz) oscillations. We hypothesized that variability quenching and induced alpha-suppression may be correlated measures that reflect the same stimulus-evoked change in neural dynamics. To test this hypothesis, we examined MEG recordings of healthy subjects viewing multiple repetitions of a full-field moving visual stimulus. We used band-stop filters to remove oscillations in specific frequency bands from the data: delta (1-4Hz), theta (4-8Hz), alpha (8-13Hz), beta (14-25Hz), and gamma (60-120Hz). When averaging the data across all subjects, removing alpha band activity entirely eliminated the variability quenching phenomenon, removing delta band activity effectively doubled the variability quenching phenomenon, and removing the other frequency bands had negligible effects. Additional analyses revealed that individual differences in the magnitude of variability quenching were strongly correlated with individual power modulations in all frequency bands except gamma (i.e., delta, theta, alpha, and beta). In contrast, individual magnitudes of variability quenching were not correlated with the timing of neural responses as measured using inter-trial phase coherence (ITPC). Taken together, these results suggest that variability quenching, as measured with EEG and MEG, stems from changes in the amplitude of neural oscillations that are not phase locked to the stimulus, primarily in the alpha

band. However, individual differences in the magnitude of variability quenching stem from power modulations in multiple frequency bands including, but not limited to, alpha suppression.

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Poster

125. Oscillations and Synchrony in the Human Brain

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Title: Beta burst rate and timing predicts action initiation and performance in the human motor cortex

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Abstract: Motor cortical beta activity (13-30 Hz) is a hallmark signature of movement, but its behavioural relevance is controversial. Recent evidence in non-human primates suggests that classical slow, spatially diffuse, trial-averaged changes in beta amplitude seen before and after movement in the primary motor cortex, may simply be a summary of underlying short-lasting, high-powered beta bursts, rather than sustained oscillations. Here we directly investigate this rapid beta burst activity in human sensorimotor cortex and relate this to classical trial-averaged accounts of beta amplitude and behaviour. Using high-precision magnetoencephalography (MEG), we quantified the subject-specific, trial-wise dynamics of beta burst activity, before and after movement onset. We show that on individual trials, beta activity has a predisposition for high powered, short lasting (~175ms) bursts which give rise to the classical event related desynchronisation / synchronisation (ERD / ERS) seen in the averaged spectral domain, in despite of a low overall (1.06 ± 0.04 bursts / sec) rate. While average beta changes are bilateral and spatially diffuse (FWHM = 30mm), individual bursts are more focal (FWHM = 16 mm) and temporally uncorrelated to bursts in contralateral motor cortex (rho=0.09). This suggests that the ERD / ERS is a spatially dispersed summation of short lasting, sporadic and more focal bursts. Conventional beta amplitude analyses average over trials and overlook these precise spatio-temporal trial-by-trial beta burst dynamics, which we here show to be a much stronger

determinant of behaviour than classical average amplitude changes. Specifically, we demonstrate that prior to movement (ie classical ERD period), burst rate is most predictive of motor initiation. Furthermore, after movement (ie, classical ERS period), it is the timing of individual bursts that most relates to performance whereby errors resulted in a delay of the first burst by ~100ms in all subjects. The behaviourally most relevant components of sensorimotor beta activity (ERD/ERS) are thus short lasting bursts of activity that are also spatially more focal than classical, trial-averaged, accounts. Moreover, we find that burst rate and timing more accurately relate to movement initiation and performance than classical amplitude changes, which therefore represents an insufficient non-dynamic summary metric of rapid burst activity. These results challenge long standing theories regarding the role of sensorimotor beta activity, and suggest the need for their updating to take account of rapidly dynamic beta burst activity.

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Poster

125. Oscillations and Synchrony in the Human Brain

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Title: Distinct deep and superficial cortical inputs cause beta bursts in human sensorimotor cortex

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Abstract: A substantial proportion of sensorimotor beta activity occurs in discrete bursts rather than sustained oscillations (Jones et al., 2009; Feingold et al., 2015; Lundqvist et al., 2016). High-power beta bursts have a stereotyped wavelet-like shape in the time domain (Sherman et al., 2016). Computational modeling and laminar recordings in rat and monkey somatosensory

cortex (Sherman et al., 2016), suggest that beta bursts are generated by strong ~50ms (beta period) input to superficial cortical layers aligned with the peak of a weaker, more prolonged input to deep layers, which combine to create the wavelet shape of the cumulative dipole measured by MEG. However, it is not clear whether this also applies to movement-related beta changes in human sensorimotor cortex. We tested this laminar account of beta burst generation in human subjects by leveraging recent developments in high precision magnetoencephalography (MEG), coupled with a new time-resolved analysis of laminar activity. The analysis builds on previous laminar MEG analyses (Troebinger et al., 2014; Bonaiuto et al., 2017, 2018) which compare the evidence for different generative models based on the white matter and pial surfaces, representing deep and superficial layers respectively, given sensor data. The new analysis involves the identification of corresponding pial / white matter source priors, and comparing the evidence for the two models in a sliding time window. The result is a temporally resolved estimate of the relative strength of superficial and deep cortical layer activity.
We first used the detailed computational model of beta burst generation (Sherman et al., 2016; Human Neocortical Neurosolver; hnn.brown.edu) to create synthetic MEG data and tested whether our new analysis could recover the simulated strong superficial layer activity at the peak of the burst and deep layer activity surrounding the burst. Our time-resolved inverse analysis was able to extract just such a temporal pattern of laminar dominance. We then tested 8 subjects during a visually cued movement paradigm using high precision, head-cast, MEG (Meyer et al., 2017). We identified beta bursts by thresholding beta power at the source level, and re-epoched the data to the each burst peak. We then averaged the time domain sensor data and analyzed the resulting waveform using our time-resolved inverse analysis. The results precisely fit the predictions of the model, demonstrating that movement-related beta bursts in human sensorimotor cortex are the result of distinct deep and superficial inputs. Further, this technique promises to allow the non-invasive testing of laminar theories of a wide variety of evoked cortical responses.

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Poster

125. Oscillations and Synchrony in the Human Brain

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Topic: B.09. Network Interactions

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Title: Brain complexity and network dynamics change during motor resonance

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Abstract: Background: Mu suppression, an index of motor resonance (MR), the activation of an observer's motor system attuned to perceived movement, has been used to study deficiency in social performance, such as those observed in autism spectrum disorder (ASD). However, mu suppression does not provide information about how nonlinear brain dynamics are affected during MR. If MR captured with electroencephalography (EEG) reflects the processing of social information, it is important to explore whether the underlying nonlinear brain dynamics change. For the current study, we hypothesized that that the complexity of the mu frequency band (8-13 Hz) would increase during mu suppression, and that this change would be frequency specific. Additionally, we sought to determine whether changes in mu complexity during MR were correlated with changes in network dynamics.
Methods: EEG was recorded from healthy participants (n=46) who performed both resting state and action-observation tasks. Baseline brain activity was measured followed by participants observing videos of hands squeezing stress balls. We used multiscale entropy (MSE) to quantify the complexity of the mu rhythm during MR, and a post-hoc graph theory analysis to explore how MR affects brain network topology.
Results: We found significant suppression of the mu rhythm, during which mu entropy was significantly increased compared to rest, while gamma, theta, and delta bands all showed decreased entropy. Resting-state EEG entropy was shown to be significantly predictive of the degree of mu suppression. We observed a decrease in the clustering coefficient in the mu and theta band over the sensorimotor area. Global efficiency significantly decreased during the task in the alpha band only. Regression analysis showed high mu entropy at baseline predicted less suppression, whereas, high global efficiency in the alpha band predicted more suppression. Task entropy was strongly correlated with alpha network efficiency during the task.
Conclusions: The current study suggests that the suppression of the mu wave during MR results in a local increase of mu entropy and decrease in the connectedness of the sensorimotor area. The decrease in the global efficiency of the alpha band may relate to a global release from inhibition. This release from inhibition may be mediated by the baseline entropy of the mu band over the sensorimotor area. The dynamical complexity and network analysis of EEG may be useful for understanding healthy biological social processing, and potentially aid in identifying the underlying biological mechanisms that drive the heterogeneity in some of the social deficiencies observed in disorders like ASD.

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Poster

125. Oscillations and Synchrony in the Human Brain

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Title: Parameterization of periodic and aperiodic human electrophysiology reveals greater between- than within-subject variability

Authors: *L. MDANDA[1], T. DONOGHUE[2], B. VOYTEK[3]
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Abstract: Oscillations are rhythmic properties of neural activity that are widely implicated in neural computation and communication. Despite their ubiquity and broad theoretical importance, they are usually examined using approaches that ignore individual variability, and with methods that may conflate the periodic and aperiodic ("1/f") components. Here, we use electroencephalography (EEG) data to characterize oscillatory characteristics within and between subjects across cognitive demands, to explore idiosyncratic variability and group level trends of oscillatory activity. We analyze 30 subjects of EEG both at rest and while performing visual psychophysics tasks. We apply a novel algorithm for parametrizing neural power spectral densities (PSDs), modeling them as a combination of an aperiodic signal and putative periodic oscillations. Using this method, we are able to measure, per subject, per condition, and per electrode, precise oscillatory features (center frequency, aperiodic-adjusted amplitude, bandwidth), for oscillation bands theta, alpha and beta, as well as the aperiodic activity, operationalized by the slope and offset of the PSD. We find that there is a high degree of individual variability of oscillatory characteristics; that those features are relatively stable within subjects within cognitive state; and that there is systematic variation between task and rest blocks. We identify spatial shifts in neural oscillatory characteristics, such as systematic drifts in band-specific peak frequencies across the anterior to posterior and medial to lateral axes, as well as shift across trial blocks (across time). We replicate and extend all of these analysis across a large, open-access dataset of EEG data (n > 100; ChildMind institute). In this larger sample, we find that oscillatory and aperiodic properties systematically vary across age, and that this partially explains individual variability in oscillatory parameters. This degree of variation is important, because typical analyses may often presume more stability and consistency than is actually present in the data; for example, when analyzing peak frequency, variation across spatial

localization and task state is often ignored, and may partially explain differing results in the literature. In sum, we show that there is a high degree of variability in oscillatory activity across subjects, we show how this variability can be captured by novel analysis methods, and suggest that more quantitative approaches should be more broadly incorporated into studies of neural oscillatory activity.

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Poster

125. Oscillations and Synchrony in the Human Brain

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Program #/Poster #: 125.15/H15

Topic: B.09. Network Interactions

Title: TMS-evoked oscillations in human cortical circuits: A search for natural frequencies

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Abstract: Previous evidence suggests that different cortical areas naturally oscillate at distinct frequencies, reflecting tuning properties of each region. Simultaneous transcranial magnetic stimulation (TMS) and electroencephalography (EEG) has been used to perturb cortical regions, resulting in an observed post-stimulation response that is maximal at the natural frequency (Rosanova et al., 2009; Ferrarelli et al., 2012). These frequencies were reported to progress from high to low in a rostro-caudal gradient across the cortex, such that a consistently evoked dominant α-band oscillations (8-12Hz) occurred in the occipital cortex (BA 19), β-band oscillations (13-20Hz) in the parietal cortex (BA 7), and fast β/γ-band oscillations (21-50 Hz) in the frontal cortex (BA 6). Thus far, literature investigating natural frequencies in cortical circuits with TMS-EEG have only been demonstrated in the left hemisphere. Here, we attempted to replicate these previous findings in the right hemisphere by employing TMS-EEG to directly perturb approximately homologous cortical areas in human subjects (N=6). Contrary to previous reports, we found limited evidence of differences in fundamental frequency between stimulation sites; while frequency-specific responses differed between sites, the same features displayed in previous studies were not replicated, such as the rostro-caudal gradient. Instead, each site possessed its own complex pattern of global and local changes in response to stimulation, with the pattern differing per subject. These findings suggest that cortical regions exhibit complex frequency-specific profiles that cannot be described by a hierarchical progression alone. Further analysis will attempt to distinguish these frequency-specific differences between site of stimulation.

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Poster

125. Oscillations and Synchrony in the Human Brain

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Title: Neural oscillation symmetry as a novel feature for decoding algorithms in brain-computer interfaces

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Abstract: In brain computer-interfaces (BCIs), a subject's brain activity is recorded and used to control an electronic device in real-time. For example, a BCI could record activity from the motor cortex of someone who lost a limb in order to control a prosthetic device. However, decoding brain signals to predict intended movement remains a challenge. Therefore, in this project, we explore the potential for novel neural features to assess their viability in decoding finger movements. We analyzed a publicly available dataset of 8 epilepsy patients with electrocorticography (ECoG) recordings performing a cued finger flexion task (Miller et al., 2009).

High gamma (80-200 Hz) power has previously been shown to be a reliable, robust signal for BCI. However, decoding accuracy using only features derived from high gamma power is not perfect, so additional features of the neural signal may be useful. Another prominent feature of motor cortical activity is the beta (13-30 Hz) oscillation. Beta power has been previously reported not to provide useful information for decoding finger movement (Chestek et al., 2013), but the beta waveform shape has not been explored. While the amplitude and frequency of neural oscillations have commonly been analyzed, oscillation waveform shape features have been more difficult to quantify. Recent methodological developments have introduced measures for rise-decay symmetry, and peak-trough symmetry (Cole & Voytek, 2018). Rise decay symmetry measures the fraction of time the oscillation spends in the rise phase, and peak-trough symmetry is a similar measure for the peak phase. The symmetry of motor cortical beta oscillations was recently shown to distinguish treatment state in Parkinson's disease, a motor disorder (Cole et al., 2017). We hypothesized that beta waveform symmetry may offer additional information to BCI decoding algorithms.

Classifiers were trained to predict when finger movement occured by using features of the ECoG recordings. Three features of brain activity were computed from each electrode by averaging over two-second intervals: 1) high gamma power, 2) beta rise-decay symmetry, and 3) beta peak-trough symmetry. We trained logistic regression models with high gamma and/or symmetry features and evaluated accuracy with 5-fold cross validation. Adding symmetry features improved the accuracy, on average, from 91% to 93%. Accuracy was particularly improved for subjects in which high gamma power offered the lowest accuracy. We also trained a logistic regression multiclass model to predict which finger moved (e.g. thumb, index, etc.), but adding symmetry features yielded no improvement.

Disclosures: Y. Yang: None. **S.R. Cole:** None. **V. Gilja:** None. **B. Voytek:** None.

Poster

125. Oscillations and Synchrony in the Human Brain

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Title: Instantaneous voltage of electroencephalographic oscillatory activity: An alternative to power and phase measurements

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Abstract: Oscillatory brain activity is usually characterized by measurements of power and phase. Recent theoretical work has suggested that oscillatory activity may be better understood by the instantaneous amplitude of oscillatory activity. Furthermore, recent experimental work using electrocorticography (ECoG) has demonstrated that the instantaneous amplitude is a better predictor of cortical excitability than either the power or the phase. At the same time, the practical impact of this finding would be greatly increased if it could be replicated with the much

more prevalent electroencephalographic (EEG) recordings from the scalp. The present study sought to test this hypothesis. We recorded EEG from 64 locations in 20 human subjects while they responded to visual stimuli with a button press. The visual stimuli were calibrated to be at perceptual threshold (i.e., 50% behavioral response rate), and were presented at one of 4 locations within foveal vision after a 1.6-3s gaze fixation period. To avoid retinal adaptation, we conducted the experiment in a dark room and presented a bright masking stimulus after every trial. We included 10% catch trials to verify subject attention (i.e., salient stimulus) and task compliance (i.e., no stimulus). In total, each subject performed 600 threshold and 120 catch trials at over 90% attention and task-compliance, respectively. We first established the neural correlates of perceived and non-perceived trials. We then determined how oscillatory power, phase or instantaneous amplitude in the alpha band (6 - 13Hz, 2 Hz window centered around individual alpha frequency) at electrode Oz varied between perceived and non-perceived trials. Our results confirm the expectation that pre-stimulus alpha power and pre-stimulus phase are different for perceived and not perceived trials ($p < 0.05$, bonf. corrected). More importantly, and in line with recent ECoG-based work, they show that the instantaneous voltage is a better neural correlate of visual perception than either alpha power or alpha phase ($p < 0.01$), and that the instantaneous amplitude provides more temporally fine-grained information about cortical excitability than oscillatory power. Finally, they show that the maximum correlation with instantaneous voltage occurs at about 50 ms after stimulus onset, which is consistent with the arrival of stimulus-related information in visual cortex as determined by single-neuron experiments in monkeys. Our results further encourage the use of the instantaneous voltage as an informative neural correlate for the study of oscillatory activity.

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Poster

125. Oscillations and Synchrony in the Human Brain

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 125.18/H18

Topic: B.09. Network Interactions

Support: NIH Intramural Research Program

Title: Oscillatory and broadband contributions to directed functional connectivity in the human cortex

Authors: *J. I. CHAPETON[1], R. HAQUE[1], S. K. INATI[2], K. A. ZAGHLOUL[1]
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Abstract: Neural oscillations are ubiquitous in the human cortex and appear at multiple temporal and spatial scales, however, the functional significance of these rhythms continues to be a topic of intense debate. One idea that has gained some traction is that oscillations may provide a gating mechanism by which the flow of information can be directed. In order to further test this idea, we analyzed intracranial EEG activity (iEEG) from 10 human participants and constructed directed functional connectivity maps for each participant. We used both time and frequency domain measures of functional connectivity in order to disentangle the contributions of narrow-band oscillations and correlated broadband activity to cortico-cortical communication. Specifically, we computed a metric of directed functional connectivity, *W*, which is based on increases in absolute correlation at consistent time delays, as well as the spectral coherence for all electrode pairs. We find that the coherence functions for pairs with a large value of *W* show a dominant peak in the 6-14Hz range. Although the peak frequency varies across subjects, within a subject the frequency of maximum coherence is consistent across time and electrode sites. This relationship between directed functional coupling and alpha coherence is further evidenced by a strong correlation between the coherence peak prominence and *W* across all participants. Along with the dominant peak, there was an overall positive shift in coherence for lower frequencies as compared to higher frequencies (>40Hz). Given the definition of coherence as a frequency domain representation of the cross-correlation function, these observations demonstrate that functionally coupled cortical regions consistently exhibit synchronous oscillations in the alpha band as well as broadband correlated activity in the 2-40 Hz range.
Next, we estimated the latency between sites using the cross-spectrum phase at the most coherent frequency and find that it is in good agreement with the preferred time delay estimated from the time domain analysis. Such phase and timing relationships are critical if these oscillations are to be used for modulating large-scale neuronal communication. Importantly, for a subset of these participants we obtained single unit activity from micro-electrode recordings, which revealed large spike-field coherence at the same frequency as the spectral coherence between the iEEG

sites. Together, our findings support the idea that coherent low frequency rhythms can provide a mechanism for the reliable transmission of neural representations encoded in correlated broadband activity over large distances.

Disclosures: J.I. Chapeton: None. **R. Haque:** None. **S.K. Inati:** None. **K.A. Zaghloul:** None.

Poster

125. Oscillations and Synchrony in the Human Brain

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 125.19/I1

Topic: B.09. Network Interactions

Title: The spatial structure of phase- and amplitude-coupling in the human brain

Authors: *M. SIEMS, M. SIEGEL
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Abstract: Correlated oscillations of neuronal activity have been proposed to facilitate and orchestrate communication between distant brain regions. There are two primary coupling modes between distant neuronal oscillations: phase-coupling and amplitude-coupling. These two coupling modes may have different neuronal origins and serve or indicate different functional roles. However, their relationship remains unclear. To address this, we combined magnetoencephalography (MEG) and distributed source-reconstruction to systematically investigate and compare the spatial structure of phase- and amplitude coupling in the human brain. We found that, if measurement reliability is accounted for, both coupling modes share strong similarities as well as frequency and connection specific differences. This suggests, that phase- and amplitude coupling at least partly result from distinct neural mechanisms.

Disclosures: M. Siems: None. **M. Siegel:** None.

Poster

125. Oscillations and Synchrony in the Human Brain

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 125.20/I2

Topic: B.09. Network Interactions

Support: DFG EXC 307

ERC StG 335880

Title: Harmonic signatures of neural oscillations in the human brain

Authors: *J. GIEHL[1,2,3], M. SIEGEL[1,2]
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Abstract: Previous investigations of cortical cross-frequency coupling have revealed prominent harmonic signatures of neural oscillations. However, it remains unclear how specific these harmonics signatures are for different brain regions and individual subjects. To address these questions, we employed resting-state MEG recordings in healthy human subjects. We identified several robust harmonic signatures of cortical resting-state oscillations that were region- and subject specific. These harmonic signatures may provide a novel spectral fingerprint of neural oscillations and open a new window onto the underlying physiological processes.

Disclosures: J. Giehl: None. **M. Siegel:** None.

Poster

125. Oscillations and Synchrony in the Human Brain

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 125.21/I3

Topic: B.09. Network Interactions

Support: DFG EXC 307
ERC StG 335880

Title: Oscillatory and fractal signal components of human resting-state MEG

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Abstract: Electrophysiological signals can be decomposed into oscillatory and non-rhythmic (1/frequency or fractal) components. Yet, little is known about the relationship of these signal components. To address this, we investigated resting state MEG recordings in humans using two separate datasets (113 total subjects). We employed a combination of source-reconstruction (beamforming), time domain orthogonalization, and irregular-resampling auto-spectral analysis (IRASA) to separate cortical oscillatory and fractal components of the MEG signal. We found a spectrally specific correlation between both signal components that was highly consistent over the cortex. Furthermore, we found similar spatial correlation structures of both signal

components. Together, our results suggest a tight coupling of fractal and oscillatory signal components in human resting-state MEG signals.

Disclosures: A. Ibarra Chaoul: None. **M. Siegel:** None.

Poster

125. Oscillations and Synchrony in the Human Brain

Location: SDCC Halls B-H

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Program #/Poster #: 125.22/I4

Topic: B.09. Network Interactions

Support: DFG EXC 307
NIMH 5R37MH087027
ERC StG 335880
DFG SI 1332/3-1

Title: Four functionally distinct cell classes can be identified from extracellular recordings in primate cortex

Authors: *C. TRAINITO[1,2,3], C. VON NICOLAI[1,2], E. K. MILLER[4], M. SIEGEL[1,2,4]
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Abstract: Brain function relies on the diversity of neuronal cell types. However, cell type information is typically lacking in extracellular recordings in behaving animals, where morphology or gene expression cannot be directly assessed. Here, we infer cell types in macaque cortex from a large sample of extracellularly recorded single-units (~2400) based on the action potential waveform. Using unsupervised clustering methods, we identified four cell classes, which consistently emerged in areas FEF, dorsolateral PFC and LIP. Cell classes with narrower waveforms (putative inhibitory interneurons) were fast-spiking, while broad-waveform units (putative excitatory cells) showed sparse, regular firing. Burst firing was prominent in one class with intermediate-broad waveform. To validate the waveform-based classification of four cell classes, we performed supervised learning of cell classes from three different sets of functional properties of neuronal activity - baseline firing statistics, response dynamics and information coding. All four classes could be significantly decoded both within and across cortical regions. Taken together, our results show that spike waveform alone can reveal a richer functional diversity of neurons than previously thought, beyond the two main excitatory and inhibitory classes.

Disclosures: C. Trainito: None. **C. Von Nicolai:** None. **E.K. Miller:** None. **M. Siegel:** None.

Poster

125. Oscillations and Synchrony in the Human Brain

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 125.23/I5

Topic: B.09. Network Interactions

Support: DFG EXC 307
DFG SFB 1233
DFG SI 1332/3-1
ERC StG 335880
NIMH 5R37MH087027

Title: Bridging the gap: Visual information in spikes, LFP, EEG and MEG

Authors: *F. SANDHAEGER[1], C. VON NICOLAI[1,2], E. K. MILLER[3], M. SIEGEL[1,2,3]
[1]Ctr. for Integrative Neurosci., [2]MEG Ctr., Univ. of Tuebingen, Tuebingen, Germany; [3]Picower Inst. Learning Memory, Massachusetts Inst. Technol., Cambridge, MA

Abstract: Multivariate classification techniques allow for extracting the information content of neural signals rather than merely measuring activity levels. However, it remains unclear what these methods measure, when applied to MEG and EEG data, and what circuit level processes their results correspond to.
To address this, we performed microelectrode recordings in several cortical areas of two macaque monkeys as well as macaque EEG and human MEG during identical visual stimulation with random dot patterns of varying colors and motion directions. We then applied identical multivariate classification analyses on all data, including source-reconstructed EEG and MEG. We found that both motion and color information were accessible in all signal types. Tuning properties and latencies of the non-invasive signals reflected those of LFP and unit activity in V4 and IT, while diverging from those in dorsal and frontal areas, suggesting that MEG and EEG signals were dominated by early visual and ventral stream sources. We confirmed this correspondence using representational similarity analysis (RSA). Source level analysis of MEG and macaque EEG revealed corresponding gradients of information content and latencies, with earlier and higher information contents in early visual areas.
In sum, we show that contents of color and motion representations are accessible to non-invasive electrophysiology, and second, how information-based methods can identify analogous properties of visual processing in signals spanning spatial scales from single units to MEG - a valuable framework for better relating human and animal studies.

Disclosures: F. Sandhaeger: None. **C. von Nicolai:** None. **E.K. Miller:** None. **M. Siegel:** None.

Poster

125. Oscillations and Synchrony in the Human Brain

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 125.24/I6

Topic: B.09. Network Interactions

Support: NIMH 5R37MH087027
ERC StG 335880
DFG EXC 307
DFG SI 1332/3-1

Title: Neuronal phase-coupling predicts neuronal coding

Authors: *M. SIEGEL[1,2,3], C. VON NICOLAI[1,2], E. K. MILLER[4]
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Abstract: Neurons encode different types of information. It is widely assumed, that this functional specificity, i.e. if and which information neurons encode, stems from both, local neuronal interactions within, as well as long-range interactions between brain regions. However, little is known about how the functional specificity of neurons is related to their local and large-scale patterns of interactions. To investigate this, we simultaneously recorded neuronal activity from six cortical regions (MT, V4, IT, LIP, PFC and FEF) of monkeys performing working-memory, visual stimulation, and decision-making tasks. We found that neuronal activity was widely phase-coupled in a frequency- and region-specific fashion. Both, local and long-range patterns of phase-coupling predicted if neurons encoded sensory, motor, memory or task information. Predictive synchronization patterns were specific for the type of information and regions at hand. Our results suggest that the functional specificity of neurons is shaped by their rhythmic phase-coupling in large-scale cortical networks.

Disclosures: M. Siegel: None. **C. von Nicolai:** None. **E.K. Miller:** None.

Poster

125. Oscillations and Synchrony in the Human Brain

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 125.25/I7

Topic: B.09. Network Interactions

Support: DFG EXC 307
ERC StG 335880

Title: Making decisions in space and time - two facets of the same neural circuit?

Authors: *D. J. HAWELLEK[1,2], M. SIEGEL[1,2]
[1]Ctr. for Integrative Neurosci., [2]MEG Ctr., Univ. Tuebingen, Tuebingen, Germany

Abstract: We continuously make decisions between behavioral options in space. For example, do I look to the right or the left when crossing a street? However, decision-making is not exclusively a spatial problem. Our behavior also depends on the ability to predict the best course of action in time. While both spatial and temporal aspects of decision-making are known to recruit distributed fronto-parietal cortex and subcortical circuits, their relationship remains unknown. We hypothesize that the identical neural system implements decision-making in space and time. To test this hypothesis, we developed a new task that allows us to dissociate spatial and temporal aspects of decision-making on a trial-by-trial basis. We recorded eye movement behavior and MEG while participants engaged in the task. Shortly after stimulus onset the direction of microsaccades predicted the later spatial choice while the rate of microsaccades and pupil dilation predicted the temporal proximity of an upcoming choice. Thus, after a brief sampling period, both, spatial and temporal predictions formed nearly simultaneously. We next mapped the information about spatial and temporal choice contained in neural activity. Alpha band (~10 Hz) activity in a distributed system with a prominent right posterior parietal cortical contribution simultaneously predicted the subjects' spatial and temporal choices seconds before the overt behavior. Our results are consistent with the idea that the computations for decisions in space and time are multiplexed and controlled by the identical distributed macro-circuit.

Disclosures: D.J. Hawellek: None. **M. Siegel:** None.

Poster

125. Oscillations and Synchrony in the Human Brain

Location: SDCC Halls B-H

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Program #/Poster #: 125.26/I8

Topic: B.09. Network Interactions

Support: Grant HBP SGA 1 - 720270
São Paulo Research Foundation, grant 2016/08263-9
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Title: Cortical complexity and cause-effect power are reduced by general anesthesia compared to wakefulness in rats

Authors: *A. ARENA[1], R. COMOLATTI[2], A. G. CASALI[2], J. F. STORM[1]
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Abstract: The study of consciousness has always been challenging mostly due to the lack of objective and robust measures of its properties. To shed light on this elusive phenomenon, the Integrated Information Theory (IIT) argues that a physical system generates consciousness as it is able to integrate information (Tononi 2004, Tononi et al. 2016). In terms of brain functioning, this is reflected in the ability to support complex activity patterns that are widely distributed among interacting neuronal ensembles (integration) and differentiated in both time and space (information content). This ability of the brain has been quantified by a new metric, the Perturbational Complexity Index (PCI; Casali et al. 2013). In agreement with IIT, this index has proved able to reliably discriminate between conscious and unconscious brain states in humans (Casali et al. 2013, Sarasso et al. 2015, Casarotto et al. 2016). Moreover, since PCI quantifies the complexity of EEG activity following a cortical perturbation, it is objective and does not require any verbal interaction, allowing it to be applied also to non-human animal models. Here, we present the first results of a study of perturbational complexity in rodents *in vivo*. Large-scale multichannel EEG activity was recorded from head-restrained rats by means of 16 epidural stainless-steel electrodes chronically implanted across the scalp, covering most of the cortex. Cortical perturbation was given by brief current pulses through a bipolar tungsten electrode placed in the right secondary motor cortex. The resulting evoked potentials were recorded from each rat in wakefulness and during general anesthesia caused by two different anesthetics (propofol and sevoflurane), allowing internal controls.Similarly to previous human studies, this method revealed in rats a loss of cortical complexity from wakefulness to general anesthesia. Moreover, the anesthetized state was associated with suppression of frequencies between 5 and 30 Hz in the event related potentials (ERPs) evoked by cortical perturbation and reduced phase-locking among subsequent ERPs, compared to wakefulness. These observations are also in line with results from humans (Pigorini et al. 2015) and may indicate an anesthesia-induced break in the cause-effect chain of neuronal events that sustains cortical integration of information during wakefulness (Tononi et al. 2016).

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Poster

126. Biology of Microglia

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 126.01/I9

Topic: B.11. Glial Mechanisms

Support: KAKENHI/16K00380 to R.K.

KAKENHI/17H01810 to I.T.

Title: Real-time electrochemical monitoring of lactic-acid released from an *in vitro* mouse brain slice preparation

Authors: *M. ZAILUDDIN[1], Y. TOJYO[1], S. RAMLI[1], M. HYODO[1], I. TAKASHIMA[2], H. KUDO[1], R. KAJIWARA[1]
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Abstract: Most of the energy in the brain is consumed by nerve cells for brain physiology and information processing. Under normal conditions, nerve cells cover their high energy demand with glucose and lactic acid (lactate). Although the role of lactate for metabolism have been much debated, research for lactate as alternative energy substrate to glucose during brain metabolism have been steadily increasing these past few years. It has been speculated that when neurons are firing at high frequencies, lactate may be a preferred energy substrate (Baltan 2015). However, real time monitoring of brain metabolism during the activation of neuron cells have not widely researched. Therefore, the role of lactate during the many state of neuronal activities remains unclear. The purpose of this research is to design the experimental system for monitoring the lactate slightly released from a living brain slice.In our previous study, we have constructed a continuous Lactate monitoring system using a micro-fluidic dual-analyte (Lactate and glucose) (Negishi et al, 2016). The system consists of a multi-analyte (lactate and glucose) biosensor, polyvinyl chloride (PVC) adhesive sheet with micro flow channel (width: 500μm) and polydimethyl siloxane (PDMS) sheet with inlet/outlet tubes for supplying artificial cerebrospinal fluid (aCSF) and experimental solutions. The biosensor has four electrodes (working electrode 1 on which lactate oxidase (LOD) is immobilized, working electrode 2 on which glucose oxidase (GOD) is immobilized, silver / silver chloride reference electrode and counter electrode). LA and glucose measured by amperometric method as a change of hydrogen peroxide which is produced by enzymatic reaction of LOD and GOD. We combined this micro-fluidic device with the interface-type chamber for electrophysiological recording. By using this system, we successfully measured the lactate signal (1.8 nA) from the brain tissue under the perfusion of aCSF. Moreover, the signal was dramatically increased to 7.7 nA when we decreased the glucose level of the aCSF (10mM to 5mM). Additionally, during the experiment the evoked field potential peak amplitude remained unchanged at 5mM glucose. These results suggest that the brain slice normally released a certain amount of lactate, and neuro-glial interaction system in the brain might be triggered by the decrease of the glucose level of the extracellular space. Since our system is capable for simultaneous recordings of lactate signal and electrical activity of neurons following electrical and/or chemical stimuli, it would be useful to investigate the relationship between metabolism and neuronal activities under various conditions.

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Poster

126. Biology of Microglia

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 126.02/I10

Topic: B.11. Glial Mechanisms

Title: Nitric oxide regulates transient receptor potential vanilloid type 2 channel trafficking in microglia

Authors: *M. J.-E. MAKSOUD[1,2], V. TELLIOS[1,2], D. AN[1], Y.-Y. XIANG[1], W.-Y. LU[1,2]
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Abstract: As primary immune cells in the central nervous system (CNS), microglia critically regulate the homeostasis and inflammatory reactions within the brain. During inflammatory events, microglia produce nitric oxide (NO) via the enzymatic activity of inducible nitric oxide synthase (iNOS). NO signalling has been implicated in the regulation of microglia proliferation and phagocytosis, and such regulations rely on the modulation of intracellular calcium (Ca^{2+}) dynamics. Previous studies have demonstrated that mouse microglia express transient receptor potential vanilloid type 1 and 2 (TRPV1 and TRPV2) channels. TRPV1/2 channels are highly permeable to Ca^{2+}. Specifically, Ca^{2+} entry through TRPV2 is not only important for initiation of phagocytosis in microglia and macrophages, but also critical for inhibition of proliferation. This study set forth to examine the role and underlying mechanism of iNOS/NO signalling in regulating TRPV2 function in the mouse microglia line BV2 cells as well as primary microglia isolated from wildtype (WT) and iNOS knockout ($iNOS^{-/-}$) mice using calcium imaging, patch-clamp recording, immunostaining and western blot. Patch clamp recordings in primary WT and $iNOS^{-/-}$ microglia, as well as BV2 microglia showed that application of the NO-donor S-nitroso-N-acetyl-D,L-penicillamine (SNAP) significantly enhanced a non-selective cation conductance that was inhibited by to ruthenium red, an inhibitor of TRPV1-3 channels. From Ca^{2+} imaging experiments, application of the TRPV1-3 agonist 2-aminoethoxydiphenyl borate (2-APB), but not the TRPV1 agonist capsaicin, elicited a rapid increase in intracellular Ca^{2+} ($[Ca^{2+}]i$) in naïve BV2 cells. Pretreatment with the iNOS inhibitor 1400W, or with a protein kinase G (PKG) inhibitor abolished this 2-APB mediated increase in $[Ca^{2+}]i$. Perfusion of 2-APB increased $[Ca^{2+}]i$ in WT microglia but not in $iNOS^{-/-}$ microglia. However, 2-APB elicited and increase in $[Ca^{2+}]i$ in $iNOS^{-/-}$ microglia pretreated with SNAP. Immunocytochemical assays displayed a significant increase in TRPV2 immunofluorescence on the plasma membrane of BV2 cells as well as WT and $iNOS^{-/-}$ microglia pretreated with SNAP in comparison to controls. The SNAP-induced TRPV2 surface expression was abolished by application of the PKG inhibitor. Taken together, these results suggest that NO enhances the trafficking of TRPV2 channels into the plasma membrane in microglia through the PKG pathway.

Disclosures: M.J. Maksoud: None. **V. Tellios:** None. **D. An:** None. **Y. Xiang:** None. **W. Lu:** None.

Poster

126. Biology of Microglia

Location: SDCC Halls B-H

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Program #/Poster #: 126.03/DP03/I11

Topic: B.11. Glial Mechanisms

Title: Systemic LPS-induced model of neuroinflammation: Ex-vivo and in-vivo 2-photon imaging of the mouse brain

Authors: *S. NEEDHAM[1], S. CAMPBELL[1], J.-S. GRIGOLEIT[1], A. CHAKRABORTY[2], L. FOURGEAUD[1], A. BHATTACHARYA[1]
[1]Janssen R&D, San Diego, CA; [2]UCSD, San Diego, CA

Abstract: The endotoxin, lipopolysaccharide (LPS) is well known to cause cytokine storm leading to systemic inflammation, including CNS (neuroinflammation). The goal of this study was to monitor neuroinflammatory phenotypes in response to systemic LPS (0.8 mg/kg, i.p.) to define a model of neuroinflammation. LPS induced changes in plasma and brain cytokines/chemokines were followed for 1, 4 and 24 hr, where robust secretion was observed from perfused brain homogenates (IL-1β, IL-6, IP-10, MCP-1, KC) at earlier time points earlier than at 24 hr. The resolution of the cytokine storm at 24 hr is consistent with resolution of 'sickness' behavior, as reported in the literature. Since microglial activation follows the initial inflammation, the objective of the study was to assess microglial activation, both *ex vivo* and *in vivo*, 2 days after a single LPS administration. FACS analysis of brain homogenates demonstrated microglial activation. Likewise, immunostaining for microglia in brain sections confirmed microglial activation (increase in microglia density & amoeboid morphology). Finally, we wanted to monitor microglia phenotype *in vivo*. This was performed by two-photon (2P) microscopy where microglia were monitored by green fluorescence ($Cx3Cr1GFP^{het}$). Imaging of microglia was performed on anesthetized mice between 50-200 µm deep in the sensory cortex through a thin skulled cranial window. By co-labeling the blood vessels using intravenous injection of a red fluorescent dye (Rhodamine Dextran), the same imaging area (using blood vessel architecture as a landmark) was used to monitor changes over time. Results were quantified using an automated image analysis software (Imaris). In-vivo 2P imaging studies were consistent with ex-vivo imaging studies confirming a clear phenotypic change in microglial morphology upon LPS that was tractable *in vivo*. The data unequivocally suggests microglial activation (M1 like) 2 days after systemic LPS challenge, a model that can be used to probe experimental compounds being developed to perturb neuroinflammation.

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Poster

126. Biology of Microglia

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Program #/Poster #: 126.04/I12

Topic: B.11. Glial Mechanisms

Support: NIH NS100294

Title: Functional interaction between the voltage-gated potassium channel Kv1.3 and purinergic P2X receptors in pro-inflammatory microglia

Authors: *H. M. NGUYEN[1], Y.-J. CHEN[1], K. WAGNER[2], B. HAMMOCK[2], H. WULFF[1]
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Abstract: Microglia play an important role in the regulation of synapse formation during early development and maintenance of neuronal health thereafter. Under pathophysiological conditions, microglia are activated by danger signals derived from local tissue damage and ischemia. Activated microglia in turn can release bioactive factors causing neurodegeneration and altered neuronal excitability. We have previously shown that brain microglia express potassium ion channels, whose differential expression profile is associated with and regulates distinct microglial activation states. For example, microglia stimulated with lipopolysaccharides (LPS) consistently display increased Kv1.3 channel expression and their treatment with the Kv1.3-selective PAP-1 inhibitor reduced proinflammatory markers such as IL-1β, iNOS and COX2. As potassium ion efflux repolarizes membrane potential, and hence determines the driving force for calcium entry, we further investigated the correlation between microglial Kv1.3 channels and P2X purinergic receptors. Using electrophysiological patch-clamp recordings, we observed that LPS upregulates Kv1.3 current expression in cultured primary microglia but has no effect on the constitutive expression of ATP-induced currents. However, LPS-stimulated cells display reduced ATP-induced signals in Fluo-4 calcium imaging experiments. This reduced calcium response may have resulted from the fact that Kv1.3-positive microglia display a more hyperpolarized membrane potential compared to Kv1.3-negative cells. Additionally, treatment with the small molecule Kv1.3 blocker PAP-1 for 24 h led to a reduced expression of both P2X mRNA and current levels, suggesting a potential functional link between Kv1.3 and P2X receptors. Since acutely isolated microglia from the brain and spinal cord of LPS-treated animals also display similar dual expression, it is of great interest to investigate further the interaction between two membrane proteins to identify new treatments for neuroinflammatory conditions such as ischemic stroke and neuropathic pain associated with peripheral nerve injury.

Disclosures: H.M. Nguyen: None. **Y. Chen:** None. **K. Wagner:** None. **B. Hammock:** None. **H. Wulff:** None.

Poster

126. Biology of Microglia

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 126.05/I13

Topic: B.11. Glial Mechanisms

Title: Systemic lipopolysaccharide (LPS) prevents microglial loss after CSF1 receptor blockade

Authors: *J.-S. GRIGOLEIT, W. A. ECKERT, III., A. BHATTACHARYA
Neurosci. TA, Janssen R&D, San Diego, CA

Abstract: Drug-induced colony-stimulating factor 1 receptor (CSF1R) blockage is now a commonly used tool to cause reversible microglia depletion in mice. However, microglial depletion is not complete with a remaining fraction of approximately 5% of the original microglia. In order to characterize this remarkably stable portion of the microglia we used fluorescent cytometry (FACS) and mass cytometry (CyTOF) in PLX3397-treated mice and found that the microglia in those animals represents a unique population different from normal microglia and bearing a lot of features usually associated with a pro-inflammatory and activated phenotype. In addition, behavioral analyses revealed subtle changes that may be interpreted as sickness behavior. Treated with a peripheral injection of LPS, (0.8 mg/kg ip) remaining microglia of depleted animals showed a much stronger inflammatory response and such-treated animals displayed a more pronounced behavioral response to LPS than control animals. Finally, we injected animals that were just at the beginning of their 1 week PLX3397 treatment with LPS and surprisingly found the resulting neuroinflammatory response to be capable of blocking the PLX-induced microglia depletion and inducing a microglial phenotype that was different from control, only LPS-treated and only PLX-treated microglia. Our observation is consistent with the emerging science that microglia do have different roles and phenotypes depending on cellular environment and disease pathology. The biology of LPS-induced protection of microglial depletion is interesting and remains to be studied in greater detail.

Disclosures: J. Grigoleit: None. **W.A. Eckert:** None. **A. Bhattacharya:** None.

Poster

126. Biology of Microglia

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 126.06/I14

Topic: B.11. Glial Mechanisms

Support: NIH Grant T32 EY007125

Title: Imaging microglial birth and maturation in the adult brain

Authors: *M. S. MENDES, A. MAJEWSKA
Neurosci., Univ. of Rochester, Rochester, NY

Abstract: Microglia are the brain's resident immune cells and have long been appreciated for their critical roles during brain injury and disease. Despite their important roles in brain health and disease, very little is known about how microglia self-maintain after they colonize the brain during development. This knowledge is fundamental to understand how microglia maintain their functions throughout the lifespan. It is known that during embryogenesis, microglia arise from yolk sac progenitors that populate the brain before the blood-brain barrier forms, and slowly mature acquiring adult gene expression in 2-3 weeks. After this, self-renewal is the only source of new microglia in the healthy adult cortex. However, the birth and maturation of self-renewing microglia in the adult brain is still poorly understood. To further explore these processes, we depleted microglia using the colony stimulating factor-1 receptor (CSF1R) inhibitor PLX5622 and used *in vivo* two-photon imaging in awake mice to track microglial repopulation and maturation. We used adult $CX_3CR1^{GFP/+}$ mice (P60+) to image microglia before depletion (control), during depletion (PLX5622), and during repopulation (control). Our data suggests that while microglia are stable in number under control conditions, during depletion microglia are rapidly lost, with the remaining microglia becoming activated and adopting a characteristic ameboid morphology. With cessation of PLX5622, microglia rapidly repopulate from existing microglia *in vivo*. We observed an increased frequency of doublet somas (where two microglial somas appear fused) which resolved into two separate cells. These newly-born microglia migrated away from each other and reached equal cell-to cell spacing similar to the rest of the microglia population within 3 days. Both newly-generated and surviving microglia adopted ramified morphologies and resumed surveillance of the brain rapidly after control chow was reintroduced, suggesting that newly born microglia mature rapidly in the adult brain to carry out their homeostatic roles. To test the functional abilities of these newly born cells, we determined that microglial depletion disrupted ocular dominance plasticity. This form of plasticity remained impaired after 7 days of repopulation when microglia appear to have fully recovered. This suggests that newly-born microglia rapidly mature morphologically, but may not be fully functional in their supportive roles in the brain. We are now in the process of investigating

whether purinergic signaling through the P2Y12 receptor and/or fractalkine signaling through CX3CR1 contribute to microglial generation and maturation in the adult brain.

Disclosures: M.S. Mendes: None. **A. Majewska:** None.

Poster

126. Biology of Microglia

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 126.07/I15

Topic: B.11. Glial Mechanisms

Support: ICMR New Delhi, 51/5/2012-BMS

Title: Changes in microglial morphology and expression of iba1 in human optic nerve with ageing

Authors: *S. GORLA, T. C. NAG, T. S. ROY
All India Inst. of Med. Science, New Delhi, New Delhi, India

Abstract: As age advances, the nervous system goes through natural changes. These changes can be loss of nerve cells, glia and in their morphology. Optic nerve (ON) shows degenerative changes with normal ageing. It is reported that there is also a moderate loss of nerve fibres in human ON with ageing. We examined the effects of ageing on the expression of ionized calcium binding adapter molecule 1 (Iba 1) in ON of 14 subjects aged between 25 and 93 years by immunohistochemistry. Human eyes with intra-orbital part of the ON were obtained from donors who had no history of ocular diseases. They were procured from the National Eye Bank, AIIMS with approval of the institutional ethics committee. Eyes of young donors below 50 years of age were treated as controls, whereas those from donors above 50 years of age were treated as aged samples. They were fixed in 4% paraformaldehyde, cryosectioned and immunolabeled for Iba1 (Abcam, UK). There is an increase in the number of microglia and their processes as age advances. But in extreme age group (> 90 years), their number is decreased, the soma size is massive and processes are fewer, and brush like. Different shapes of microglia such as triangular, rod, rhomboid and droplet were seen in different age groups. Rod shape is prominent in young ON, which changes to rhomboid shape as age advances.

Disclosures: S. Gorla: None. **T.C. Nag:** None. **T.S. Roy:** None.

Poster

126. Biology of Microglia

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 126.08/I16

Topic: B.11. Glial Mechanisms

Support: UC Berkeley Summer Undergraduate Research Fellowship

Title: Control of microglial polarization by the endocannabinoid system

Authors: *D. J. ARAUJO[1], A. SINHA[1], K. TJOA[1], K. SAIJO[1,2]
[1]Dept. of Mol. and Cell Biol., Univ. of California, Berkeley, Berkeley, CA; [2]Helen Wills Neurosci. Inst., Berkeley, CA

Abstract: Microglia are the resident immune cells of the central nervous system where they respond to injury and infection. However, exaggerated increases in microglial activity are associated with neuropsychiatric disorders. The endocannabinoid system (ECS) modulates microglial polarization which in turn impacts other microglial processes such as cytokine production, migration, and phagocytosis. Thus, leveraging the ECS may represent a novel therapeutic approach for certain neuropsychiatric conditions. Cannabinoid receptor 1 (CB_1) and CB_2 are the major receptors of the ECS in the brain, with CB_1 being widely expressed and CB_2 being largely restricted to microglia. In this proposal, we set out to test the hypothesis that loss of CB_2 will lead to aberrant microglial polarization and thus aberrant microglial activity associated with neuropsychiatric disorders. In order to accomplish this, we used the CRISPR-Cas9 system to generate a SIMA9 (spontaneously immortalized microglia) CB_2 knockout cell line. Here we report the effects of CB_2 loss on the normal immune response of microglia. Future experiments will focus on detailing the signaling cascades controlling these processes via the use of next-generation sequencing. The results of this study may lead to improved treatments for conditions characterized by microglial hyperactivity.

Disclosures: D.J. Araujo: None. **A. Sinha:** None. **K. Tjoa:** None. **K. Saijo:** None.

Poster

126. Biology of Microglia

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 126.09/I17

Topic: B.11. Glial Mechanisms

Support: NIH R21-NS096334-01A1
NIH R01-AG051437-01
5T32AG052354-02

Title: Microglia progenitor cells in the mouse brain express Prominin-1 (CD133)

Authors: *K. E. PRATER[1], M. S. ALOI[2], W. SU[1], S. L. DAVIDSON[1], G. A. GARDEN[1]
[2]Pathology Department, Neurol. Dept., [1]Univ. of Washington, Seattle, WA

Abstract: Microglia-mediated neuroinflammation is critical to CNS development, injury, and age-related diseases. While microglia are self-renewing, the precise nature of population(s) capable of generating new microglia remains controversial. Recent studies proposed that new microglia are derived from division of adult microglia, but some data suggests the existence of a committed microglia progenitor cell. Data in support of an adult microglia progenitor cell population suggest that microglia depletion leads to repopulation by proliferating cells that express markers observed on other progenitor cell populations (e.g. nestin). However, cell-surface markers expressed by microglia progenitors have not been reported. Here, we demonstrate that Prominin-1 (CD133) is a surface marker of a microglia progenitor population. CD133 is a known marker of multi-potent astrocytic and neural stem cells. We identified CD133 as differentially expressed by microglia progenitors compared to mature microglia using RNA sequencing. To validate CD133 as a surface marker of microglia progenitors, we isolated a population of cells with surface expression of both CD133 and CD45 from brains of C57Bl/6J wild-type mice by *ex vivo* fluorescence activated cell sorting (FACS) and confirmed gene expression consistent with myeloid identity by qPCR. We developed a fate mapping approach to definitively prove that CD133 expressing cells can become microglia. Transgenic mice with a Cre reporter (TdTomato) were crossed with CD133 promoter-tamoxifen inducible Cre mice. Bigenic heterozygous animals were treated with tamoxifen at 10 weeks of age, and TdTomato expressing cells analyzed by flow cytometry. We observed TdTomato positive CD11b+/CD45^{int} microglia at 5 months of age. This indicates that under naïve conditions, CD133+ cells generate microglia in the mouse brain. We observed that CD133 expressing cells are present only in the cell population adherent to the flask surface in a cortical mixed glia culture system. In contrast, mature floating microglia express CD45 and CD11b but do not express CD133. We isolated CD133+ cells from the adherent monolayer using FACS and placed them in culture with D10C + MCSF for seven days. We observed that new microglia expressing CD11b and CD45^{int} were generated from this CD133 expressing population. The newly generated microglia demonstrate nearly identical expression of mature microglia marker genes. These findings demonstrate the existence of a microglia progenitor cell population that expresses CD133 as a surface marker in both neonatal and adult brain allowing us to better understand microglia population dynamics in uninjured adult brain.

Disclosures: K.E. Prater: None. **M.S. Aloi:** None. **W. Su:** None. **S.L. Davidson:** None. **G.A. Garden:** None.

Poster

126. Biology of Microglia

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 126.10/J1

Topic: B.11. Glial Mechanisms

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Université Laval
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M.E.T. is a Canada Research Chair (Tier 2) in Neuroimmune Plasticity in Health and Therapy

Title: Identification of a dark microglia specific marker

Authors: *M.-K. ST-PIERRE, S. BELHOCINE, M. CARRIER, D. GOSSELIN, M.-E. TREMBLAY
Neurosciences, Univ. Laval, Quebec, QC, Canada

Abstract: Dark microglia, a recently discovered subtype of microglia, have been associated with ultrastructural alterations caused by oxidative stress, as well as down-regulation of the homeostatic proteins CX3CR1, P2RY12, and IBA1. These cells were found to be abundant in mouse models of psychiatric (schizophrenia, chronic stress-induced depression) and degenerative (Alzheimer's) diseases. Predominantly localized in the vicinity of blood vessels, this microglial subtype was observed in some brain regions including the hippocampus. Although dark microglia can be identified by electron microscopy due to their electron dense cytoplasm and nucleoplasm, the impossibility to study these cells with other techniques has left many questions unanswered. Therefore, our goal was to identify a marker that labels these cells selectively, in order to analyze their molecular signature, regional density, localization, morphology, dynamics, and ultrastructure in a myriad of pathologies. The specificity of a putative dark microglia marker was assessed using correlative immunocytochemical light and electron microscopy. Positive cells were observed during postnatal development and in $CX3CR1^{-/-}$ mice. These cells were then sorted with flow cytometry using five P14 $CX3CR1^{-/-}$mice, Furthermore, series of brain sections providing a non-biased representation of the brain of male and female $CX3CR1^{-/-}$ mice were imaged using a slide scanner to reveal the distribution of dark microglia across postnatal development, adulthood and aging. Our preliminary results indicated that dark microglia are found in unexpected regions such as the corpus callosum and the dorsal hippocampal commissure. Moreover, flow cytometry analysis revealed that these cells compose 2.5% of the total microglial population during postnatal development in fractalkine signaling-deficient mice.

The identification of a dark microglial marker will allow for a high-throughput characterization of these cells. While dark microglia were associated with an upregulation of complement receptor 3 (CD11b) and appear to be involved in synaptic modulation, in our ultrastructural analyses, future studies establishing their transcriptome using RNA sequencing will be essential to determine their function in the central nervous system.

Disclosures: M. St-Pierre: None. **S. Belhocine:** None. **M. Carrier:** None. **D. Gosselin:** None. **M. Tremblay:** None.

Poster

126. Biology of Microglia

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 126.11/J2

Topic: B.11. Glial Mechanisms

Title: Quantitative, live-cell kinetic analysis of microglial function and morphology

Authors: *A. C. OVERLAND, J. N. RAUCH, L. OUPICKA, D. M. APPLEDORN
Essen Biosci. Inc, Ann Arbor, MI

Abstract: Microglia, the resident immune cells of the central nervous system (CNS), play significant roles in the regulation of CNS homeostasis and in the management of tissue response to inflammatory or pathological insults. In the healthy unperturbed brain, microglia impact synaptic remodeling and turnover of dendritic spines through the removal of damaged or unnecessary neurons or synapses and are the first line of defense against pathogens through regulation of innate and adaptive immune responses. Upon insult, disease or stress, microglia can transform into an ‘activated’ state with altered phenotype and macrophage-like immune functions, including cytokine release and increased phagocytosis. Despite the importance of microglia in CNS regulation and disease, limited tools and in vitro model systems exist to enable optimizing, monitoring, and analyzing functional and morphological changes of these cells. In this study, we present data outlining optimization of plating conditions for in vitro microglial cultures, exemplified by the use of immortalized microglial cell lines (BV-2, HMC3 and C8-B4). We also evaluated the ability of BV-2, HMC3 and C8-B4 cells to phagocytose pHrodo-labeled *E. coli* bioparticles and apoptotic Neuro2A cells using a quantitative, live-cell imaging approach with the IncuCyte S3®. Activation of Toll-like receptor 4 (TLR-4) via the Gram-negative bacterial endotoxin lipopolysaccharide (LPS) resulted in variable microglial activation as measured by an altered phagocytic response. The ability of BV-2 cells to phagocytose apoptotic Neuro2A cells was diminished by pretreatment with an inhibitor of vitronectin receptors (Cilengitide) as well as an inhibitor of actin polymerization (cytochalasin D). Cilengitide treatment did not alter the ability of BV-2 cells to phagocytose *E. coli* bioparticles. Morphology, viability and proliferation were also monitored in real time over the course of the experiments.

Proliferation of BV-2 cells was decreased with cytochalasin D treatment while remaining unaffected by Cilengitide. These results highlight the use of a quantitative, live-cell imaging approach with the IncuCyte S3® to monitor microglial function and morphology over the course of assay optimization and experimentation, thus allowing for improved developmental and pharmacological characterization.

Disclosures: **A.C. Overland:** A. Employment/Salary (full or part-time):; Essen Bioscience. **J.N. Rauch:** A. Employment/Salary (full or part-time):; Essen BioScience. **L. Oupicka:** A. Employment/Salary (full or part-time):; Essen BioScience. **D.M. Appledorn:** A. Employment/Salary (full or part-time):; Essen BioScience.

Poster

126. Biology of Microglia

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 126.12/J3

Topic: B.11. Glial Mechanisms

Support: NIH Grant R01 EY019277
NIH Grant T32 CM07356

Title: Characterization of P2Y12 expression throughout the brain during post-natal development

Authors: ***B. S. WHITELAW**[1], A. K. MAJEWSKA[2]
[1]Univ. of Rochester, Rochester, NY; [2]Univ. of Rochester Med. Ctr., Rochester, NY

Abstract: Microglia, the resident immune cells of the central nervous system, perform a diverse array of functions to modulate neural activity, including phagocytosis of apoptotic neurons, modification of synapses during development, and provision of neurotrophic support. The microglial-specific receptors CX3CR1 and P2Y12 have been implicated in a number of neurodevelopmental processes, including neurotrophic support, synaptic pruning, and microglial migration. We sought to characterize the expression patterns of P2Y12 in microglia throughout the brain during postnatal development, in order to determine in which developmental processes microglial P2Y12 may be involved. We performed immunohistochemical staining of P2Y12 in brain tissue at several ages in post-natal development, using the Cx3cr1-GFP heterozygous mouse line, in which all microglia are labeled with GFP. This approach allowed for the determination of P2Y12 expression and overall microglial distribution and morphology with a high degree of spatial resolution, necessary for delineating differences between neighboring brain regions. We have found region-specific differences in microglial colonization of the brain during early postnatal development. Specifically, microglia are not present in the cerebellar gray matter (granular and molecular layers) until after the first week of post-natal development. In the cortex, ramified microglia expressing P2Y12 are present within the gray matter during the first

post-natal week, but microglial density varies between cortical regions. We have also identified two distinct populations of microglia during the first post-natal week: high-density clusters of amoeboid-shaped cells that do not express P2Y12 located in the white matter tracts of cortex and cerebellum; and ramified P2Y12-expressing cells that are more characteristic of adult microglia present in cortical and subcortical areas. We are in the process of determining the molecular pathways that establish these microglial behaviors. These region-specific differences in microglial colonization and phenotype may provide a basis to understand the diverse functions of microglia in development.

Disclosures: B.S. Whitelaw: None. **A.K. Majewska:** None.

Poster

126. Biology of Microglia

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 126.13/J4

Topic: B.11. Glial Mechanisms

Support: WMU Internal Funds

Title: Microglial proliferation patterns following damage to the olfactory bulb in adult zebrafish

Authors: *S. R. VAR[1], C. A. BYRD-JACOBS[2]
[1]Biol. Sci., [2]Dept Biolog Sci., Western Michigan Univ., Kalamazoo, MI

Abstract: The plasticity of the zebrafish olfactory system serves as a useful model for examining immune cell response after injury. Microglia are the resident immune cells of the CNS that respond to damage by phagocytizing neuronal debris. We previously demonstrated the time course of the microglial response to olfactory deafferentation and direct injury in adult zebrafish; however, it is unclear whether the response is from proliferation of resident microglia or migration from other areas. We hypothesize that after damage, there will be a gradual increase in resident microglia from other parts of the brain, followed by the influx of macrophages from the periphery, rather than localized cellular proliferation. A direct lesion injury to the olfactory bulb in the whole fish was compared to direct lesion of the isolated brain in culture removed of all afferent input. The 4C4 antibody was used to label microglia. Proliferating cell nuclear antigen (PCNA) antibody was used as a marker of cell proliferation. At 4h after a direct lesion injury to the olfactory bulb in whole fish, there were few PCNA-immunoreactive (-ir) profiles in the bulbs, microglia were transitioning to amoeboid profiles in both the ipsilateral and contralateral bulbs, and there appeared to be a decrease in PCNA/4C4 double staining compared to controls. At 12h, there was an increase in transitioning microglial profiles in the ipsilateral bulb and few-to-no PCNA/4C4 double-stained profiles compared to controls. At 24h, transitioning microglial profiles slowly decreased to near-control levels, and there was a small increase in PCNA/4C4-ir

profiles compared to previous time points. At all time points there were few PCNA/4C4-ir profiles in either bulb, suggesting that local proliferation does not appear to be a major contributor to the microglial response. With direct lesion injury in the isolated brain, there was a significant increase in activated microglia after 1, 4, and 12h in culture; however, the response in isolated brains showed significantly fewer microglia when compared to direct injury in the whole fish. After 4h in culture, there appeared to be a greater number of PCNA/4C4-ir profiles compared to 4h after injury in the whole fish. At 12h in culture, there appeared to be few-to-no PCNA/4C4-ir profiles compared to controls. These results suggest that microglia can respond to damage without afferent input or peripheral influence, but only up to a certain time after injury when further proliferation from migrating peripheral macrophages is required. Further work is required to explore the microglial proliferation patterns and the potential role of these immune cells in recovery and regeneration after injury.

Disclosures: S.R. Var: None. **C.A. Byrd-Jacobs:** None.

Poster

126. Biology of Microglia

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 126.14/J5

Topic: B.11. Glial Mechanisms

Title: Phenotypic comparison of microglia activated by TLR3 and TLR4 agonists

Authors: *Y. HE, N. TAYLOR, A. BHATTACHARYA
Neurosci., Janssen Res. & Develop. LLC., San Diego, CA

Abstract: Microglia are CNS resident innate immune cells that become activated in response to bacteria or viruses. TLRs are a conserved diverse receptor family that drives innate immune responses following the interaction with pathogen-associated molecular patterns (PAMPs). Among them, TLR3 and TLR4 recognize viral dsRNA Poly(I:C) and bacterial endotoxin LPS, respectively. So far, only a few studies have investigated the effects of TLR3 and TLR4 in microglia. However, the phenotypic differences between TLR3- and TLR4-activated primary microglia have not been fully examined yet. To this end, we compared microglial responses upon Poly(I:C) and LPS stimulation by detecting various phenotypes ranging from morphology, proliferation, gene expression, secretion, chemotaxis, and phagocytosis. Results showed that LPS changed microglial morphology from ramified to amoeboid shape, while Poly(I:C) induced more thick branches. Accompanied with morphological changes, the number of microglial cells increased concentration-dependently in response to LPS but not to Poly(I:C). Although both LPS and Poly(I:C) enhanced the expression of pro-inflammatory genes such as *Nos2*, *Tspo*, *Tnfa*, *Il6*, and *Il1b* in microglia, Poly(I:C) induced much higher expression of type I interferon than LPS. By using the Luminex platform, we measured 38 secreted proteins and found that more proteins

with higher amount were secreted in LPS-treated microglia than in Poly(I:C)-treated cells. In addition, we compared chemotaxis and phagocytosis in microglia after exposure to LPS and Poly(I:C) by using Incucyte, an automated live cell analysis system. To our surprise, in contrast to Poly(I:C) that slightly suppressed chemotaxis of microglia upon C5a, LPS strongly enhanced that function. Furthermore, we applied pHrodo-synaptosome to assess microglial phagocytosis, which may mimic *in vivo* system. Interestingly, both LPS and Poly(I:C) inhibited the up-take of synaptosomes by microglia. Taken together, despite that both Poly(I:C) and LPS stimulated microglial activation, the phenotypes differed in various aspects, which may be due to distinct downstream pathways upon TLR3 and TLR4 activation.

Disclosures: Y. He: None. **N. Taylor:** None. **A. Bhattacharya:** None.

Poster

126. Biology of Microglia

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 126.15/J6

Topic: B.11. Glial Mechanisms

Support: NIH U01MH105989

Title: Heterogeneity of primary human microglia in normal development and disease

Authors: *G. SCHMUNK[1], A. BHADURI[1], E. DI LULLO[1], T. O. SHARF[2], S. CHO[2], T. NOWAKOWSKI[3]
[1]Regeneration Med., Univ. of California, San Francisco, San Francisco, CA; [2]Univ. of California, Berkeley, Berkeley, CA; [3]Regeneration Med., Univ. of California San Francisco, San Francisco, CA

Abstract: In the developing human brain, microglia are a molecularly and morphologically heterogeneous population, interacting with different cell types and undergoing complex transformation programs in a region- and state-specific manner. Using single-cell RNA sequencing (scRNA-seq) in combination with live cell imaging, we explored microglial heterogeneity to define molecular signatures dictating microglia transformation in normal brain development and progression to disease.
Our research provides comprehensive transcriptional profiles of microglia at the single-cell level during peak neurogenesis and during pathological transformation in response to the brain inflammatory responses, and identifies novel markers of microglial activation and maturation. Together, these findings will lead to a better understanding of microglial heterogeneity, help to refine existing iPSC-derived microglia models, and provide insight into how perturbations of microglia normal function might contribute to neurological disorders.

Disclosures: G. Schmunk: None. **A. Bhaduri:** None. **E. Di Lullo:** None. **T.O. Sharf:** None. **S. Cho:** None. **T. Nowakowski:** None.

Poster

126. Biology of Microglia

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Program #/Poster #: 126.16/J7

Topic: B.11. Glial Mechanisms

Support: Garfield Weston Foundation
CIHR

Title: Identifying morphological changes in microglia and astrocytes through an unbiased machine learning protocol

Authors: *J. SILBURT[1], S. HEINEN[2], K. A. MARKHAM-COULTES[2], K. HYNYNEN[3], I. AUBERT[2]
[1]Lab. Med. and Pathobiology, Univ. of Toronto, Toronto, ON, Canada; [2]Sunnybrook Res. Inst., Toronto, ON, Canada; [3]Med. Biophysics / Physical Sci., Univ. of Toronto / Sunnybrook Res. Inst., Toronto, ON, Canada

Abstract: Microglia and astrocyte activation occurs along a gradient and leads to the adoption of new functions (e.g. phagocytosis), and the secretion of pro- or anti-inflammatory molecules, and/or growth factors. Depending on the type or severity of the brain pathology, activated microglia and astrocytes can help clear or exacerbate pathology; halt or promote regeneration. Activated microglia and astrocytes demonstrate characteristic morphological changes (e.g. phagocytic microglia de-ramify). Considering the diverse gradient of microglia and astrocyte activation states, we developed a machine learning approach to identify morphological changes associated with glial activation in response to focused ultrasound (FUS). FUS has been shown to transiently activate microglia and astrocytes, contributing to the clearance of amyloid-beta pathology (Jordão et al, 2013). Here we characterized the morphological profile and the time-course of glial activation in response to FUS. Our approach utilizes a single factor, Iba1 or GFAP, to label microglia or astrocytes respectively, and characterizes distinct classes of microglia and astrocytes, based on complexity and immunofluorescence features. In 3.5 month old C57bl/6 mice, we applied FUS unilaterally to the hippocampus, and measured microglia and astrocyte morphology changes at 1, 4, 7, 10, and 30 days. We demonstrate our approach can more sensitively identify microglia and astrocyte morphological changes compared with conventional methodologies; detecting significant changes up to 10D post-FUS. By 30D microglia and astrocyte morphology has largely returned to a baseline phenotype. Our data indicate that morphological changes in microglia and astrocytes are present in overlapping spatial clusters. We furthermore identified two subtypes of microglia; a highly de-ramified

subset characterized by an enlarged soma and reduced branching, and a neighbouring network of microglia with more subtle changes in complexity. We conclude that our approach can substantially improve the sensitivity and precision of identifying morphologically distinct microglia and astrocytes. The application of this unbiased machine learning protocol will facilitate further research in the field to study nuanced microglia and astrocyte morphologies and which can relate to activation.

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Poster

126. Biology of Microglia

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Program #/Poster #: 126.17/J8

Topic: B.11. Glial Mechanisms

Support: NIH Grant R01HL136395
NIH Grant R01NS097590

Title: Microglial LRP1 promotes microglial activation and neuro-inflammation in the spinal dorsal horn following peripheral nerve injury in mice: Role of LRP1 shedding

Authors: *C. BRIFAULT[1], W. M. CAMPANA[2], S. L. GONIAS[1]
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Abstract: Activation of microglia and neuro-inflammation in the spinal dorsal horn have been implicated in development of neuropathic pain. However, the molecular mechanisms responsible for microglial activation remain unclear. We have uncovered a novel pathway in microglia that controls microglial activation. The pathway involves the endocytic and cell-signaling receptor, LDL Receptor-related Protein-1 (LRP1). In microglia, membrane-anchored LRP1 exerts anti-inflammatory activity. However, when these cells are exposed to pro-inflammatory stimuli, LRP1 is shed from microglial cell-surfaces, and a soluble product (shed LRP1/sLRP1) is generated that is potently pro-inflammatory.
Herein, we investigated the role of LRP1 shedding from microglia *in vivo* and its association with neuro-inflammation in a mouse model of peripheral nerve injury. Three days after partial sciatic nerve ligation (PNL), expression of LRP1 was increased in activated microglia in the spinal dorsal horn. However, in mice in which *LRP1* is conditionally deleted in microglia, under the control of the *LysM* promoter or the *Cx3cr1* promoter, microglial activation was decreased in the spinal dorsal horn following PNL. Expression of the pro-inflammatory mediators: TNFα; IL-1β; and IL-6 also was decreased in these mice. Furthermore, we demonstrated that purified sLRP1 induced expression of mRNAs encoding pro-inflammatory mediators in cultures of

microglia, isolated from the brain or the spinal cord.
Collectively, our results demonstrate that following peripheral nerve injury, microglial LRP1 regulates changes in gene expression and cell physiology in the spinal dorsal horn. We hypothesize that LRP1 deficiency in microglia prevents LRP1 shedding, which attenuates neuro-inflammation. Given the important role of neuro-inflammation in driving neuropathic pain, inhibiting LRP1 shedding from microglia emerges as a potential therapeutic strategy for attenuating neuropathic pain following peripheral nerve injury.

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Poster

126. Biology of Microglia

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 126.18/J9

Topic: B.11. Glial Mechanisms

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Title: Selective deletion of Na^+/H^+ exchanger in $Cx3cr1^+$ microglia stimulates white matter myelination and improves post-stroke function recovery

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Abstract: Activation of microglia, the main innate immune cells in the brain, is involved in brain injury and tissue repair after ischemic stroke. Na^+/H^+ exchanger isoform-1 (NHE1) protein regulates microglial intracellular pH (pH_i), free radical superoxide production, and cell migration. We established *Cx3cr1-Cre^{ER};Nhe1$^{f/f}$* mouse line which allows for cell-type specific deletion of the *Nhe1* gene in microglia. In this study, we investigated effects of selective deletion of microglial *Nhe1* on neuroinflammation, white matter demyelination, and neurological dysfunction after ischemic stroke. Corn oil (3.75 ml/kg body weight/day, i.p.) or tamoxifen (Tam, 75 mg/kg body weight/day at a concentration of 20 mg/ml in corn oil, i.p.) was injected for 5 consecutive days to induce Cre recombinase-mediated gene deletion. A 30-day waiting period was given for clearance of Tam effects and turnover of *Cx3cr1$^+$* monocytes. Ischemic stroke was induced by transient middle cerebral artery occlusion (tMCAO). Compared to the oil-

treated control (Con) mice, the Tam-mediated knockout (KO) mice exhibited comparable infarct volume at day 2 and day 14 post-stroke, however, they displayed faster neurological function recovery during the 14-day recovery period. The ischemic *Nhe1* KO brains contained higher numbers of APC^+ mature oligodendrocytes and enhanced white matter remyelination with increased MBP expression at day 14 post-stroke, despite of initial loss of MBP expression at day 3 post-stroke. Interestingly, microglial *Nhe1* KO mice showed decreased $CD11b^+/CD45^{low-med}/P2RY12^+$ microglial population with a switch from pro-inflammatory to anti-inflammatory phenotypes at the early phase post-stroke, which is correlated to accelerated functional recovery. Taken together, our study demonstrated that microglia played an important role in neuroinflammation, white matter demyelination, and post-stroke neurological dysfunction. Activation of microglial NHE1 protein is critical for this process. NHE1 protein emerges as a potential therapeutic target for neuroinflammation and white matter tissue repair after ischemic stroke.

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Poster

126. Biology of Microglia

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Shenzhen Peacock Plan

Title: The origins of repopulated microglia in the brain and retina

Authors: *B. PENG[1], Y. HUANG[1], Z. XU[1], S. XIONG[1], G. QIN[2], F. SUN[1], J. WANG[1], Y.-X. LIANG[3], T. WU[1], K.-F. SO[3], G. HU[1], T.-F. YUAN[4], Y. RAO[3]
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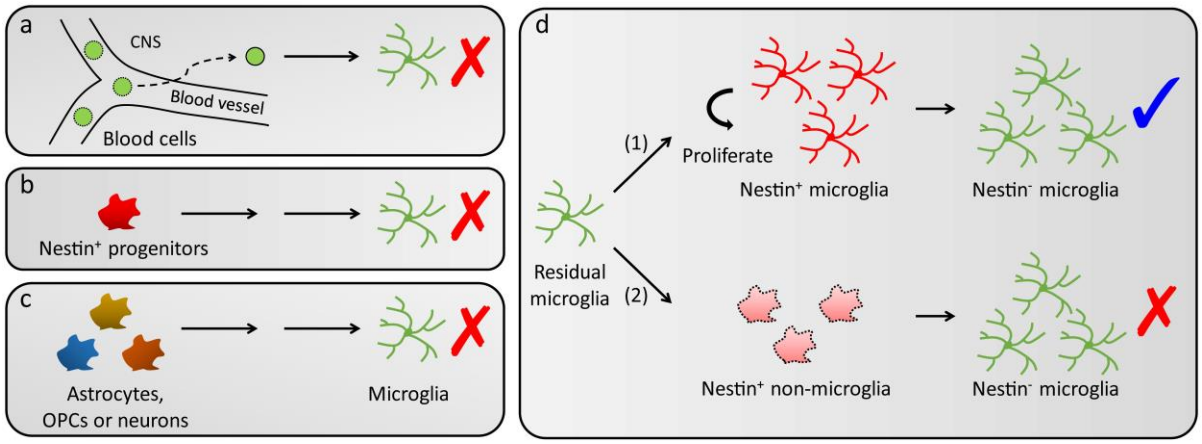
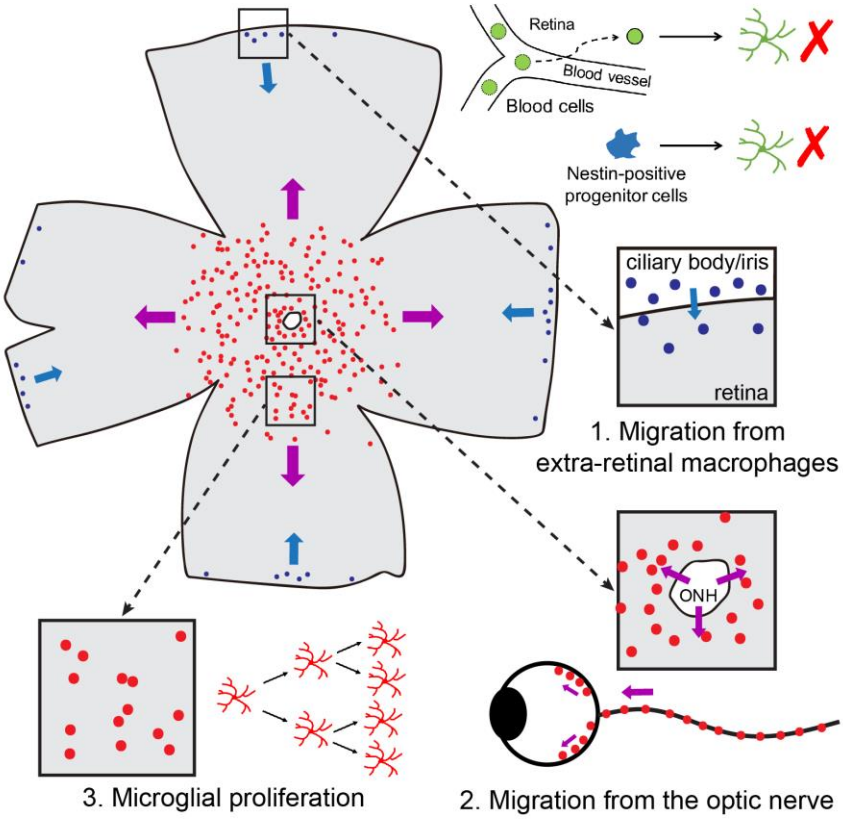
Abstract: New-born microglia rapidly replenish the whole brain after selective elimination of most microglia (>99%) in adult mice. Previous studies reported that repopulated microglia were largely derived from microglial progenitor cells expressing Nestin in the brain. However, the origin of these repopulated microglia has been hotly debated. In this study, we investigated the origin of repopulated microglia by fate mapping. We first excluded the blood origin of repopulated microglia via parabiosis. With different transgenic mouse lines, we then demonstrated that all repopulated microglia were derived from the proliferation of the few surviving microglia (<1%). Though with a transient pattern of Nestin expression in newly forming microglia, none of repopulated microglia was derived from Nestin-positive non-microglial cells. In summary, we conclude that repopulated microglia are solely derived from residual microglia rather than de novo progenitors, suggesting for the absence of microglial progenitor cells in the adult brain.



In addition, we investigated the origins of repopulated microglia in the retina and found that the repopulated retinal microglia were not derived from the residual microglia in the retina. Instead, they had two distinct origins: the center-emerging microglia were derived from residual microglia in the optic nerve and the periphery-emerging microglia were derived from macrophages in the ciliary body/iris. Therefore, we identified novel origins of retinal microglia.



Disclosures: B. Peng: None. **Y. Huang:** None. **Z. Xu:** None. **S. Xiong:** None. **G. Qin:** None. **F. Sun:** None. **J. Wang:** None. **Y. Liang:** None. **T. Wu:** None. **K. So:** None. **G. Hu:** None. **T. Yuan:** None. **Y. Rao:** None.

Poster

126. Biology of Microglia

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Program #/Poster #: 126.20/J11

Topic: B.11. Glial Mechanisms

Support: NIH Grant U01 AG052460

Title: Creation and characterization of a novel microglial BV2 cell line expressing the human EP2 receptor

Authors: *A. BANIK, A. ROJAS, D. CHEN, R. DINGLEDINE, T. GANESH
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Abstract: In rats and mice, inhibition of the EP2 receptor for PGE2 by selective small molecule antagonists leads to a beneficial outcome in the days following status epilepticus (SE). Conditional EP2 ablation restricted to myeloid cells recapitulated many beneficial outcomes, implicating microglia or monocytes in the deleterious effects after SE (see Varvel et al. poster this meeting). Using rat primary microglial cultures, we have recently shown that EP2 signaling pathways regulate the classical activation and death of microglia. To explore the role of EP2 activation in microglia and to screen small molecule compounds that suppress microglial EP2 activation, we created a murine BV2 microglial cell line that stably expresses human EP2 receptors. We compared the characteristics of this cell line to those of primary microglia when treated with lipopolysaccharide (LPS), and EP2 agonists and antagonists. The hEP2 receptor in BV2-hEP2 cells responds to EP2 activation with a robust increase in cellular cAMP, whereas the parent BV2 cells were unresponsive. BV2-hEP2 cells displayed a dose dependent cAMP elevation when exposed to the selective EP2 receptor agonist ONO-AE1-259-1 (EC_{50} 0.56 nM), and this response was competitively inhibited by TG4-155, a selective EP2 antagonist. Similar to rat primary microglial cells, the BV2-hEP2 cells displayed a rapid and robust induction of a small group of inflammatory mediators (COX-2, IL-1β, TNFα, IL-6) following treatment with LPS (100 ng/ml). These effects were modulated by EP2 activation similar to primary microglia, and treatment with TG4-155 alleviated the inflammatory modulation in these cells. Like primary microglia in culture, BV2 cells are also able to phagocytose fluorescent-labeled latex beads. The presence of the hEP2 receptor in BV2 cells strongly reduced phagocytosis by 85% compared to their parent cells. Taken together these results indicate that we have successfully created a stable microglial cell line that expresses human EP2 receptors, and that EP2 activation plays a critical role in the immune regulation and phagocytic ability of these cells.

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Poster

126. Biology of Microglia

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Program #/Poster #: 126.21/J12

Topic: B.11. Glial Mechanisms

Support: NIH Grant NS076620

Title: Mechanisms of ischemia/reperfusion-induced microglial interferon signaling

Authors: *J. R. WEINSTEIN, C. LEE, R. V. LEE, A. MCDONOUGH
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Abstract: Innate immune signaling is important in the pathophysiology of ischemia/reperfusion-induced injury and recovery. Several lines of evidence support a central role for microglia, the resident immune cells in the central nervous system, in the physiological and neuroimmunological response to ischemia/reperfusion (stroke). Recent work has identified Toll-like receptors (TLRs) and type I interferon (IFN) signaling in both ischemia/reperfusion-induced brain injury and ischemic preconditioning-mediated neuroprotection. Using microarray and qRT-PCR analyses, we have previously demonstrated that both ischemia/reperfusion *in vivo* and ischemia/reperfusion-like conditions *in vitro* (hypoxia-hypoglycemia followed by normoxia/normoglycemia or H/H-N/N) induce broad and robust expression of interferon stimulated genes (ISGs) in microglia. ISG responses were completely dependent on expression of type 1 IFN receptor IFNAR1 both *in vitro* and *in vivo,* and dependent on TLR4 *in vitro*. In addition we have previously demonstrated in primary microglia cultures *in vitro* that both H/H-N/N and IFN-beta induce rapid phosphorylation of signal transducer and activator of transcription 1 (STAT1), a downstream kinase in the canonical type 1 IFN signaling pathway. Here we use flow cytometry to further characterize the phosphorylation of STAT1 in cultured wild-type primary microglia in response to H/H-N/N or type I IFNs with an emphasis on dose response and temporal kinetics. Next, using primary microglia derived from $TLR4^{-/-}$ and $IFNAR1^{-/-}$ mice, we quantify the extent to which phosphorylation of STAT1 is dependent on TLR4 and type 1 IFN signaling. We then investigate the ability of TLR4 agonists (including endogenous ones) to induce phosphorylation of STAT1 in wild-type, $IFNAR1^{-/-}$ and $STAT1^{-/-}$ microglia in vitro. We then use qRT-PCR with a panel of ISG-targeted primer/probe sets to determine the extent to which H/H-N/N-, type 1 IFN- and TLR4 agonist-induced microglial ISG responses are dependent on STAT1 phosphorylation. Finally, we use our mouse middle cerebral artery occlusion/reperfusion (MCAO/R) model to investigate the impact of genetic deficiency of STAT1 on the ischemia/reperfusion-induced microglial ISG response *in vivo*. Taken together, results from these studies suggest novel ischemia/reperfusion-induced pathways for type 1 IFN signaling in microglia.

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Poster

126. Biology of Microglia

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Program #/Poster #: 126.22/J13

Topic: B.11. Glial Mechanisms

Support: NIH Grant RF1AG051496

Title: Defining a novel population of complement-expressing $CD11b^{+}CD11c^{+}$ myeloid cells in aging and neurodegenerative diseases

Authors: *H. YANG, L. C. GRAHAM, A. M. REAGAN, G. R. HOWELL
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Abstract: Neuroinflammation and neurovascular unit (NVU) decline are hallmarks of brain aging leading to cognitive decline and increased risk for neurodegenerative diseases. As part of the innate immune system, the complement cascade has been strongly implicated in the aging brain and in neurodegenerative diseases. Targeting complement components such as C1Q or C3 protects the aging brain from neuronal deficits and cognitive decline. Our previous data showed that NVU decline correlates with an increase in C1qa-expressing myeloid cells in aging and can be exacerbated by a chronic consumption of a western diet. However, the direct impact of complement activation on the NVU is not well understood. In this study, we aimed to determine the effect of complement activation on NVU decline in aging, diet, and in neurodegenerative diseases. We show that aged mice and western diet-induced obese mice deficient in *C1qa* are protected from NVU decline compared to *C1qa* sufficient mice. To more precisely characterize the complement-expressing myeloid cells in aging brains, fluorescent activated cell sorting was used to profile two myeloid cell subpopulations: $CD45^{lo}CD11b^{+}$ cells (expected to comprise primarily resident microglia) and $CD45^{hi}CD11b^{+}$ cells (expected to include the majority of infiltrating monocytes/macrophages). The percentage of $CD45^{hi}CD11b^{+}$ cells increased in the brains of aging and western diet-fed mice compared to young controls. These two subpopulations of cells were further characterized by RNA-seq followed by various gene set enrichment analyses. Both subpopulations expressed similar levels of complement components C1qa, C1qb, C1qc as well as myeloid cell markers Itgam and Trem2. However, $CD45^{hi}CD11b^{+}$ cells expressed higher levels of downstream complement components such as C2, C3 and C4b compared to $CD45^{lo}CD11b^{+}$ cells. In addition, $CD45^{hi}CD11b^{+}$ cells also expressed higher levels of *Cd11c*, *Trem1* and *Mmp9*. Other studies have shown that elevated levels of MMP9 can affect NVU integrity, resulting in loss of basement membrane and functional tight junctions. These potentially damaging, complement-producing, CD11b/CD11c double positive myeloid cells are also prevalent in the brains of mouse models of AD, as well as in optic nerves of a mouse model of glaucoma. Collectively, our data suggest this subpopulation of myeloid cells play a major and as yet, unexplored role in aging brains and in multiple neurodegenerative diseases.

Disclosures: H. Yang: None. **L.C. Graham:** None. **A.M. Reagan:** None. **G.R. Howell:** None.

Poster

126. Biology of Microglia

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Program #/Poster #: 126.23/J14

Topic: B.11. Glial Mechanisms

Support: 1R01MH107730-01A1

Title: Microglia regulates cognitive flexibility: The role of the nuclear GAPDH cascade

Authors: *A. RAMOS[1], N. J. ELKINS[2], T. PALEN[3], B. LEE[4], K. ISHIZUKA[5], A. SAWA[6]
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Abstract: Behavioral flexibility is one of the most important behavioral constructs that is required for adaptability. This behavior is affected under stress conditions, and its disturbance is involved in many neuropsychiatric disorders, in particular schizophrenia (SZ). Excess oxidative stress has been proposed to play a role in the pathology of neuropsychiatric disorders. Our lab has studied a novel signaling cascade that is triggered upon oxidative stress: it is nuclear GAPDH (N-GAPDH) cascade, in which a specific pool of GAPDH is posttranslationally modified and translocated to the nucleus where it regulates gene transcription. We propose the present study based on the hypothesis that the N-GAPDH cascade might play a role in regulating behavioral inflexibility. There is a potent compound that specifically blocks the N-GAPDH cascade without disturbing glycolytic activity of GAPDH [(1R, 3R)-1,3-dymethyl-2-propargyl-1,2,3,4-tetrahidrosiquinoline] ("RR").
In a clinical study, we found an increase of celular autofluorescence (AF) in blood cells from SZ patients, compared with those from health controls, and is negatively correlated with the behavioral flexibility assesed by the Wisconsin Card Sorting Test (WCST). The pharmacological intervention of the pathological AF by "RR" has indicated the N-GAPDH cascade may underlie the cellular and clinical phenotypes. To validate this notion at the mechanistic level, we have used an animal model that displays cognitive inflexibility and inflammation/excess oxidative stress (LPS-treated mice). In this model, the N-GAPDH cascade was selectively activated in cortical microglia. We demonstrated the causal role of N-GAPDH cascade in the behavioral deficits by pharmacological intervention with "RR". Through CHIP-seq analysis, we have pinned down the HMGB pathway as a candidate mechanism that links the microglial changes to neuronal deficits via the interaction of HMGB and neuronal NMDA-R. We also observed augmented AF in the blood cells of LPS mice. Finally, to genetically intervene with the N-GAPDH cascade, we generated a conditional knock-in mouse in which the N-GAPDH pathway is silenced, whereas glycolytic activity is intact (GAPDH-K225A$^{f/f}$).
The main contributions of the present study are (1) we propose a solid mechanism that explains how oxidative stress elicits changes in the context of glia-neuron interactions and how these changes might lead to behavioral inflexibility, (2) we propose autofluorescence as a molecular based biomarker for behavioral inflexibility considering that reflects the activation of N-GAPDH cascade in blood cells.

Disclosures: A. Ramos: None. **N.J. Elkins:** None. **T. Palen:** None. **B. Lee:** None. **K. ishizuka:** None. **A. Sawa:** None.

Poster

126. Biology of Microglia

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Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 126.24/K1

Topic: B.11. Glial Mechanisms

Support: NIH Grant MH094268

Title: Early maternal immune stress diminishes microglia responsiveness and dopamine receptor 2 medium spiny neuron connectivity

Authors: *L. N. HAYES[1], K. AN[2], E. VINCENT[3], M. PARANJPE[2], M. KIM[2], A. J. CHANG[1], C. V. DIAZ[2], L. A. GOFF[1], A. SAWA[2]
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Abstract: Several clinical studies demonstrated immune dysfunction in the pathophysiology and pathogenesis of psychiatric disorders, such as schizophrenia. Cerebrospinal fluid and peripheral blood from psychosis patients had increased levels of immune cytokines. Furthermore, epidemiologic data showed prenatal infection led to an increased risk for schizophrenia. However, it is unclear if early immune stress is mechanistically related to the disease pathology. We hypothesize early immune activation targets microglia leading to impaired microglia functions, which impacts neural circuitry and ultimately adult behavior. To address this question, we used the maternal immune activation (MIA) mouse model of psychiatric disorders with an immunologic etiology. We stimulated MIA and control mice with lipopolysaccharide (LPS) *in vivo* and measured microglia activation using RNA sequencing. Contrary to our expectation, we found a blunted immune response program and increased metabolic pathways in MIA microglia. These results indicate that MIA microglia have a long-term memory of the early priming stimulus. We confirmed these findings using an *in vitro* microglia model and indeed found decreased cytokine secretion after LPS stimulation both in embryonic and adult microglia especially in the striatum. Next, we evaluated the LPS response of bulk tissue isolated from frontal cortex and striatum. Interestingly, we found an increase in interleukin-6 expression in frontal cortex and striatum of MIA mice, consistent with molecular findings in patients. We next aimed to determine how the blunted microglia reactivity could impact neuronal function. Since we found susceptibility of the striatal microglia, we evaluated physiology of synapses in striatal medium spiny neurons. We found MIA mice showed decreased frequency of the spontaneous excitatory postsynaptic current and increased paired pulse ratio only in dopamine receptor 2 medium spiny neurons (D2R-MSN). These results suggested a decrease in glutamatergic presynaptic input on to D2R-MSNs. The decreased connectivity of the striatal MSN may contribute to the behavioral deficits of novelty driven social recognition and hypersensitivity to

amphetamine observed in these mice. We are now identifying markers for these MIA primed microglia and specific gene programs susceptible to long-term blunting by evaluating the developmental profile of MIA microglia. Altogether, these results demonstrated 1) MIA acts as a priming stimulus that leads to long-term innate immune memory, 2) the MIA priming induced a hypo-activation of microglia reactivity, and 3) this impacted specifically D2R-MSN circuitry.

Disclosures: L.N. Hayes: None. **K. An:** None. **E. Vincent:** None. **M. Paranjpe:** None. **M. Kim:** None. **A.J. Chang:** None. **C.V. Diaz:** None. **L.A. Goff:** None. **A. Sawa:** None.

Poster

126. Biology of Microglia

Location: SDCC Halls B-H

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Program #/Poster #: 126.25/K2

Topic: B.11. Glial Mechanisms

Support: Nancy Lurie Marks Family Foundation
Robert E. Landreth and Dona Landreth Family Foundation
NIH Training Grant 5T32GM008541

Title: Renewal of microglia corrects maternal inflammation-induced morphological and transcriptomic microglial abnormalities in mouse offspring

Authors: *H. YEH[1,3], S. IKEZU[3], M. WOODBURY[3], A. VAN ENOO[3], S. SIVAKUMARAN[3], C. HOLLAND[2], T. GUILLAMON-VIVANCOS[2], A. YOSHII-KITAHARA[3], Z. RUAN[3], J.-C. DELPECH[3], M. B. BOTROS[3], A. DESANI[1], S. MANIMARAN[3], O. BUTOVSKY[7], W. E. JOHNSON[4], M. MEDALLA[5], J. I. LUEBKE[5], T. IKEZU[6]
[1]Pharmacol. & Exptl. Therapeut., [2]Boston Univ., Boston, MA; [4]Computat. Biomedicine, [5]Anat. & Neurobio., [6]Pharmacol. and Neurol., [3]Boston Univ. Sch. of Med., Boston, MA; [7]Neurology/ARCND, Brigham and Women's Hosp., Boston, MA

Abstract: Central immune dysfunction has been implicated in the pathogenesis of neurodevelopmental disorders. Maternal immune activation (MIA) due to viral infection during pregnancy is associated with increased risk of autism or schizophrenia. As brain immune cells, microglia survey the immune landscape and maintain homeostasis. Early prenatal exposure to elevated levels of cytokines from maternal infection leads to abnormal microglial function that contributes to aberrant brain development of progeny, although the biological mechanism of microglial involvement has not been clearly defined. We hypothesize that MIA alters microglial morphology and function, and that renewal of microglia can restore their homeostatic function in offspring. Here, we show that MIA alters microglial gene expression and morphological phenotype in the cortex of MIA offspring. MIA microglia have increased branching complexity and interactions with dendritic spines of intrinsically bursting pyramidal neurons in layer V of

the medial prefrontal cortex. RNA-sequencing analysis of acutely isolated microglia revealed enriched neuritogenic signaling pathways and down-regulated interferon-gamma signaling. Renewal of microglia by treatment of MIA offspring with colony stimulating factor 1 inhibitor reversed the MIA microglial phenotype by normalizing branching complexity and reverting transcriptional alterations. This was validated by intrathecal injection of interferon-gamma, which reduced hyper-ramification of MIA microglia and demonstrated its role as an upstream regulator in mediating the effect of MIA in microglia. Using dual pulse labeling of newly repopulated cells with 5-bromo-2'-deoxyuridine (BrdU) and 5-Ethynyl-2´-deoxyuridine (EdU), we show that MIA microglia repopulate from a quiescent pool that is distinct from the pool of the saline microglia repopulation. These data demonstrate that maternal immune challenge results in aberrant microglial phenotype lasting into adulthood, and reveals the potent effect of microglial depletion and repopulation on correcting the MIA phenotype of microglia.

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Poster

126. Biology of Microglia

Location: SDCC Halls B-H

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Program #/Poster #: 126.26/K3

Topic: A.01. Neurogenesis and Gliogenesis

Support: NSF IOS 1557451

Title: Ablation of microglia has region-specific effects on cell death in the neonatal mouse brain

Authors: *A. J. JACOBS, N. G. FORGER
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Abstract: Post-mitotic cell death is a key process in brain development and occurs mainly during the first postnatal week in mice. Microglia, the resident immune cells of the brain, have recently been implicated to play an active role in developmental neuronal cell death, but the data are contradictory. Some studies suggest that microglia promote neuronal cell death, while others find that microglia are neuroprotective. These different outcomes may be related to the brain regions analyzed, age differences, or other features of experimental design. In this study, we selectively eliminated microglia in newborn mice and examined the effect on cell death in 7 brain regions of each animal. C57BL/6 mice received intracerebroventricular injections of clodronate liposomes or vehicle liposomes on postnatal days (P) 0 and P1 and brains were

collected 24 h after the last injection. Alternate sections were processed for immunohistochemical detection of ionized calcium-binding adapter molecule 1 (Iba1) and activated caspase-3 (AC3) to label microglia and dying cells, respectively. Clodronate liposomes caused a significant, ~50%, reduction in microglia across all brain regions analyzed. Cell death was significantly reduced in clodronate-treated pups in the medial septum and medial amygdala. Conversely, cell death was significantly increased in clodronate-treated pups in the oriens layer of the hippocampus. In other regions, there was no significant effect on cell death. These results suggest that microglia promote developmental cell death in some brain regions, while preventing it in other regions of the same animal. Microglia are the main source of pro-inflammatory cytokines in the brain, and cytokines have been liked to neuronal cell death. To identify potential mechanisms for the observed region-specific effects of microglia, we injected a second cohort of mice as described above, and selectively micropunched hippocampal, septal and amygdalar areas. We are examining the expression of pro- and anti-inflammatory cytokines to determine whether cytokine expression is affected by microglial reduction and, if so, whether this differs by brain region.

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

Poster

126. Biology of Microglia

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Program #/Poster #: 126.27/K4

Topic: B.11. Glial Mechanisms

Support: NIH Grant R01AG041944

Title: Age and infection interact to produce unique gene expression profiles in hippocampi

Authors: *N. TANAKA, B. METENKO, H. E. ANNI, S. L. PATTERSON
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Abstract: Aging increases the risk of precipitous cognitive declines after an immune challenge, such as a surgery, injury or infection. This medical condition (sometimes termed delirium) is usually temporary, but its occurrence is associated with a greater risk of eventually developing dementia. The underlying molecular mechanisms remain unclear, but rodent models have begun to provide some clues. 24-month-old Fischer Brown Norway (F344xBN) rats are generally healthy agers with no significant physical or cognitive deficits. However, in response to a peripheral immune challenge (an intraperitoneal injection of *E. coli*), they mount an exaggerated and prolonged inflammatory reaction in the brain. Four days after infection, even though they have recovered from the acute symptoms of illness (fever or loss of appetite), these aging animals still show deficits in a long-term memory task (contextual fear conditioning), elevated

levels of hippocampal IL-1β protein, reductions in hippocampal area CA1 theta burst-evoked late-phase long-term potentiation (L-LTP, a memory-related form of long-lasting synaptic plasticity), and decreased levels of the mature brain-derived neurotrophic factor (mBDNF) protein isoform (required for long-term memory and L-LTP) in hippocampal synaptoneurosomes. Also, levels of specific BDNF mRNA transcripts (involved in local protein synthesis to stabilize long-lasting memory-related plasticity) are reduced in hippocampal tissues from aging animals at 4 days after infection. Moreover, levels of BDNF mRNA transcripts containing a long 3' untranslated region (thought to be trafficked to dendrites for local protein synthesis) and the protein coding domain (common to all the mRNA isoforms of BDNF) are both reduced in hippocampal synaptoneurosomes of these animals. In contrast, their younger (3-month-old) counterparts display little or no impairment of these parameters at the same 4-day time point. In this study, a large-scale genomic assay was performed using whole hippocampal homogenates to further investigate the effects of aging and infection on more than 700 genes related-to inflammation and neuronal structure/function. We found that a recent immune challenge produces different gene profiles in hippocampal tissues from aging and young animals. Levels of mRNAs involved in microglial activation and cytokine signaling are increased in aging *E. coli* animals. Genes related to angiogenesis (VEGF signaling), autophagy, neurotrophic factor signaling (BDNF signaling) and synaptic transmission are uniquely altered in these animals.

Disclosures: N. Tanaka: None. **B. Metenko:** None. **H.E. Anni:** None. **S.L. Patterson:** None.

Poster

126. Biology of Microglia

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 126.28/K5

Topic: B.11. Glial Mechanisms

Support: CIHR

Title: TLR4-mediated increase of microglial glycolysis inhibits expression of LTP through IL-1b

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Abstract: Microglia are critical for maintaining brain health. However, during immune activation, they also contribute to altered brain function, neurotoxicity, and degeneration. In peripheral immune cells, activating stimuli increase glycolysis, while anti-inflammatory polarization enhances oxidative phosphorylation. Therefore, we investigated whether microglia also become dependent on glycolysis following an immune challenge, and whether blocking the

glycolytic pathway weakens pro-inflammatory responses. Here, we establish the use of fluorescence lifetime imaging (FLIM) of endogenous NADH to investigate the metabolic state of microglia and neuropil in acute hippocampal slices. While neuropil energy rapidly declines in conditions of 0 mM glucose, microglia appear metabolically flexible and are able to maintain oxidative phosphorylation. This unique NADH FLIM signal in microglia suggests that they are capable of utilizing multiple metabolic sources. To investigate whether the relative activity of glycolysis and oxidative phosphorylation pathways regulate microglial immune activation or quiescence, we used lipopolysaccharide (LPS) stimulation of TLR4 receptors in microglial cultures. This treatment increased production of the pro-inflammatory cytokine, interleukin-1b (IL-1b), which was blocked by the glycolysis inhibitor 2-deoxy-D-glucose, or by addition of cell-permeable a-ketoglutarate. In acute hippocampal field recordings, LPS stimulation and IL-1b release impairs long term potentiation. This effect can be rescued either by the IL-1b receptor antagonist or by inhibiting glycolysis. These results suggest a link between inflammation and cognitive deficit, and implicate cellular metabolism as a potential mediator of microglial function.

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Poster

126. Biology of Microglia

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 126.29/K6

Topic: B.11. Glial Mechanisms

Support: William Paterson University
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CONACYT # CVU332502/232728
KAKENHI 18023009

Title: Identification of cannabinoid CB2 receptor neuro-immune crosstalk following conditional deletion of type 2 cannabinoid receptors in microglial and dopamine neurons

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Ababa, Ethiopia; [6]NIDA/IRP, Baltimore, MD; [7]Psychiatry, Harvard Med. Sch., Belmont, MA; [8]Univ. of Yamanashi, Chuo, Yamanashi, Japan; [9]NIA-NIH, Baltimore, MD

Abstract: CB1 and CB2 cannabinoid receptors (CB1Rs, CB2Rs) are expressed in neurons and neuroglia cells and CB2Rs are enhanced during inflammation. The hypothesis that CB2R neuro-immune signaling is involved in mouse models of CNS function was examined in these studies. We created and generated Cx3cr1-*Cnr2* and DAT-*Cnr2* conditional knockout (cKO) mice with deletion of CB2Rs in microglia and dopamine (DA) neurons. Immunoblotting, immunohistochemistry, gene expression profiling, behavioral assessment in models of traumatic brain injury (TBI), chronic mild stress (CMS), drug abuse, and assessment of the microbiota of these CB2R cKO mice and their wild type (WT). RNAscope *in situ* hybridization (ISH) with CB2R mRNA and tyrosine hydroxylase (TH) probes for DAT-*Cnr2* cKO mice; and Cd11b, CB2R mRNA and vGluT2 probes for CX3cr1-*Cnr2* cKO mice were used to validate the deletion of CB2Rs in DA neurons and microglia. We report that TH immune-reactivity is enhanced in DAT-*Cn2* but reduced in Cx3cr1-*Cnr2* cKO mice whereas mDAT is significantly reduced in DAT-*Cnr2* but unaffected in the Cx3cr1-*Cnr2* cKO mice compared to the WT. There was distinct expression of TNF-α following deletion of CB2Rs that is enhanced in both microglia and in DA neurons in the prefrontal cortex. The Cx3cr1-*Cnr2* showed little to no difference compared to the WT in the TBI model. Quantification of the relative abundance of *Akkermansia muciniphila* in the gut-microbiome showed high variability, both within and between DAT-*Cnr2* and Cx3cr1-*Cnr2* cKO mice. Microglia activation was detected by Cd11b in the dentate gyrus in DAT-*Cnr2* and Cx3cr1-*Cnr2* cKO mice that were enhanced after7 weeks of CMS paradigm. Neurodevelopmental and behavioral characterization after the deletion of CB2Rs in microglia and DA neurons demonstrates distinct patterns in motor function and emotionality tests. DAT-*Cnr2* cKO mice exhibit a hyperactive phenotype compared with the Cx3Cr1-*Cn2* cKO and WT mice. In the plus-maze test DAT-*Cnr2* cKO mice were less aversive to the open arms than the Cx3cr1-*Cnr2* cKO mice whereas in the tail-suspension test, depression-like behavior was provoked in both genotypes with Cx3cr1-*Cnr2* cKO mice more sensitive than DAT-*Cnr2* cKO mice. The behavioral effects of psychostimulants like Khat (300 mg/kg) enhanced locomotor activity of DAT-*Cnr2* cKO mice compared to Cx3cr1-*Cnr2* cKO mice that were not different from the WT mice. The mixed cannabinoid agonist WIN55212-2 reduced locomotor activities in all the genotypes. In conclusion investigation of CB2R neuro-immune signaling in gut-brain-axis will contribute to understanding psychiatric and neurological disorders that are associated with neuroinflammation.

Disclosures: E.S. Onaivi: None. **A. Canseco-alba:** None. **B.D. Sanabria:** None. **H. Zhang:** None. **M. Eita:** None. **T. Rohani:** None. **R. Bernadin:** None. **M. Zamora:** None. **E. Dennis:** None. **S. Gomez:** None. **B. Kibret:** None. **M. Chung:** None. **N. Schanz:** None. **S. Sgro:** None. **C.M. Leonard:** None. **P. Tagliaferro:** None. **K. Martin:** None. **S. Bierbower:** None. **J. Lee:** None. **E. Engidawork:** None. **E.L. Gardner:** None. **Z. Lin:** None. **H. Ishiguro:** None. **Z. Xi:** None. **Q. Liu:** None.

Poster

127. Microglia in Disease

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 127.01/K7

Topic: B.11. Glial Mechanisms

Support: Grants-in-Aid for scientific research (17K16736) from the Ministry of Education, Culture, Sports, Science and Technology of Japan

Title: Microglial activation in spinal anterior horn after peripheral nerve injury: Is the activation detrimental or neuroprotective?

Authors: *T. NISHIHARA[1], J. TANAKA[2], T. YOROZUYA[3]
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Abstract: It is known that microglial cells are activated in the posterior horn (PH) after peripheral nerve injury and engage in the pathogenesis of neuropathic pain. Although most pain researches have addressed activated microglia in PH, activation of microglia is apparent in the anterior horn (AH) as well.
In the present study, we compared the activated microglia in AH and PH from both the morphological and functional aspects after chronic constriction injury (CCI) of the left sciatic nerve. The activation of microglial cells in AH and PH were analyzed with immunohistochemical staining and quantitative real-time RT-PCR at day 7 after CCI. Microglial cells in the left AH showed enlarged but flattened shape while intimately attached to and surround motoneurons. By contrast, those in the left PH displayed amoeboid-like shape and their attachment to neurons was not apparent. Confocal laser scan microscopy revealed that activated microglia in AH bore $CD68^{+}$ phagosomes co-localized with synaptophysin, whereas those in PH co-localized with myelin basic protein, suggesting that both microglia in AH and PH phagocytosed synapses and myelin sheaths, respectively. We prepared cDNA from four parts (left AH, left PH, right AH and right PH) of the spinal cord after CCI. CD11b and F4/80 mRNA expression was significantly higher in the left AH and PH than that in the right. The expression of the those mRNA in the left PH was higher than in the left AH. mRNA for Compliments C1q and C3 was also expressed at higher levels in the left PH than that in the left AH. Arg1 and hepatocyte growth factor (HGF) mRNA was increased in left PH.
These results suggest that microglial cells in the AH and the PH may be activated in different manners. Activated microglia in PH in the injured side appeared more phagocytic than those in AH while eliminating myelin via a compliments-dependent manner. Microglia in the injured AH may engage in synaptic elimination while attaching to motoneurons.

Disclosures: J. Tanaka: None. **T. Yorozuya:** None.

Poster

127. Microglia in Disease

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 127.02/K8

Topic: B.11. Glial Mechanisms

Title: Differentiation of microglia and macrophages in traumatic brain injuries in terms of aggravation of neural tissues

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Abstract: Although both resident microglia and blood-borne macrophages may be responsible for aggravation of traumatic brain injury (TBI), the differences in their roles in the TBI pathogenesis have not been well described. This is because resident microglia and blood-borne macrophages in injured brain are difficult to be distinguished with each other due to their many common characteristics. However, the identification of microglia-specific markers and the use of flow cytometry have made it possible to discriminate these types of cells. In this study, we analyzed the features of blood-borne macrophages, and activated and resting microglia in and around lesions of a rat TBI model by employing flow cytometry and immunohistochemistry. We evaluated oxidative injury in the TBI model by determining 8-hydroxy-2′-deoxoguanosine level. Flow cytometry analyses revealed that macrophages generated far more mitochondrial reactive oxygen species (ROS) than activated or resting microglia. Dihydroethidium staining also showed that macrophages but not microglia were the major source of ROS in TBI lesions. Furthermore, sorted macrophages expressed NADPH oxidase 2 (NOX2), inducible nitric oxide synthase (iNOS) and IL-1b at higher levels than microglia. By contrast, microglia expressed TGFb1, IL-6 and TNFa at higher levels than macrophages. Macrophages expressed CD68, a phagocytic marker, at a higher level, and SIRP1a, a don’t-eat-me signal, at a lower level than microglia. These results indicate that macrophages are the major aggravating cell type in TBI lesions, in particular during the acute phase. On the other hand, microglia may even play favorable roles, because there are reports that TNFa and IL6 also have neuroprotective effects. Therefore, therapeutic interventions to prevent infiltration and activation of macrophages in the acute phase may be promising as a treatment of TBI.

Disclosures: N. Abe: None. **J. Tanaka:** None. **M.E. Choudhury:** None. **H. Yano:** None. **T. Yorozuya:** None.

Poster

127. Microglia in Disease

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Program #/Poster #: 127.03/K9

Topic: B.11. Glial Mechanisms

Support: Grants-in-Aid for scientific research (17K11576) from the Ministry of Education, Culture, Sports, Science and Technology of Japan

Title: The comparison of microglial phagocytosis between carbon monoxide poisoning and hypoxemic hypoxia

Authors: *K. SEKIYA, T. NISHIHARA, N. ABE, T. YOROZUYA
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Abstract: Carbon monoxide (CO) poisoning causes delayed neurologic syndrome called delayed encephalopathy (DE). However, the detailed pathophysiological mechanisms of this syndrome have not been clarified. Brain hypoxia also induces delayed post-hypoxic leukoencephalopathy which is similar to CO-induced DE.
In the brain, microglial cells constitutively search the abnormality of tissue integrity. In case of neuronal damage, they are activated and clear the debris, which is essential for the remyelination and nerve regeneration. However, by contrast, inflammation by activated microglia is also detrimental to the nerves. As one of the mechanisms for pathogenesis of DE, the contribution of the microglial activation is reported.
In the present research, we aimed to clarify the pathophysiological mechanisms of CO-induced DE and to compare the differences with hypoxemic hypoxia in terms of inflammation and responses of microglial cells.
We randomly assigned rats to 3 groups; control group (Air group), CO exposure group (CO group), and hypoxia exposure group ($LowO_2$ group), assessed their cognitive function, and analyzed their hippocampus.
As the results, cognitive dysfunction was observed only in CO group after 7days post exposure (dpe). In hippocampus, myelin basic protein (MBP) was significantly decreased at 7dpe both in CO group and $LowO_2$ group, which was continuous until 21days in CO group. However, microglial activation was not observed and phagocytic function was decreased in CO group. By contrast, microglial cells were activated and mRNA of CD68, a phagocytic marker, was increased in $LowO_2$ group. Flow cytometric analysis revealed that the percentage of $NeuN^+$ neuronal cells and $CD11b^+$ microglial cells in alive cells were significantly decreased only in CO group at 7dpe.
These data indicate that both CO and hypoxemic hypoxia induces demyelination and neuronal

cell death, however, the condition of microglia is different between in two conditions; CO decreases microglia and impairs phagocytic function but hypoxemic hypoxia activates microglia. The decrease and dysfunction of microglial cells delay the debris clearance. Demyelination, neuronal cell death, and microglial phagocytic dysfunction may collaborate for CO-induced DE.

Disclosures: K. Sekiya: None. **T. Nishihara:** None. **N. Abe:** None. **T. Yorozuya:** None.

Poster

127. Microglia in Disease

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 127.04/K10

Topic: B.11. Glial Mechanisms

Title: Serum amyloid A primes microglia for ATP-dependent interleukin-1β release

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Abstract: The acute-phase response is a systemic reaction to environmental/inflammatory insults and involves production of acute-phase proteins, including serum amyloid A (SAA). Interleukin-1β (IL-1β), a master regulator of neuroinflammation produced by activated inflammatory cells of the myeloid lineage, in particular microglia, plays a key role in the pathogenesis of acute and chronic diseases of the peripheral nervous system and CNS. IL-1β release is promoted by ATP acting at the purinergic P2X7 receptor (P2X7R) in cells primed with Toll-like receptor (TLR) ligands. The present study was undertaken to examine the ability of ATP to promote the intracellular production, and release, of IL-1β from cortical and cerebellar microglia stimulated with Apo-SAA, and the involvement of P2X7R, TLR4, and TLR2. Purified (>99%) microglia cultured from neonatal rat cortex and cerebellum were first primed with the putative TLR4/TLR2 agonist SAA (recombinant human Apo-SAA) or the established TLR4 agonist lipopolysaccharide (LPS), followed by addition of ATP. Expression of genes for the NLRP3 inflammasome, IL-1β, tumor necrosis factor-α (TNF-α), and SAA1 was measured by quantitative Real-Time polymerase chain reaction (q-PCR). Intracellular and extracellular amounts of IL-1β were determined by ELISA. Apo-SAA stimulated, in a time-dependent manner, the expression of NLRP3, IL-1β, and TNF-α in cortical microglia, and produced a concentration-dependent increase in the intracellular content of IL-1β in these cells. A 2-h 'priming' of the microglia with Apo-SAA, followed by addition of ATP for 1 h, resulting in a robust release of IL-1β into the culture medium, with a concomitant reduction in its intracellular content. The selective P2X7R antagonist A740003 blocked ATP-dependent release of IL-1β. Microglia prepared from rat cerebellum displayed similar behaviors. As with LPS, Apo-SAA up-regulated SAA1 and TLR2 mRNA, and down-regulated that of TLR4. LPS was less efficacious than Apo-SAA, perhaps reflecting an action of the latter at both TLR4 and TLR2. The TLR4

antagonist CLI-095 fully blocked the action of LPS, but only partially that of Apo-SAA. Although the TLR2 antagonist CU-CPT was inactive against Apo-SAA, it also failed to block the TLR2 agonist Pam3CSK4. Microglia are central to the inflammatory process and a major source of IL-1β when activated. P2X7R-triggered IL-1β maturation and export is thus likely to represent an important contributor to this cytokine pool. Given that SAA is detected in Alzheimer disease and multiple sclerosis brain, together with IL-1β-immunopositive microglia, these findings propose a link between P2X7R, SAA and IL-1β in CNS pathophysiology.

Disclosures: L. Facci: None. **M. Barbierato:** None. **M. Zusso:** None. **P. Giusti:** None. **S.D. Skaper:** None.

Poster

127. Microglia in Disease

Location: SDCC Halls B-H

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Program #/Poster #: 127.05/K11

Topic: B.11. Glial Mechanisms

Title: Neurogenesis in adult hippocampus is affected in rats with delayed carbon monoxide encephalopathy via glial cells

Authors: *S. OCHI[1], K. SEKIYA[2], N. ABE[2], T. NISHIHARA[2], J.-I. IGA[1], S.-I. UENO[1]
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Abstract: Delayed carbon monoxide (CO) encephalopathy may occur following recovery after several weeks from acute CO poisoning. However, the mechanism of delayed neuronal injury remains unknown. Previously, we reported that we made the rat model of delayed CO encephalopathy and showed cognitive impairment and hippocampal cell death, especially the lesions of dentate gyrus. It has been well known that neurogenesis containing the proliferation of neural precursor cells occurs in the dentate gyrus of adult hippocampus and may be associated in the modulation of glial cells. Therefore, we hypothesized that neurogenesis in the dentate gyrus may be affected in delayed CO encephalopathy.
In the current study, we investigated the effect of delayed neuronal CO poisoning on neural precursor cells and glial cells employing immunohistochemistry and flow cytometry in the hippocampus of CO model rats and control rats.
Wistar male rats (6 weeks old) were exposed to 1000ppm CO for 40 min and then 3000 ppm for 20 min until they lost consciousness, and then they were removed to breathe room. If they didn't lose consciousness for this 60 min, rats were exposed to 10000ppm until they lost consciousness. Behavioral effects on learning and memory function were measured by the passive-avoidance test between control and CO treated rats until 3 weeks. The latencies were significantly shorter in the CO models. Immunohistochemical analyses revealed cell numbers in Sex determining region

Y-box 2 (SOX2) positive cells and microglia tended to decrease CO models less than controls in the lesions of dentate gyrus. Especially, a lot of morphologically abnormal microglia was found in the lesion. On the other hand, astrocytes tends to increase CO models more than controls in the lesions of dentate gyrus. Flow cytometry analyses revealed that the cell numbers of microglia were significantly reduced in CO models. These results suggested that delayed CO encephalopathy may occur the abnormalities in glial cells such as microglia and astrocytes, and reduce neural precursor cells. Thus, the impairment of neural precursor cells via abnormalities of glial cells may be affected in the mechanism of delayed neuronal injury.

Disclosures: S. Ochi: None. **K. Sekiya:** None. **N. Abe:** None. **T. Nishihara:** None. **J. Iga:** None. **S. Ueno:** None.

Poster

127. Microglia in Disease

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Topic: B.11. Glial Mechanisms

Support: NIH Grant NS092938

Title: Metabolic stress leads to reduced microglial functionality in a model of Leigh Syndrome

Authors: *R. S. MCCAIN, JR, G. G. PIROLI, H. SMITH, N. FRIZZELL
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Abstract: Leigh Syndrome is a subacute necrotizing encephalopathy stemming from defects in the mitochondrial electron transport chain with bilateral lesions of the basal ganglia and brainstem that show accumulation of activated microglia. Knockout(KO) of NADH dehydrogenase [ubiquinone] iron-sulfur protein 4 (Ndufs4), an assembly protein for Complex I of the electron transport chain, models Leigh Syndrome in mice and shows significant increases in microglia in lesioned areas of the brainstem. While some microglial activation leads to debris removal, excessive chronic activation may be harmful. The reductive stress associated with Ndufs4 KO leads to an increase in Krebs cycle metabolites, including fumarate, which can irreversibly react with free protein thiols by succination and lead to altered protein function. We examined the extent of protein succination in lipolysaccharide (LPS) activated microglial models and assessed the association between metabolic stress and altered function in the Ndufs4 KO model. WT and Ndufs4 KO microglia were isolated and stimulated with LPS for up to 24 hrs. Comparative analyses were performed with additional microglial models including peritoneal macrophages and the highly aggressive proliferating immortalized rat microglial (HAPI) cell line.) Significant increases in protein succination and IL-1β (a pro-inflammatory cytokine) were detected by immunoblotting across all sample types treated with LPS. In addition, Ndufs4 KO

macrophages had diminished LPS-induced increases in IL-1β levels and altered succination patterns. *In vitro* mitochondrial and glycolytic stress tests showed increased levels of glycolytic activity, decreased basal and maximal mitochondrial respiration, and decreased ATP production in Ndufs4 KO macrophages compared to WT controls using a Seahorse XF24 analyzer. Analysis of phagocytosis showed decreased uptake of fluorescently labelled latex beads in activated Ndufs4 KO macrophages compared to WT macrophages and unstimulated controls. In summary, we demonstrate that activated microglial models demonstrate intrinsic increases in protein succination. An altered succination profile in the Ndufs4 KO mouse is associated with decreased mitochondrial metabolism, altered inflammatory profiles and phagocytic function that may indicate reduced microglial functionality in Leigh Syndrome.

Disclosures: R.S. McCain: None. **G.G. Piroli:** None. **H. Smith:** None. **N. Frizzell:** None.

Poster

127. Microglia in Disease

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Program #/Poster #: 127.07/K13

Topic: B.11. Glial Mechanisms

Support: KAKENHI 17H04714

Title: Effects of valproate prenatal exposure on developmental microglial activity and neural circuit formation

Authors: *Y. ISHIHARA, T. HONDA, R. TANIGUCHI, N. ISHIHARA, T. YAMAZAKI
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Abstract: Sodium valproate (VPA) has been widely used for generalized epilepsy. However, it is reported that treatment with VPA during gestation can cause cognitive dysfunction and/or autistic behavior in child. Recently, growing evidence shows that microglia, an immune cell in the brain, are involved in developmental neural circuit formation by engulfing dispensable synapse having low activity. The purpose of this study is to reveal the mechanism of abnormal behavior induced by prenatal VPA exposure, focusing on microglial action in the developmental stage. ICR mice were orally administered with VPA at 800mg/kg on embryonic day 11 (E11). Behaviors were examined by open field test, Y-maze test, social interaction test and marble burying test. Microglial activity was measured by Iba1/CD68 staining as well as levels of proinflammatory cytokine mRNA. The number of synapse was evaluated by PSD95 staining. Minocycline, an inhibitor of microglial activation was administered with lactation from postnatal day 1 (P1) to P20. Phagocytic assay was done with BV-2, a mouse microglial cell line. There was no change in locomotor activity between vehicle and VPA-treated groups, while VPA-

treated mice showed deficit in alternation, decreases in contact with a novel mouse and increment of burying behavior at the age of 5 to 6 weeks compared with vehicle-treated mice, indicating impairment of working memory, abnormal social behavior and symptom of repetitive behavior, respectively by VPA exposure. VPA increased mRNA expression of interleukin 1β, and induced enlargement of soma area and up-regulation of CD68 expression in microglia at the hippocampus of P10 mice. VPA exposure during gestation also reduced the number of PSD95 puncta in the CA1 region of hippocampus on P10. Therefore, microglia activated by VPA might be involved in excess engulfment of synapse during development. Treatment with minocycline suppressed microglial activation as well as decreases in PSD95 puncta elicited by VPA. Minocycline improved decreases in contact with a novel mouse and increment of burying behavior, but showed no effect on deficit in alternation. Treatment with VPA showed no change in proinflammatory cytokine expression and phagocytosis in BV-2 cells activated by lipopolysaccharide or ATP. These results suggest that prenatal VPA exposure causes abnormal behavior by microglial activation and subsequent excess engulfment of synapses and that VPA can indirectly activate microglia such as epigenetic modification.

Disclosures: Y. Ishihara: None. **T. Honda:** None. **R. Taniguchi:** None. **N. Ishihara:** None. **T. Yamazaki:** None.

Poster

127. Microglia in Disease

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Program #/Poster #: 127.08/K14

Topic: B.11. Glial Mechanisms

Support: AA020023
AA020024
AA019767

Title: Targeting tlr7 and ifn-gamma reverses down-regulation of trem2 mrna induced by ethanol and hdac inhibitor tsa

Authors: *J. Y. ZOU, L. COLEMAN, F. CREWS
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Abstract: The triggering receptor expressed on myeloid cells 2 (TREM2) is predominantly expressed by microglia in CNS and implicated in microglial homeostasis, neuroinflammation and neurodegeneration. However, ethanol and epigenetic regulation of TREM2 expression remains unclear. The present study investigated the effects of ethanol and HDAC inhibitor TSA on TREM2 mRNA expression in hippocampal-entorhinal cortex (HEC) slice cultures. We first determined cell-type expression of TREM2 in this model. Immunohistochemistry staining show

predominant co-expression of TREM2 and Iba-1+ microglia; RT-PCR analysis indicated that TREM2 mRNA was eliminated when microglia were depleted (Control 100±12; M-depletion 16±3), and fully recovered after new microglia repopulated for 14 days (Control 100±8; M-repopulation-14D 155±10). These data confirm that TREM2 is expressed predominantly in microglia in HEC slices. Treatment of HEC slices after 11DIV with ethanol (100mM) for 4 days significantly reduced TREM2 mRNA expression by 20-40% at different time point. TLR7 signaling has been engaged in ethanol-induced neuroimmune activation and neurotoxicity. Similarly, activation of TLR7 with agonist Imiquimod drastically reduced TREM2 expression by 66% and 97% with Imiquimod treatment at concentration 2.5ug/ml and 5ug/ml respectively. To test if TLR7 signaling is involved in ethanol down-regulation of TREM2, HEC slices were transfected with TLR7 siRNA (100 and 200nM) for 24hr and followed by ethanol treatment for 72hrs. The results revealed that TLR7 knockdown completely reversed ethanol-induced reduction of TREM2 mRNA expression. Interestingly, both ethanol and TLR7 agonist Imiquimod increased expression and micropartical release of NF-kB sensitive miR-34a. In addition, inhibition of miR-34a abolished ethanol down-regulation of TREM2 mRNA expression. Furthermore, we investigated epigenetic regulation of TREM2 by treating HEC slices with HDAC inhibitor TSA. TSA reduced TREM2 mRNA expression in HEC slices in concentration-dependent manner (250-5000nM). However, TSA-induced down-regulation of TREM2 is not associated with miR-34a but with significant increased IFN-gamma and miR-155. Administration of IFN-gamma neutralizing antibodies (2ug/ml) significantly reversed TSA reduction of TREM2 mRNA level in this model. Together, our data reveal different mechanism underlying ethanol and TSA regulation of TREM2 gene expression.

Disclosures: J.Y. Zou: None. **L. Coleman:** None. **F. Crews:** None.

Poster

127. Microglia in Disease

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 127.09/K15

Topic: B.11. Glial Mechanisms

Support: 17H03988
17H05738
18H05114

Title: Thermosensitive TRPV4 mediates social defeat stress-induced behaviors in mice

Authors: *Y. HOSHI[1], K. SHIBASAKI[2], R. KOYAMA[1], Y. IKEGAYA[1]
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Abstract: Social defeat stress is known to increase the core body temperature of stressed animals; however, it remains unknown whether and how the stress-induced elevations in the core body temperature modify animal behaviors. Here we used a mouse model of social defeat stress and examined the relationship between the core body temperature and stress-induced behaviors. Social defeat stress induced depressive- and anxiety-like behaviors in mice, as assessed by the tail suspension and elevated plus maze tests. The rectal temperature of stressed mice was significantly increased compared to control. This elevation in the rectal temperature was maintained at least for four weeks after the final stress experience. Next, we examined the cellular and molecular mechanisms how the stress-induced hyperthermia modifies the behaviors of stressed mice, specifically focusing on the involvement of transient receptor potential vanilloid 4 (TRPV4), a thermosensitive channel which is highly expressed in the brain. We found that stress-induced depressive or anxiety-like behaviors were not observed in TRPV4 knockout (KO) mice, however, stress-induced hyperthermia was maintained in TRPV4KO mice. These results suggest that TRPV4 mediates stress-induced behaviors, probably by temperature elevation. Because stress could modulate the function of the brain-resident immune cell microglia, we immunohistochemically investigated the microglial property in stressed mice using immunohistochemistry. We found that the expression of microglial marker, Iba1 and the activated microglial marker, CD68 was upregulated in the hippocampus of stressed mice compared to control. In contrast, stress-induced microglial activation was not observed in TRPV4KO mice. These results suggest the possible involvement of hyperthermia and TRPV4 in stress-induced behaviors.

Disclosures: Y. Hoshi: None. **K. Shibasaki:** None. **R. Koyama:** None. **Y. Ikegaya:** None.

Poster

127. Microglia in Disease

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 127.10/K16

Topic: B.11. Glial Mechanisms

Support: MOST106-2320-B-039-019

Title: Acupuncture attenuates bile acid-induced itch and spinal microglial activation in mice

Authors: *Y.-H. CHEN[1], Y.-C. LEE[2], C.-H. LIN[3], S.-Y. HUNG[1], H.-Y. CHUNG[1], S.-T. LUO[1], I. MACDONALD[1], Y.-T. CHU[1], P.-L. LIN[4]
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Abstract: Cholestatic hepatobiliary disease is frequently associated with pruritus, which is difficult to manage. In this study, we examined the effects of manual acupuncture (MA) at

particular acupoints on scratching behavior in mice injected with pruritogens deoxycholic acid (DCA) or 5'-guanidinonaltrindole (GNTI, a kappa opioid receptor antagonist). MA at Hegu (LI4) and Quchi (LI11) acupoints significantly attenuated DCA- and GNTI-induced scratching. MA at non-acupoints did not affect scratching behavior. GNTI- and DCA-induced scratching behavior was reduced by an intraperitoneal injection of minocycline (a microglial inhibitor). Western blot analysis revealed increases in spinal cord expression of ionized calcium-binding adapter molecule 1 (Iba1) and tumor necrosis factor-alpha (TNF-α) after DCA injection as compared with saline injection; MA at LI4 and LI11 reduced these DCA-induced changes. DCA injection was also associated with Iba1-positivity with thick processes emanating from enlarged cell bodies in the spinal cord dorsal horn, which was attenuated with MA pretreatment. Clearly, microglial activation and TNF-α play important roles in DCA-induced itch and these effects were reduced by MA. MA may therefore be an effective treatment for cholestatic pruritus.

Disclosures: Y. Chen: None. **Y. Lee:** None. **C. Lin:** None. **S. Hung:** None. **H. Chung:** None. **S. Luo:** None. **I. MacDonald:** None. **Y. Chu:** None. **P. Lin:** None.

Poster

127. Microglia in Disease

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 127.11/K17

Topic: B.11. Glial Mechanisms

Support: University of Sussex - Research Training and Support Grant
Sussex Neuroscience 4 Year PhD Program

Title: Investigating the region-specific neurovascular and microglial effects of short-term high fat diet exposure

Authors: *D. CLARKE, H. CROMBAG, C. N. HALL
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Abstract: Obesity, a major risk factor for type-2 diabetes, heart disease[1], mental illness[2], and dementia[3], is associated with diets high in fat and sugar. Components of these diets can induce food-seeking behaviours[4], disrupt energy homeostasis mechanisms[5], and induce rapid deficits in hippocampal function: Rats are impaired on spatial- but not object- dependent memory after 6 days of high fat/high sugar diet access[6], and impaired in their context-specific operant responding on a renewal task after two weeks of high fat diet[7] (HFD). We sought to reproduce these results in mice, and found impaired object-in-context recognition after two weeks of HFD, and deficits in a hippocampal-dependent Pavlovian discrimination task after 30 days. To determine what underlies these behavioural deficits, we are investigating region-specific effects of short-term HFD exposure in mice. Experiments utilise confocal microscopy to image

hypothalamic, hippocampal, and cortical regions in fluorescently labeled brain slices, as well as two photon imaging and combined haemoglobin spectroscopy/laser Doppler flowmetry in awake, behaving, animals with cranial windows over visual cortex. Mice are given *ad libitum* access to either a control or HFD, and sacrificed after 14 days of diet exposure for immunofluorescence experiments, or imaged at various timepoints up to 8-weeks in the *in vivo* experiments. Mice vary in age and sex. Initial immunofluorescence results showed that, across all brain regions, HFD reduced vascular perfusion and vascular radius, and increased microglial activation as assessed from microglial density and morphology. However these results failed to replicate in a subsequent study, suggesting HFD-induced changes at 14 days are variable. To interrogate the time course of any changes in more physiological conditions, we are using *in vivo* measurements of microglial morphology, motility, and vascular functionality. Preliminary results suggest that microglial motility and neurovascular coupling are unaffected up to 30 days following HFD exposure, although HFD-induced changes in microglial morphology begin to appear at this timepoint. Current work is focused on increasing our sample size, but in the future we would like to track these changes over longer time periods, investigate blood-brain barrier permeability *in vivo*, and repeat these measurements in mice with chronic hippocampal cranial windows.
1[*Obesity*, **22**, S40 (2014)] **2**[*Int J Obesity*, **32**, 558 (2008)] **3**[*Neurology*, **76**, 1568-1574 (2011)] **4**[*Neurosci Biobehav R*, **32**, 20-39 (2008)] **5**[*Physiol Behav*, **83**, 573-578 (2004)] **6**[*Psychol Sci*, **26**, 1947-1957 (2015)] **7**[*Behav Neurosci*, **126**, 493-498 (2012)]

Disclosures: D. Clarke: None. **H. Crombag:** None. **C.N. Hall:** None.

Poster

127. Microglia in Disease

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 127.12/K18

Topic: B.11. Glial Mechanisms

Title: Anthocyanin oligomers counteracts ischemic and oxidative insults to retinal cells and lipid peroxidation to brain membranes

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[2]Ophthalmology, St. Mary's Hospital, , Col. of Medicine, The Catholic Univ. of Korea,, Incheon, Korea, Republic of

Abstract: Purpose : The purpose of the present study was to determine whether the flavonoid, anthocyanin oligomers is effective at blunting the negative influence of ischemia/reperfusion to the rat retina *in situ* and of various insults to a retinal precursor cells (R28) in culture.
Methods : R28 in culture were given one of three different insults, light (1000 lux for 2 days),

hydrogen peroxide (200 µM H_2O_2 for 24 hours) or serum deprivation (48 hours). Cell survival and reactive oxygen species (ROS) formation was assayed. Lipid peroxidation assay was used to compare the antioxidant capacity of anthocyanin oligomers. In animal studies, anthocyanin oligomers was administered intraperitoneally just before and after an ischemic insult and ischemia was delivered by raising the intraocular pressure above the systolic blood pressure(50 min). Retinas were analysed for the localisation of various antigens and retinal extracts were also analysed for various mRNAs.
Results : Ischemia/reperfusion to the retina affected the localisation of Thy-1 and choline acetyltransferase (ChAT) and the content of various proteins (optic nerve and retina) and mRNAs (retina). Importantly, anthocyanin Oligomers statistically blunted most of the effects induced by ischemia/reperfusion. Only the increase in caspase-8 caused by ischemia/reperfusion was unaffected by anthocyanin oligomers treatment. Anthocyanin oligomers also attenuated significantly the negative insult of light, hydrogen peroxide and serum withdrawal to R28 cells. In the lipid peroxidation studies, anthocyanin oligomers was also found to be equally effective as EGCG to act as an antioxidant. Significantly, the negative insult of serum withdrawal on R28 cell survival was blunted by anthocyanin oligomers but not by EGCG revealing the different properties of the two flavonoids.
Conclusions : Our results demonstrates the powerful antioxidant characteristics of anthocyanin oligomers. Together with anti-apoptotic effects of anthocyanin oligomers suggest its potential use for the treatment of various ocular diseases such as glaucoma, where oxidative stress has been suggested to be involved in many aspects of pathogenesis.
This study demonstrates the anti-oxidative properties of anthocyanin oligomers using both in vivo and in vitro studies. It is of an interest that the anti-oxidative properties of anthocyanin oligomers were comparable to EGCG, suggesting that anthocyanin oligomers can be used to treat various ocular diseases where oxidative stress plays a major role.

Disclosures: B. Lim: None. **K. Kang:** None.

Poster

127. Microglia in Disease

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 127.13/L1

Topic: B.11. Glial Mechanisms

Support: ERC grant 725563

Title: Acetaminophen rescues microglial defects and cognitive impairment in the dp16 murine model of down syndrome

Authors: *B. PINTO[1,2], A. PETRETTO[3], M. BARTOLUCCI[3], L. E. PERLINI[1], L. CANCEDDA[1,4]

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Abstract: Microglia are the main immune cells of the brain and play important roles in mechanisms essential for cognitive functions. Down syndrome (DS) is caused by the presence of a supernumerary chromosome 21 (Chr21), and it represents the most frequent cause of genetic intellectual disability. Interestingly, some of the genes located on Chr21 are essential for the function of the immune system. However, it is not known whether microglial defects are involved in the cognitive deficits associated with DS. Here, we investigated the presence of microglial alterations and their implication in cognitive impairment in the DP16 mouse model of DS. At postnatal day 22, we found enlarged cell body and decreased ramifications in microglia of DP16 hippocampi, indicative of an activated state. Accordingly, we also found increased levels of pro-inflammatory cytokines in DP16 hippocampi. In addition, DP16 mice showed lower levels of drebrin and kalirin, two proteins important for dendritic spine function and morphology. Remarkably, depletion of defective microglia in DP16 mice by PLX3397 treatment was able to fully rescue cognitive defects in DP16 mice, as measured by the Novel Object Recognition (NOR) and Object Location (OLT) test. Notably, treatment with the commonly used non-steroidal, anti-inflammatory drug acetaminophen (APAP) rescued microglia morphology and the cognitive performance of the DP mice. Interestingly, PLX3397 as well as APAP treatment rescued the alteration in drebrin and kalirin levels, suggesting that DP16 animals may have dendritic spine alterations that depend on the defective microglia. Altogether, our data suggest an involvement of microglia in the cognitive impairment observed in adult DS animals, possibly mediated by defective dendritic spines. Moreover, our study identifies microglia as a new target for safe therapeutic intervention by a common anti-inflammatory drug to rescue cognitive disabilities in individuals with DS.

Disclosures: B. Pinto: None. **A. Petretto:** None. **M. Bartolucci:** None. **L.E. Perlini:** None. **L. Cancedda:** None.

Poster

127. Microglia in Disease

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 127.14/L2

Topic: B.11. Glial Mechanisms

Title: Inhibition of ATGL reduces inflammation in LPS-activated microglial cells

Authors: *A. I. MACHUCA PARRA, R. MANCEAU, D. RODAROS, C. LAURENT, N. ARBOUR, S. FULTON, T. ALQUIER
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Abstract: Microglia, the intrinsic immune system of the brain, is the primary cell population responding to insults that alter brain homeostasis generating different molecular and morphological profiles: the classically activated M1-type and the alternatively activated state M2-type. During inflammation, large lipid droplets (LDs) are actively formed and constitute sites for the synthesis and storage of various inflammatory mediators in many cell types, including neurons and microglia. Specific glycerolipid lipases surrounding LDs catalyze the hydrolysis of triglycerides (TG) to generate mono-(MAG), di-acylglycerol (DAG), and fatty acids (FA). The Adipose Triglyceride Lipase (ATGL), which catalyzes the hydrolysis of triglycerides, plays a key role in lipid homeostasis by regulating glycerolipid metabolism in several cell types. However, the role of ATGL and LDs in microglial cell function and neuroinflammation is unknown. We found that ATGL is enriched in FACS-purified microglial cells from adult mouse brain and is expressed in postnatal microglial cells in culture. Primary microglia cultures derived from mouse pups (P2) and BV2 cells were treated with Atglistatin (a specific ATGL inhibitor) to determine the effect of ATGL inhibition on apoptosis and cell viability measuring cell health and viability, and pro-inflammatory responses induced by LPS. The expression of different inflammatory markers was measured by qRT-PCR. A significant decrease in apoptosis ($p<0.0002$) and cytotoxicity ($p<0.0004$) with Atglistatin was observed. ATGL expression was decreased ($p<0.001$) in the presence of LPS. Expression levels of IL-6 ($p<0.0001$) and MCP-1 ($p<0.0001$) were decreased in the presence of Atglistatin under inflammatory conditions but not for IL-1β, TNF-α, and NF-κB. With ORlistat, a general lipase inhibitor, expression levels of IL-1β ($p<0.0001$), IL-6 ($p<0.0001$), and NF-κB ($p<0.0001$), but not MCP-1 and TNF-α, were significantly decreased under inflammatory conditions. We propose that ATGL regulates inflammatory responses in LPS-stimulated microglia, suggesting a significant role of ATGL during inflammation *in vitro*. Ongoing experiments are aimed at assessing the role of ATGL in TG metabolism, LDs dynamics, and inflammation processes *in vivo* using genetic loss-of-function models.

Disclosures: A.I. Machuca Parra: None. **R. Manceau:** None. **D. Rodaros:** None. **C. Laurent:** None. **N. Arbour:** None. **S. Fulton:** None. **T. Alquier:** None.

Poster

127. Microglia in Disease

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 127.15/L3

Topic: B.11. Glial Mechanisms

Support: University of Arizona College of Nursing Emmons

Title: Spatial and sex differences in microglia responses after ischemic stroke in mice: Evaluating morphology, phagocytosis and Iba1+/TMEM119- cells

Authors: *H. MORRISON[1], K. YOUNG[2]
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Abstract: Ischemic stroke is an acquired brain injury with sex and age dependent outcomes. The influence of sex and menopause on neuroinflammatory mechanisms of ischemic brain injury is unclear. Microglia phagocytosis is necessary for debris clearance and wound healing but is potentially detrimental in excess. A sex-specific and fine-tuned phagocytic response from microglia may be tissue sparing post-stroke. Therefore, we investigated the effects of sex and, to model post-menopause, female ovarian failure (FOF) on microglia phagocytosis after focal ischemic stroke in mice. Male, female diestrus (FD) and FOF mice underwent either 60 min. of middle cerebral artery occlusion and 24 hours of reperfusion or a sham surgery (N = 6-10/group). Brain tissue was collected for immunohistochemical staining. Microglia were visualized using antibodies against ionized calcium-binding adapter molecule (Iba1), complement receptor 3 (CR3), and transmembrane protein 119 (TMEM119). Microglia were imaged using confocal microscopy to obtain photomicrographs in four regions for analysis: sham and ipsilateral regions distal (ID), proximal (IP) and the edge of infarcted area (IN). Analyses were carried out in coronal sections at 0 Bregma, where the infarct area was similar among groups ($p < 0.4$). Microglia phagocytosis was operationalized with multiple measures of the phagocytic process: microglia morphology (skeleton and fractal analysis), CR3 area/cell, phagocytic capacity (% cells with multiple phagosomes), phagocytic ratio (phagocytic cells:total cells) and phagosome area. All evaluators were blinded to experimental conditions, with data tested using two-way ANOVA with Tuckey's post-hoc. Evidence of increased microglia phagocytosis is spatially dependent in the ipsilateral hemisphere ($F_{(3,70)} > 13.92$ $p < 0.05$) and different among sex groups ($F_{(2,70)} > 2.1$; $p < 0.05$). Microglia in the IN region were morphologically distinct, most notably in the FOF group ($p < 0.05$). Therefore, using TMEM119, we tested the hypothesis that although all cells in this region were ramified to some extent, they may be infiltrating (Iba1+/TMEM119-) rather than resident cells (Iba1+/TMEM119+). Iba1+/TMEM119+ cells were decreased by > 50% ($F_{(2,74)} = 126.2$ $p < 0.0001$, vs. sham) in proximity to the infarct and more prevalent in the FD mice ($p < 0.05$). However, contrary to our hypothesis, Iba1+/TMEM119- cells weren't limited to the infarct edge but also present in the distal region where microglia morphologies remained highly ramified. We illustrate spatial and sex differences in microglia phagocytosis. To distinguish highly phagocytic microglia as infiltrating cells requires more investigation.

Disclosures: K. Young: None.

Poster

127. Microglia in Disease

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 127.16/L4

Topic: B.11. Glial Mechanisms

Support: CIHR
HSFC
NSERC

Title: Suppressing interferon gamma reinvigorates microglial responses and repair in the diabetic brain

Authors: *C. E. BROWN[1], S. TAYLOR[2], E. MEHINA[4], E. R. WHITE[3], K. P. DOYLE[5], P. L. REESON[2]
[1]Island Med. Program, [3]Div. of Med. Sci., [2]Univ. of Victoria, Victoria, BC, Canada; [4]Univ. of Calgary, Calgary, AB, Canada; [5]Dept. of Immunobiology, Univ. of Arizona, Tucson, AZ

Abstract: Microcirculatory damage is a common complication for those with vascular risk factors such as diabetes. In order to resolve vascular insults, the brain's immune cells (microglia) must rapidly envelop the site of injury. Currently it is unknown whether type 1 diabetes, a condition associated with chronic immune system dysfunction, alters microglial responses to damage and what mechanisms are responsible. Using *in vivo* two-photon microscopy, we show that microglial envelopment of laser induced cerebral microbleeds is diminished in a hyperglycemic mouse model of type 1 diabetes, which could not be fully rescued with chronic insulin treatment. Microglia were important for vessel repair since reduced microglial accumulation in diabetic mice or near complete depletion in healthy controls was associated with greater secondary leakage of the damaged vessel. Broadly suppressing inflammation with dexamethasone (DEX) in diabetic mice but not healthy controls, significantly enhanced microglial responses to microbleeds and attenuated secondary vessel leakage. These enhancements were associated with changes in interferon gamma (IFNγ) signalling since DEX suppressed abnormally high levels of IFNγ protein levels in brain and blood serum of diabetic mice. Further, blocking IFNγ in diabetic mice with neutralizing antibodies normalized microglial responses and gene expression of chemotaxis-related purinoceptor P2YR12, as well as mitigated secondary leakage. These results suggest that abnormal IFNγ signalling disrupts microglial activation in the diabetic brain, and that immunotherapies targeting IFNγ can stimulate microglial repair of damaged vessels.

Disclosures: C.E. Brown: None. **S. Taylor:** None. **E. Mehina:** None. **E.R. White:** None. **K.P. Doyle:** None. **P.L. Reeson:** None.

Poster

127. Microglia in Disease

Location: SDCC Halls B-H

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Program #/Poster #: 127.17/L5

Topic: B.11. Glial Mechanisms

Support: Canadian Institute of Health Research (CIHR)
Canada Research Chair in Neuroimmune Plasticity in Health and Therapy
Fonds de recherche du Québec – Santé (FRQS)
Fondation du CHU de Québec

Title: Microglial alterations in the striatum of control compared to parkinsonian monkeys with and without L-Dopa treatment

Authors: *C. LECOURS, M.-K. ST-PIERRE, L. CANTIN, M. PARENT, T. P. DIPAOLO, M.-E. TREMBLAY
Univ. Laval, Quebec, QC, Canada

Abstract: Parkinson's disease (PD) is characterized by the progressive loss of dopamine neurons located in the *substantia nigra pars compacta*, which innervates the striatum. Consequently, this degeneration leads to a decrease of striatal dopamine content. In this study, *post-mortem* analyses of microglia were performed on female cynomolgus monkeys (*Macaca fascicularis*), under normal condition and PD pathology, modeled by systemic administration of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). A MPTP group chronically treated with the dopamine precursor, L-3,4-dihydroxyphenylalanine (L-Dopa), which developed dyskinesias, was also included. Transverse brain sections taken through the striatum were immunolabeled with an antibody directed against the microglial marker Iba1 and subsequently analyzed by light and electron microscopy. At the light microscopy level, quantitative analysis with an unbiased stereological approach reveals an increased density of Iba1-positive cells in the putamen of MPTP monkeys, compared to healthy controls, indicating microglial proliferation. Moreover, morphological analyses show increased arborization areas in MPTP-lesioned monkeys, suggesting exacerbated microglial interactions with surrounding neurons and synapses. Both the increased number and arborization of microglia were normalized following L-Dopa treatment. There were no significant changes in microglia soma size between experimental groups. At the electron microscopy level, we observed a decrease in the association of Iba1-positive microglial cell bodies with surrounding cellular elements undergoing degradation in the extracellular space in MPTP monkeys, that was normalized following L-Dopa treatment. Finally, dark microglia making extensive contacts with synapses were observed in the MPTP group. We hypothesize that these cells contribute to the remodeling of neuronal circuits that is known to occur in PD. More in-depth analyzes are underway to provide additional insights into the implication of microglia in PD neuropathological process.

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Poster

127. Microglia in Disease

Location: SDCC Halls B-H

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Topic: B.11. Glial Mechanisms

Support: H26-Nanchitou (Nan)-Ippan-07
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Title: Novel disease-modifying anti-rheumatic drug iguratimod suppresses chronic experimental autoimmune encephalomyelitis by down-regulating activation of macrophage/microglia through an NF-kB pathway

Authors: *G. LI[1], R. YAMASAKI[2], M. FANG[1], K. MASAKI[1], J.-I. KIRA[1]
[2]Neurolgoy, [1]Kyushu Univ., Fukuoka-Shi, Japan

Abstract: Objective: We aimed to elucidate the effects of iguratimod, a widely used anti-rheumatic drug with no severe side effects, on chronic experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS).
Methods: In vivo: Iguratimod was orally administered to C57BL6 mice immunised with myelin oligodendrocyte glycoprotein peptide 35-55 from the time of immunisation or from the time after the onset of EAE clinical symptoms. Clinical score and body were recorded, and pathological and flow cytometric analysis were applied at the end of experiment. In vitro: Peritoneal macrophage or microglia isolated from adult mice CNS were used for stimulation with LPS or LPS+IGU.
Results: Iguratimod was found to markedly reduce the clinical severity of acute and chronic EAE. Pathologically, iguratimod treatment significantly reduced demyelination and infiltration of CD3+ T, F4/80+, and CD169+ cells into the spinal cord, and suppressed macrophage/microglia activation in the parenchyma at the acute and chronic stages compared with vehicle treatment. Therapeutic administration of iguratimod after the onset of clinical symptoms significantly ameliorated the clinical severity of chronic EAE and reduced demyelination, T helper (Th)1/Th17 cell infiltration, macrophage/microglia activation, and nuclear factor (NF)-κB p65 and cyclooxygenase-2 expression in the spinal cord. In vitro, iguratimod treatment inhibited nuclear translocation of NF-κB p65 and down-regulated pro-inflammatory responses in macrophages and microglia..

Conclusion: Our results suggest that iguratimod ameliorates acute and chronic EAE by suppressing inflammatory cell infiltration and immune cell activation, partly through inhibition of NF-κB p65, supporting the therapeutic potential of this drug for not only acute, but also chronic MS.

Disclosures: G. Li: None. **R. Yamasaki:** None. **M. Fang:** None. **K. Masaki:** None. **J. Kira:** None.

Poster

127. Microglia in Disease

Location: SDCC Halls B-H

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Program #/Poster #: 127.19/L7

Topic: B.11. Glial Mechanisms

Support: MOST 106-2320-B-039-019

Title: The protective effects of acupuncture against the microglia activation in the brainstem induced by dental pulp injury

Authors: *S. S. BALLON ROMERO[1,2], Y.-H. CHEN[2], L. CHEN[2], S.-Y. HUNG[2]
[2]Acupuncture Dept., [1]China Med. Univ., Taichung, Taiwan

Abstract: Background: The dental pulp injury (DPI) model type III in rodents resembles irreversible pulpitis in humans; both are characterized by spontaneous pain, hyperalgesia, allodynia, inflammatory pain and necrosis that inducing microglial activation in the trigeminal subnucleus caudalis (Vc). The standard treatment for pain in irreversible pulpitis is the NSAID ibuprofen, which is associated with multiple adverse effects. Notably, acupuncture or electroacupuncture (EA) can provide faster, prolonged pain relief with fewer side effects than ibuprofen in patients with irreversible pulpitis profile. We sought to determine the effects of EA in a DPI model in mice, specifically focusing on neuroinflammation and microglial activation in the Vc.
Methods: Using a DPI type III model of irreversible pulpitis, we assessed the effects of pain via burrowing behavior and examined the analgesic effects of ibuprofen and EA. Immunohistochemistry (IHC) and Western blot protocols monitored microglial activation and cytokine expression.
Results: DPI significantly decreased burrowing activity from Day 1 to Day 7, but not on Day 14. Ibuprofen and EA at local acupoints (ST6 and ST7) reversed this effect on Days 1, 3 and 7, while EA at distal acupoints (LI4 and LI11) was effective on Days 3 and 7 only. Sham EA had no such effects. IHC results demonstrated thicker cell processes emanating from enlarged $Iba1^+$ cell bodies after DPI on Days 14, 21 and 28 compared with $Iba1^+$ cell morphology in the control group. On Day 21, EA at ST6 and ST7 as well as LI4 and LI11 acupoints appeared to be

associated with smaller cell bodies that had thinner processes compared with $Iba1^+$ cells in the sham EA and DPI-only groups. Western blot analyses revealed DPI-induced increases in Iba1 and TNF-α expression in the Vc. Ibuprofen and EA at local and distal acupoints significantly reduced these increments in $Iba1^+$ and TNF-α expression.
Conclusion: DPI is associated with microglial activation and increased TNF-α expression in the Vc, which persists following pain resolution. EA at local and distal acupoints provides pain relief and appears to alleviate DPI-induced neuroinflammation in the Vc.
Keywords: Dental Pulp Injury, Trigeminal Subnucleus Caudalis, Burrowing, Microglia, Ibuprofen, Electroacupuncture.

Disclosures: S.S. Ballon Romero: None. **Y. Chen:** None. **L. Chen:** None. **S. Hung:** None.

Poster

127. Microglia in Disease

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 127.20/L8

Topic: B.11. Glial Mechanisms

Support: NIH AG050854
NIH AG053719
NIH AG054937

Title: Roles of exosomal microRNAs derived from inflammatory macrophages in Alzheimer's disease

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Abstract: Almost all highly ranked risk factors for Alzheimer's disease (AD) are characterized by systemic inflammation. Genome-wide association studies on late-onset AD have identified a dozen genetic risk variants that are involved in immune/inflammatory responses, highlighting the importance of immune cells in the pathogenesis of late-onset AD. However, the mechanisms by which peripheral inflammation increases the risk of AD are elusive. Exosomal microRNAs (miRNAs) have important roles in cell-to-cell communication involved in numerous physiological and pathological processes such as modulation of immune cell function and metabolism, leading to acute or chronic inflammation. Exosomes carrying miRNAs, which are shed by peripheral inflammatory cells including macrophages, circulate in the blood, cross the blood-brain barrier, and release miRNAs into cells in the central nervous system (CNS). This process may cause inflammatory responses in microglia in CNS. In current study, BV2

microglial cells were treated with exosomes derived from Raw 264.7 macrophages stimulated with or without lipopolysaccharide (LPS) and, then, stimulated with LPS, 24 hours after exosome treatment. We found that exosomes derived from LPS-stimulated macrophages can induce LPS tolerance in recipient BV2 microglia. We further found that exosomes derived from LPS-stimulated macrophages contain increased levels of several miRNAs including miR-146a, which can induce LPS tolerance. Our data suggest that exosomes derived from inflammatory macrophages can modulate microglial responses to LPS in vitro. Currently, we are studying the effects of over- and under-expression of miR-146a in AD mouse models.

Disclosures: J. Yang: None. **L.M. Wise:** None. **R. Lalonde:** None. **K. Fukuchi:** None.

Poster

127. Microglia in Disease

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 127.21/L9

Topic: B.11. Glial Mechanisms

Support: NIH Grant NS088627
NIH Grant DE025689

Title: Microglial voltage-gated proton channel Hv1 contributes to secondary damage following spinal cord injury

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Abstract: Traumatic injury to the spinal cord initiates a series of destructive cellular processes that exacerbate tissue damage at and beyond the original site of injury. Microglia, as the resident macrophages of the central nervous system, are one of the first responders to spinal cord injury (SCI) and aggravate damage by involving in oxidative stress responses and pro-inflammatory cascades. Voltage-gated proton channel Hv1 is necessary for NADPH oxidase-dependent reactive oxygen species (ROS) production by microglia. However, it is unclear whether microglial Hv1 contributes to secondary damage in the context of SCI. With the aim of studying the role of Hv1 in microglia activation, ROS production, pro-inflammatory response, neuronal cell death, and demyelination, we utilized a moderate spinal cord contusive injury model with adult wild-type and Hv1-/- mice. Our results showed that loss of Hv1 reduced microglial activation following spinal cord injury. Moreover, deficiency of Hv1 alleviated oxidative stress-mediated secondary injury, as well as inflammatory response. After SCI, Hv1-/- mice exhibited

increased neuronal survival, white matter sparing, and improved locomotive recovery. Together, our results demonstrated the critical role of microglial Hv1 in the secondary damage after SCI and thus shed light on the possibility of new treatment for SCI by blocking Hv1.

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Poster

127. Microglia in Disease

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 127.22/L10

Topic: B.11. Glial Mechanisms

Support: NIH DA031777
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American Pain Society
Damon Runyon Cancer Research Foundation

Title: Divergent transcriptional responses of microglia during opioid exposure and neuropathic pain

Authors: *E. I. SYPEK[1], H. COLLINS[2], A. T. BOURDILLON[2], B. A. BARRES[2], C. J. BOHLEN[2], G. SCHERRER[2]
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Abstract: Microglia, immune cells within the central nervous system, respond to a host of insults and diseases by taking on an altered histological appearance that is used to identify their activated state. This phenomenon has been observed during both peripheral nerve injury associated chronic pain and opioid-induced hyperalgesia (OIH), suggesting that these two states of heightened pain sensitivity rely on similar microglial mechanisms. Recently, we demonstrated that mu opioid receptors (MORs) expressed by primary afferent nociceptors are required for the initiation of morphine tolerance and OIH, and that microglial activation by opioids does not require MORs (Corder et al., 2017). To characterize these pronociceptive microglial responses further, we conducted RNA sequencing on microglia acutely isolated from the spinal cord of adult mice following either chronic morphine treatment or several sciatic nerve injuries (complete transection, chronic constriction, and spared nerve injury). We found that in the setting

of injury, an early common proliferative response dominated, but later time points differed in the magnitude and the type of response. Strikingly, our analysis revealed that the most significant transcriptional changes occur prior to the full development of histological markers of activation. Most importantly, and in sharp contrast with nerve injury conditions, we observed little to no transcriptional variance following chronic morphine treatment, despite established histological changes. Along with highlighting a discrepancy between histological markers of activation and transcriptional response, our results suggest that neuropathic pain and OIH rely on distinct microglial mechanisms and caution against a universal signature of microglia activation.

Disclosures: E.I. Sypek: None. **H. Collins:** None. **A.T. Bourdillon:** None. **B.A. Barres:** None. **C.J. Bohlen:** None. **G. Scherrer:** None.

Poster

127. Microglia in Disease

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 127.23/L11

Topic: B.11. Glial Mechanisms

Support: Brain and Behavior Research Foundation
Canadian Institutes of Health Research

Title: Alterations in fractalkine signaling and microglial activity in bipolar disorder

Authors: *S. L. HILL, C. HERCHER, L. SHAO, C. L. BEASLEY
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Abstract: Bipolar disorder is a chronic psychiatric disorder characterized by periods of elevated mood and depression. Evidence from genetic studies, as well as observations of altered peripheral cytokine expression, have led to the hypothesis that inflammation or altered immune function contribute to the pathophysiology of the disorder. However, the degree to which immune activation impacts brain function in bipolar disorder remains to be established. Microglia, the brain's resident immune cells, are critical for mounting the neuro-immune response and shaping synaptic connectivity. Evidence suggests that fractalkine signaling plays a critical role in the management of microglial function, including modulation of microglial activation and regulation of synaptic pruning and plasticity. Intriguingly, we have previously identified decreased density of primed/activated microglia in postmortem brain tissue from individuals with bipolar disorder. We hypothesize that microglial changes may be regulated by alterations in fractalkine signaling. Levels of the fractalkine receptor CX3CR1 were quantified in human postmortem brain tissue from individuals with bipolar disorder (n=34), schizophrenia (n=35) and controls (n=35) by Western blotting. Correlations between CX3CR1, microglial measures, and levels of the putative microglial activity marker translocator protein (TSPO) were

examined. CX3CR1 protein levels were lower in both bipolar disorder and schizophrenia relative to controls, although only at the trend level. CX3CR1 protein levels correlated with density of non-ramified (primed and activated) and vascular microglia, but not with density of ramified (resting) microglia, suggesting that fractalkine-CX3CR1 signaling may impact microglial activation. A correlation between CX3CR1 protein expression and levels of the synaptic protein SNAP25 protein was also observed, implicating the signaling pathway as a potential means through which microglia modulate synaptic connectivity. Finally, our results are not associated with sex, age, body mass index, medication use, or serum CRP, a marker of systemic inflammation. Future studies will further investigate the role of fractalkine-CX3CR1 signaling in modulating microglial activity in bipolar disorder.

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Poster

127. Microglia in Disease

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 127.24/L12

Topic: B.11. Glial Mechanisms

Title: Glial cell morphology and delta power suppression mediate the antidepressant like effects of Interferon gamma

Authors: *K. SCHULTZE[1], B. TORRES[3], E. A. AQUINO[3], R. M. HINES[2], D. J. HINES[2]
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Abstract: Immune dysfunction has recently been highlighted as a common mechanism behind multiple neuropsychiatric disorders. Major depressive disorder (MDD), characterized by persistently low mood, is often associated with loss of interest in activities, and effects on appetite and sleep patterns. Major depressive disorder is also associated with alterations in the relationship between pro- and anti- inflammatory cytokines. In addition to their role in immune signaling, cytokines have been shown to modulate synaptic signals, physically alter circuits, and regulate behavioral outputs from the nervous system. Interferon-γ (IFN-γ) is a cytokine that has been implicated in innate and adaptive immunity, as well as regulating complex nervous system properties such as social behavior. In order to further elucidate the role of IFN-γ in the intersection between immune and nervous system signaling, it is important to identify its role in sleep and disease states, such as in major depressive disorder. Using murine models we administered INF-γ through an intracerebroventricular cannula and found glial cell morphology changes that occurred rapidly. Analysis of EEG also highlighted specific power changes at a similar time frame. EEG analysis also indicated that IFN-γ induced alterations to sleep architecture. Behavioral analysis using the forced swim task showed surprisingly rapid and long-

lasting anti-depressant-like effects. These findings suggest that INF-γ is an important regulating molecule in the symptoms associated with depressive-like behaviors. These findings could potentially lead to novel therapeutics to treat MDD in humans.

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Poster

127. Microglia in Disease

Location: SDCC Halls B-H

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Program #/Poster #: 127.25/L13

Topic: B.11. Glial Mechanisms

Support: CART Fund
NSF Grant DGE-1745038

Title: The circadian clock REV-ERB proteins regulate neuroinflammation

Authors: *P. GRIFFIN, J. DIMITRY, B. LANANNA, C. NADARAJAH, M. ROBINETTE, M. COLONNA, E. MUSIEK
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Abstract: Disturbance of circadian rhythms is a common symptom of many neurodegenerative diseases, though the mechanisms linking circadian clock dysfunction to neurodegeneration remain unknown. Deletion of the master circadian clock gene *Bmal1* causes massive gliosis and synaptic degeneration in mice, though the mechanisms mediating this process are largely unknown. Herein we provide evidence that REV-ERBα and β, orphan nuclear receptors which are directly transcriptionally regulated by the core clock and which participate in circadian clock function, are potent regulators of neuroinflammation in mice. Deletion of either REV-ERBα or β results in spontaneous hippocampal astrocyte and microglial activation, reminiscent of that seen in *Bmal1* KO mice. REV-ERBα KO mice also exhibit an exaggerated hippocampal inflammatory response when injected systemically with the inflammogen lipopolysaccharide (LPS). Simultaneous knockdown of both REV-ERBα and β also induces inflammation in in mixed glial cultures. This proinflammatory environment adversely impacted neuronal function, as REV-ERBα KO mice had diminished cortical resting-state functional connectivity. Because REV-ERBα/β are nuclear receptors, they are amenable to pharmacologic manipulation. We found that pharmacologic activation of REV-ERBs with the small molecule agonist SR9009 protected primary cortical neurons from oxidative stress in vitro, and blunted the neuroinflammatory response to LPS in the hippocampus of wild type mice, suggesting that SR9009 could modulate neuroinflammation and promote neuronal survival. Taken together, our results suggest that REV-ERBs may serves as a molecular link between the core circadian clock and neuroinflammation,

and that pharmacological targeting of REV-ERBs may represent a novel neuroprotective strategy.

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Poster

127. Microglia in Disease

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 127.26/L14

Topic: B.11. Glial Mechanisms

Support: R01NS100090
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Armbrust Endowment

Title: Mitochondrial impairment induces epigenetic histone modifications in microglia: Relevance to neuroinflammation in Parkinson's disease

Authors: *A. G. KANTHASAMY, M. HUANG, A. CHARLI, S. SARKAR, J. LUO, H. JIN, V. ANANTHARAM, A. KANTHASAMY
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Abstract: Chronic neuroinflammation mediated by resident microglia and mitochondrial dysfunction are key pathophysiological contributors to many neurodegenerative diseases, including Parkinson's disease (PD). Growing evidence also suggests that histone modifications (e.g., acetylation and methylation) and their role in epigenetic dysregulation and chromatin remodeling are beginning to emerge as key players in the pathogenesis of PD. We recently reported that impaired mitochondrial function can augment the pro-inflammatory cascade in microglial cells. However, the histone modifications central to mitochondrial defects during microglial activation are less well understood. In this study, we first performed Cell Signaling Technology's AcetylScan proteomics assay to identify key changes in histone acetylation in mitochondrially impaired N27 dopaminergic neuronal cells. Exposure of N27 cells to the classic mitochondrial complex-1 inhibitory pesticide rotenone (1 μM for 3 h) conferred an approximately 99.2-fold increase in the levels of H3K27 acetylation, indicating that mitochondrial impairment can lead to the epigenetic modification of histones in dopaminergic cells. H3K27 acetylation has been linked to active gene transcription and antagonism of methylated H3K27-associated gene repression. To further study whether mitochondrial defects induce histone modifications during microglial activation, we treated a microglial cell line with 1

μM rotenone for 3 h and probed for the levels of H3K27 acetylation by Western blotting. Similar to the response in N27 cells, H3K27 acetylation significantly increased in rotenone-treated microglia. Furthermore, using an ELISA-based histone H3 modification multiplex assay, we found that all H3K4 methylation levels (mono-, di- and tri-methylation) for this important repressive marker were altered by rotenone treatment. Interestingly, expression of the microglial histone deacetylases HDAC3 and HDAC6 were also modulated after rotenone treatment. Finally, using a Seahorse XFe24 bioanalyzer to assess the bioenergetics of mitochondria isolated from rotenone-treated brain slice cultures confirmed that acute rotenone treatment (1 μM for 6 h) inhibited mitochondrial respiration. Together, these data suggest that mitochondrial impairment in microglia induces epigenetic histone modifications, which may subsequently lead to aberrant gene expression, culminating in PD-related neuroinflammatory processes. Support: R01NS100090, R01NS088206 and R01ES027245, Eugene and Linda Lloyd Endowment and Armbrust Endowment.

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Poster

127. Microglia in Disease

Location: SDCC Halls B-H

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Program #/Poster #: 127.27/L15

Topic: B.11. Glial Mechanisms

Support: NIH NINDS Grant KNS094547A
FAER MRTG-BS
Stanford Anesthesia Grant

Title: Temporal control of microglial reactivity reveals sex-independent functional contribution of microglia to long-lasting allodynia

Authors: E. S. HAIGHT[1], T. FORMAN[1], Y. TAKEMURA[2], D. CLARK[1], *V. L. TAWFIK[3]
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Abstract: Introduction: Complex regional pain syndrome (CRPS) is a form of chronic pain affecting the limbs, often after trauma such as fracture or minor surgery. Pain is ultimately mediated by neurons, which are heavily influenced by circulating and resident innate immune cells including monocyte-lineage cells peripherally as inflammatory monocytes and centrally as microglia activated in part through TLR4. Previous work at limited time points suggested that microglia contribute to male allodynia only. We therefore sought comprehensive evaluation of

microglial involvement in CRPS progression in male and female mice to better understand whether these cells contribute to allodynia in both sexes and can be modulated to alter the post-injury pain trajectory.
Results: We used a mouse model of traumatic CRPS involving unilateral tibial fracture followed by three weeks of casting. Upon cast removal, both male and female mice developed allodynia, edema, unweighting and increased temperature of the injured hind paw. Within the spinal cord, microglial expression of CD11b and TLR4 increased as early as 3 days post-fracture in males and persisted for up to 7 weeks. In contrast, increases in microglial expression of CD11b and TLR4 were delayed until three weeks post-fracture in females, but persisted for as long as 20 weeks. In order to elucidate any time-dependent, microglia-specific contributions, we performed timed depletion of microglia using a cre-lox system. Microglial depletion in male mice at the time of cast removal caused an immediate decrease in nociceptive responses with effects outlasting the time to microglial repopulation. Microglial depletion in female mice at the time of cast removal caused a delayed and persistent reversal of nociceptive responses. We then sought to examine whether specific inhibition of microglial activation via TLR4 can produce similarly improved pain trajectories. Microglia-specific TLR4 depletion resulted in decreased allodynia and CRPS-like changes in males, albeit to a lesser degree than that seen with microglial depletion with results in female delayed and less robust.
Conclusions: These data indicate that microglia mediate nociception in both male and female mice after injury, however the involvement may occur in a delayed fashion in females and may be less dependent on TLR4. This suggests that specifically timed modulation of CNS microglia, at least in part through TLR4, can restore proper microglial function and reduce pain after injury in both males and females and that multiple mechanisms are involved in microglial activation.

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Poster

128. Neuro-Oncology

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Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 128.01/L16

Topic: B.13. Neuro-Oncology

Support: PAPIIT Grant IA201215

Title: ER beta and PKC alfa play an important role in medulloblastoma development

Authors: *R. HERNÁNDEZ, A. GONZÁLEZ-ARENAS
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Abstract: Medulloblastoma is the most common malignant pediatric brain tumor according to the World Health Organization (WHO). It is known that estrogen receptor beta (ERβ) is important for development and maturation of cerebellar granular cells, that places it as a possible factor associated to medulloblastoma biology. In addition to this, it has been reported that activation of PKCα in medulloblastoma cell lines induces an increase in cell proliferation. Furthermore, *In silico* studies have shown that PKCα is able to phosphorylate ERβ with high probability. Importantly, there are studies that indicate both proteins are expressed in all medulloblastoma subgroups.
The aim of this work was to study the interaction between ERβ and PKCα and its role in medulloblastoma development using a medulloblastoma cell line, Daoy.
To study if PKCα activation induces ERβ phosphorylation, cell cultures were treated with TPA (PKCα activator). Using inmunoprecipitation, western blot and inmunofluorescence assays, we observed a basal association between ERβ and PKCα. Also, we observed a significant increase in the ERβ phosphorylation and translocation of PKCα to the nucleus after TPA treatment. Taken together, these results suggest that PKCα plays a role in the phosphorylation and possible activation of ERβ in DAOY cell line.
To evaluate the role of ERβ and PKCα in cell proliferation, cell cultures were treated with DPN (ERβ agonist) and with TPA. Using trypan blue exclusion method and BrdU assay, we observed that ERβ activation increases proliferation after 72 hours of treatment. This effect was blocked by an ERβ antagonist. Although TPA treatment had no effect in cell proliferation, classic PKC inhibition caused a diminishment in this cellular process compared with TPA treatment at 48 and 72 hours. We found that PKCδ is abundantly expressed, which may counteract PKCα activation. These results together suggest that ERβ activation induces proliferation whereas PKCα activation is important to maintain this cellular process in DAOY cells. All experiments were repeated at least three times.
Our results suggest that PKCα plays a role in ERβ activation, which in turn contributes to maintain cellular proliferation. However it is necessary to study other PKCs roles on this cellular process.

Disclosures: R. Hernández: None. **A. González-Arenas:** None.

Poster

128. Neuro-Oncology

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 128.02/L17

Topic: B.13. Neuro-Oncology

Title: Calmodulin: Biomarker and therapeutic target to challenge drug resistance in neuroblastoma

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Abstract: The identification of new diagnostic biomarkers and therapeutic targets are important for the development of new and more efficient therapeutic strategies but also to develop new or less toxic strategies to make chemotherapy more effective. Neuroblastoma is still a major challenge, especially as high risk neuroblastoma patients' show poor survival, often due to acquired drug resistance. Calcium/calmodulin signaling is most important in neuronal processes regulating learning and memory. However, recent scientific evidence indicates that a deregulation of the calcium/calmodulin signaling also plays an important role in cancer related processes including cancer progression and its response to chemotherapy. Therefore, more scientific determination is needed to understand the physiological and molecular changes that occur upon acquired drug resistance. Here we propose that the calcium/calmodulin signaling changes in drug resistant neuroblastoma cells. These molecular changes might impact the physiology of the cancer cells. Furthermore, specific molecular markers related to chemotherapy resistance can be used in clinical applications such as in diagnostic or therapy of patients. A pharmacological approach integrating transcriptomics, epigenomics and other molecular investigations targeting physiological processes related to neuroblastoma chemotherapy resistance will identify the role of adaptive and/or toxic physiological and molecular processes that de-/regulate neuroblastoma cell survival and will open new avenues for improved diagnostics and chemotherapy for neuroblastoma.

Disclosures: D. Busselberg: None.

Poster

128. Neuro-Oncology

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 128.03/L18

Topic: B.13. Neuro-Oncology

Title: Combination strategies with epigenetic modulators in cisplatin based neuroblastoma chemotherapy

Authors: *D. BUSSELBERG[1], E. VARGHESE[1], A. M. FLOREA[2]
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Abstract: Neuroblastoma is a childhood cancer, constitutes 7% of all childhood cancers and is often difficult to treat. Cisplatin (CDDP) is commonly used as chemotherapeutic drug for NB treatment. CDDP interfere with the DNA replication machinery and modulates intracellular

calcium concentration $[Ca^{2+}]_i$.. $[Ca^{2+}]_i$ homeostasis is important in various cell physiological process like proliferation, apoptosis, cell cycle progression and others. Deregulated $[Ca^{2+}]_i$ has been described in several cancers including neuroblastoma. Epigenetic factors (DNA methylation status, histone modification and miRNA) play an important role in neoplastic development and hence modulating epigenetic changes can improve the treatment and survival. Here we target $[Ca^{2+}]_i$ signaling and b) modulate epigenetic mechanism for enhancing CDDP mediated cytotoxicity. Nevertheless, it is not understood how epigenetic mechanisms, such as DNA methylation and chromatin modification influence the effect of CDDP cytotoxicity. Additionally we use CDDP in combination with epigenetic modulators such as the DNA methylation inhibitor 5-Aza-2′-deoxycytidine (5-AZA) and histone deacetylase inhibitor Trichostatin A (TSA) for neuroblastoma (SHSY-5Y) chemotherapy. **Method**: Cytotoxicity is assessed using trypan blue dye exclusion assay and changes in $[Ca^{2+}]_i$ were recorded using fluorescence microscopy. Cells were loaded with the calcium sensitive dye Fluo-4 AM. Images were captured using BX51 Olympus microscope, with xcellence rt software. **Results**: A concentration dependent CDDP response in SH-SY5Y cell death correlated with increased $[Ca^{2+}]_i$ with maximum toxicity at 10 µM. 5-AZA and TSA induce cytotoxicity in SH-SY5Y human neuroblastoma cells as single drugs or in combination with cisplatin (CDDP). Both drugs showed a time- and concentration-dependent increase of cytotoxicity. TSA (1 µM) was more toxic than 5-Aza (50-200µM). In addition 5-AZA and TSA interfered with calcium homeostasis. TSA and 5 AZA suppressed the CDDP induced increase of $[Ca^{2+}]_i$ and increased cell death was observed in TSA and 5 AZA pretreatment cells than CDDP alone treated cells. **Conclusion**: Combination treatment with epigenetic modulators enhanced the cytotoxicity of cisplatin based chemotherapy in neuroblastoma cells.

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Poster

128. Neuro-Oncology

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Topic: B.13. Neuro-Oncology

Support: National Research Foundation of Korea (NRF), funded through the Ministry of Science, ICT South Korea (2017R1C1B2008643)

Title: RNA binding protein HuD/ELAVL4 modulates mTORC1 activity in response to stress in neuroblastoma

Authors: *K. BISHAYEE[1], K. HABIB[2], A. SADRA[2], A. SZABO[3], S. HUH[2]
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Abstract: Neuroblastoma originates from neuroblast cells (primitive nerve cells) and is the most common extracranial solid tumor of childhood with various clinical courses ranging from metastatic disease to spontaneous regression. In neuroblastoma cells, proper fine-tuning of mTORC1 activity/signaling is required for optimal cell growth and survival. Here we show that RNA binding protein HuD/ELAVL4 is overexpressed in neuroblastoma cells and its expression correlates with the aggressiveness of cancer. In its pathway, HuD/ELAVL4 stabilizes GRB-10 mRNA. Furthermore, GRB-10 deactivates mTORC1 after binding to it in a phosphorylation-dependent manner. This results in initiation of the autophagy-survival signals following various stress conditions. Silencing/knockdown of HuD/ELAVL4 under both normal and stress conditions decreases autophagy and reduces cell viability. This implies that HuD/ELAVL4 is important in preserving the growth-survival balance in the cancer cell. Activation of mTORC1 by activated Rheb (Rheb S16H) also downregulates HuD/ELAVL4 levels by inducing miR375 through mTORC1-neuroD1 signaling. Here, we propose HuD/ELAVL4 as being an important modulator of mTORC1 signaling with mTORC1 being a modulator of cell survival-autophagy and required for protecting the cancer cells under different stress conditions. As such, HuD/ELAVL4 could be a potential therapeutic target in the treatment of neuroblastoma.
General Significance
HuD/ELAVL4 is overexpressed in aggressive/stage 4S neuroblastoma. HuD/ELAVL4 produces the survival signal in these cells via downregulating the mTORC1 activity and initiating autophagy. Silencing of HuD under normal and stress conditions reduces the viability of neuroblastoma cells. Thus we propose that downmodulation of HuD/ELAVL4 could provide a potential therapeutic benefit in aggressive neuroblastoma cases.
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Poster

128. Neuro-Oncology

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 128.05/M2

Topic: B.13. Neuro-Oncology

Support: NRF Grant 2017R1A5A2015391
NRF Grant 2016R1C1B2008772

Title: Glioma stem cells are sensitive to high-dose vitamin C-driven DNA damage

Authors: *T.-J. KIM[1], J.-S. BYUN[1], H. KWON[2], D.-Y. KIM[1]
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Abstract: Increasing evidence suggests that many chronic diseases such as diabetics, atherosclerosis, cardiovascular diseases, chronic inflammation, cancer, neurodegenerative diseases, and aging are highly related to oxidative stress. As a powerful first-line antioxidant, vitamin C protects cells from oxidative damage by inhibiting production of free radicals. Interestingly, however, high-dose vitamin C often shows cytotoxicity in some contexts. Especially on cancerous cells, high levels of vitamin C shows cytotoxicity through generating excessive ROS and blocking the energy homeostasis. Although the double-sided character of vitamin C has been extensively studied in many cell types, there is little research on the consequence of vitamin C treatment in normal and cancer stem cells. Here, we identified that high-dose of vitamin C suppresses the sphere-forming ability of neural stem/progenitor cells (NSPCs) and induced expression of apoptotic genes through depletion of GSH and NAD+. Compared to differentiated cells, undifferentiated NSPCs are more sensitive to high-dose vitamin C probably due to enhanced expression of Glut1 and Glut3. Based on our result that undifferentiated cancer stem cells showed higher DNA damage signals upon high-dose vitamin C treatment than differentiated cells did, high-dose vitamin C may be used to block tumor recurrence through eradicating cancer stem cells.

Disclosures: T. Kim: None. **J. Byun:** None. **H. Kwon:** None. **D. Kim:** None.

Poster

128. Neuro-Oncology

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 128.06/M3

Topic: B.13. Neuro-Oncology

Support: NRF grant / 2017R1A5A2015391
NRF grant / 2016R1C1B2008772

Title: Oncogenic role of C17orf62 in glioma cell survival

Authors: *S. M. LEE, J. SUNG, T.-J. KIM, M. KANG, D.-Y. KIM
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Abstract: Glioblastoma multiforme (GBM) is the most common primary malignant brain tumour in adults with a median survival of approximately 15 months and less than 5% of patients alive at 5 years. Despite multimodal therapies including surgical resection, radiation, and chemotherapy treatment, malignant gliomas still account for the majority of brain cancer related

deaths. Therefore, identification and validation of molecular biomarkers of glioma is critical, and extremely required. Although a couple of molecular markers, including MGMT, 1p/19q, IDH, EGFR, p53, PI3K, Rb, and RAF, have been suggested, molecular mechanisms of those genes are not fully understood yet. Here, we demonstrate new roles of uncharacterized gene C17orf62 in glioma cell survival. We first identified that C17orf62 mRNA expression is significantly increased in glioblastoma, compared to normal brain. Gene manipulation against C17orf62 through the CRISPR/Cas9 system showed that C17orf62 silencing caused higher accumulation of p53 protein under stress condition, leading to upregulated cell death. Mechanistically, we suggest that C17orf62 induces p53 expression in two different way. One is by activating p38 MAPK-p53 axis, and the other is by elevating the level of hnRNP Q which positively regulates p53 mRNA translation.

Disclosures: S.M. Lee: None. **J. Sung:** None. **T. Kim:** None. **M. Kang:** None. **D. Kim:** None.

Poster

128. Neuro-Oncology

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 128.07/M4

Topic: B.13. Neuro-Oncology

Title: The diagnosis of neurologic immune related adverse events (nIRAEs) in patients treated with immune checkpoint inhibitors: A single institution retrospective analysis

Authors: *J. N. HOLDER[1], R. MALANI[2], A. HAGGIAGI[2], Y. SHAMES[3], C. MAHER[1], S. BRIGGS[1], M. CALLAHAN[1], B. SANTOMASSO[2]
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Abstract: BACKGROUND
Immune checkpoint inhibitors (ICIs) have transformed the management of multiple cancers. With broadening use, a greater appreciation of rare, but potentially serious side effects termed nIRAEs is coming into focus. This study seeks to review the incidence and clinical manifestations of patients (pts) who developed nIRAEs with ICIs.
METHODS
An IRB approved retrospective study was conducted to identify pts who developed nIRAEs after being treated with ICIs from 01/01/2010 - 08/31/2017. We collected the clinical, radiologic and pathologic features of pts who developed nIRAEs.
RESULTS
A total of 5369 instances of treatment with ICIs were identified in 4869 pts. Among these individuals, nIRAEs developed in 81, affecting both the central and peripheral nervous systems. The diagnosis was based on the temporal relationship between ICIs and appropriate workup which may have included electrophysiologic (EP) studies, neuroimaging and cerebrospinal fluid

(CSF) analysis. The various neurologic phenotypes observed in pts included: Sensory Neuropathy (27), Aseptic Meningitis (16), Myopathy (16), Encephalopathy (15), Guillain-Barre (GBS) like (6), Mononeuritis (4), Myasthenia Gravis (MG) (5), Paraneoplastic Syndromes (5), Retinopathy (3), Autonomic Neuropathy (2), PRES (2), Vasculitis (2), and Brachial Plexitis (1). Of the 27 pts who developed a sensory neuropathy, 18 completed additional EP studies which supported this diagnosis. All pts who developed a GBS like syndrome completed neuroimaging, CSF analysis and EP studies. Five pts. had elevated CSF protein and 4 had a lymphocytic pleocytosis. Of the 5 pts that developed MG, 4 completed EP testing as well as serum antibody analysis. Myopathy/Myositis developed in 16 pts and an elevated creatine phosphokinase was found in 8. Eleven pts had normal neuroimaging and 12 had EP studies that were abnormal. Sixteen pts developed aseptic meningitis. Twelve pts had CSF analysis, which in 9 pts demonstrated a lymphocytic pleocytosis. All 14 pts who developed encephalopathy had CSF analysis, which in 13 pts revealed a lymphocytic pleocytosis. They also had work up which included an MRI Brain as well as EEG monitoring in 11 pts. We found that 62 pts (77%) patients had improvement or resolution of their symptoms with holding ICI and/or immunosuppression.
CONCLUSION
Neurologic irAEs can manifest in a variety of ways after treatment with ICIs and may require an extensive evaluation to accurately diagnose and treat pts. While pts have a chance of developing nIRAEs, it is important to note that most events improved or resolved. Prompt identification and treatment may help with the quick resolution of symptoms.

Disclosures: J.N. Holder: None. **R. Malani:** None. **A. Haggiagi:** None. **Y. Shames:** F. Consulting Fees (e.g., advisory boards); Novartis. **C. Maher:** None. **S. Briggs:** None. **M. Callahan:** 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.; Bristol-Myers Squibb. Other; Bristol-Myers Squibb. **B. Santomasso:** 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.; Juno Therapeutics, Kite Pharma. F. Consulting Fees (e.g., advisory boards); Juno Therapeutics, Kite Pharma.

Poster

128. Neuro-Oncology

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 128.08/M5

Topic: B.13. Neuro-Oncology

Support: NIH Grant CA200624

Title: Implications of PDL1 up-regulation by MLN4924 in glioma treatment

Authors: *N. FILIPPOVA, X. YANG, Z. AN, L. PEREBOEVA, L. B. NABORS
Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: MLN4924, a pharmacological inhibitor of NEDD8 E1 activation enzyme, is currently considered as an attractive treatment for brain tumors. MLN4924 can cross the blood-brain barrier and exhibits strong effectiveness towards tumors with an overactivated protein neddylation pathway, *in vitro,* and *in vivo*. However, targeting of the *neddylation* pathway with MLN4924 treatment stabilized the hypoxia-inducible factor 1A (HIF1A), which is one of the main transcriptional enhancers of the immune checkpoint molecule PDL1 (programmed death ligand-1) in cancer cells. The engagement of MLN4924 treatment with an anti-tumor immune axis is currently unexplored. Our work seeks to evaluate an impact of MLN4924 treatment on PDL1 expression in gliomas, in vitro, and in vivo. We found that MLN4924 exhibited strong cytotoxicity towards PDGx and established glioma cell lines, *in vitro*. The IC50s were 0.3$\pm$0.2 uM (n=4), 2.7$\pm$1 uM (n=6), 3$\pm$2 uM (n=3), 3$\pm$1 uM (n=4), 2.9$\pm$0.5 uM (n=4), 0.8$\pm$0.2 uM (n =4), 0.2$\pm$0.1uM (n=4) for LN221, U251, U87, XD456, JX10, XD456-stem, X14P-stem cell lines, respectively, after treatment with MLN4924 for 5 days. However, we also confirmed a significant enhancement of HIF1A protein levels in all evaluated PDGx and established glioma cell lines after treatment with MLN4924, 1uM for 5 days. HIF1A accumulation was accompanied by a significant increase of PDL1 in mRNA and protein levels. The average enhancements of PDL1/18S mRNA ratio after MLN4924 treatment compared to untreated cells were 8$\pm$3, 25$\pm$5, 5$\pm$1, 8$\pm$3, 4.5$\pm$1 folds for U251, Ln229, U87, XD456, JX6 cell lines, respectively, based on three experiments. To check whether the HIF1A/PDL1 axis may contribute to the decrease of MLN4924 performance, *in vivo*, we evaluated MLN4924 action on PDL1 expression in immunocompetent glioma mice model. The GL261 cells have been injected intracranially into C57BL/6 immunocompetent mice, and tumors have been removed and analyzed from treated with MLN4924 and untreated (as the control) groups. Also, CSF samples have been collected by the puncture of the cisterna magna at the endpoint of the experiment, and PDL1 concentration in the samples has been determined by using ELISA PDL1 kit. We detected an enhancement of PDL1 concentration in tumor and CSF samples from mice treated with MLN4924 compared to the PDL1 values in mice from untreated group. Therefore, we predict that a blockage of PD1/PDL1 interaction during MLN4924 treatment may significantly improve the efficiency of MLN4924 therapy, *in vivo*, via reduction of immune system evasion by cancer cells and promotion of anti-tumor immunity.

Disclosures: N. Filippova: None. **X. Yang:** None. **Z. An:** None. **L. Pereboeva:** None. **L.B. Nabors:** None.

Poster

128. Neuro-Oncology

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 128.09/M6

Topic: B.13. Neuro-Oncology

Support: Northwestern Medicine Foundation Grant

Title: Preoperative venous lactate predicts metabolic alterations in a subset of gliomas - a prospective clinical, radiographic and biochemical study

Authors: *O. H. KHAN[1], J. NOLT[1], J. P. COUSINS[2], S. AGNIHOTRI[3]
[1]Neurosurg., Northwestern Med. - Chicago, Warrenville, IL; [2]Univ. of Missouri, Columbia, MO; [3]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Lactate, a by-product of glycolysis, has been well established as a marker of poor tissue perfusion. Elevated lactate production is observed in tumor glycolysis known as the Warburg effect. We have previously shown that serum lactate correlated with brain tumor grade. In this prospective study we aimed to determine if the preoperative serum lactate correlated with preoperative MR spectroscopy and in lactate levels in the fresh frozen tissue samples. Twenty-one glioma patients (13 male, 8 female) ages 34 – 86 underwent craniotomy at a single institution by lead author. Tumor pathology revealed a Glioblastoma (n=16), grade II (oligodendroglioma n=1) and Grade III Glioma (anaplastic astrocytoma n=4). Preoperative spectroscopy was performed on 18 patients. A fellowship trained neuro-radiologist (JPC) was blinded to the serum and tissue lactate levels and graded the spectroscopy lactate levels as low or elevated. There was direct correlation of spectroscopy tissue lactate levels with serum lactate levels. Pre-operative serum lactate (range 6.6- 29.9 mg/dl) was directly correlated with the fresh frozen tissue lactate levels (range 0.1 – 0.39 ug/mg; Pearson r=0.6 p = 0.0021). This study supports our theory that serum lactate correlates with spectroscopy and tissue lactate levels.

Disclosures: O.H. Khan: None. **J. Nolt:** None. **J.P. Cousins:** None. **S. Agnihotri:** None.

Poster

128. Neuro-Oncology

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 128.10/M7

Topic: B.13. Neuro-Oncology

Support: R.S. received a fellowship by CNR-IBB, Catania, Italy

Title: MT3 expression and function in glioma cells

Authors: R. SANTANGELO[1], E. RIZZARELLI[2], *A. G. COPANI[1]
[1]Univ. of Catania, Dept. Drug Sci., Catania, Italy; [2]Univ. of Catania, Dept. Chem. Sci., Catania, Italy

Abstract: Glioma, as other cancer types, have an elevated cellular zinc turnover that may be attributed to their malignancy (Takeda et al., Cancer Res. 2001). Consequently, zinc transporters are dysregulated in human glioma and, specifically, ZIP 4 is associated with higher grade of gliomas and reduced overall survival (Lin et al., Neuro-Oncology 2013).
We have investigated the repertoire of zinc transporters (ZIP and ZNT) and zinc-binding proteins (metallothioneins, MT) in human glioma cell lines of different grade, including 132N1 astrocytoma cells, A172 cells (non-metastatic grade III), U373 cells (metastatic grade III) and U87 cells (grade IV). We have profiled by real-time PCR the following gene products: ZIP1, ZIP4, Znt1, ZnT10, MT1A, MT1B, MT1E, MT1F, MT1G, MT1X, MT2A and MT3). Interestingly, these cells lines reproduce some of the expression gene profiles observed in glioma tumor samples, including a malignancy-related overexpression of ZIP4 and downregulation of ZnT10 (Lin et al., Neuro-Oncology 2013).
Metallothioneins are especially interesting because are associated with resistance to cytotoxic drugs (Maier et al., Acta Neuropathol. 1997), and have functions other than the strict regulation of intracellular zinc (Hidalgo et al., Brain Res Bull. 2001). We found that highly expressed MT3 mRNA was related to malignant cell phenotype. Intriguingly, in the highest-grade glioma U87 cells, MT3 mRNA expression was not regulated by proliferative stimuli, and it was not dependent on intracellular levels of zinc either. MT3 silencing somewhat halted U87 cell proliferation at the S phase, without affecting cell survival. The alkylating drug, temozolomide (TMZ), was used for comparison. TMZ (100 μM for 96h) induced a massive accumulation of glioma cells in the S and G2 fractions of the cell cycle, consistent with a slowing of the S phase and a G2/M arrest, but not significant death. MT3 silencing favored the occurrence of TMZ-induced apoptosis in U87 cells, and modified TMZ-induced cell cycle perturbations. Specifically, cell cycle analysis suggested that apoptotic cells were coming from the S phase. Overall, our data indicate that MT3 contributes to the proliferation status of high-grade glioma cells and their resistance to current alkylating drugs.

Disclosures: R. Santangelo: None. **E. Rizzarelli:** None. **A.G. Copani:** None.

Poster

128. Neuro-Oncology

Location: SDCC Halls B-H

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Program #/Poster #: 128.11/M8

Topic: B.13. Neuro-Oncology

Support: R01 NS088648A
Barrow Neurological Foundation

Title: Assessing acute responses to ionizing radiation of human glioma stem-like cells *in vivo*

Authors: *C. LO CASCIO[1], E. LUNA MELENDEZ[2], R. FIORELLI[1], S. V. MEHTA[1]
[2]Neurobio., [1]Barrow Neurolog. Inst., Phoenix, AZ

Abstract: Glioblastoma (GBM) is the most common and lethal primary malignant brain tumor affecting the adult population. The current standard-of-care treatment is ineffective and fails to significantly prolong survival. Moreover, these invasive tumors display extensive intratumoural heterogeneity and resistance to radio- and chemotherapy, posing a major clinical challenge due to inevitable tumor recurrence. Several studies have reported that following exposure to aggressive treatment regimens, GBMs frequently shift their biological features upon recurrence and acquire a more resistant phenotype. However, the temporal dynamics and molecular mechanisms that facilitate GBM recurrence are still poorly understood and have been studied primarily *in vitro.* Considering the unchanged dismal prognosis for GBM patients, there is a need to understand, at a systems level, how plastic processes (molecular switches) in glioma stem-like cells (GSCs) may drive tumor progression and adaptability in GBM. The objective of our study is to determine how GSCs temporally adjust their expression profile and phenotype in response to ionizing radiation *in vivo* using several patient-derived xenograft (PDX) models of GBM. The tumor-bearing mice were treated with single or multiple doses of ionizing radiation to assess acute and long-term responses to treatment respectively. Using immunohistochemical methods, we assessed changes in the expression of GBM subclass markers, stemness and differentiation markers, and DNA damage/repair proteins across the entire tumor population over time. Furthermore, to understand how the PDX tumors respond to radiation at a molecular level, we employed proteomic and genomic analyses to determine alterations in cellular signaling pathways and transcriptional programs necessary for GSC self-renewal, invasion and growth. We demonstrate that GSCs undergo a phenotypic and genotypic shift in response to radiation that results in modulation of the cellular behavior under adverse conditions. Our results suggest that this acute response allows GSCs to enter a transient state that favors GSC adaptability and resistance to therapy.

Disclosures: C. Lo Cascio: None. **E. Luna Melendez:** None. **R. Fiorelli:** None. **S.V. Mehta:** None.

Poster

128. Neuro-Oncology

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 128.12/M9

Topic: B.13. Neuro-Oncology

Title: Study on the glioblastoma cells and extracellular matrix interactions using nanofibrous scaffolds containing tissue-derived matrix

Authors: *B. LUO[1], C. JIA[2], J. WANG[2], F. PARNIAN[2], H. WANG[2]
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Abstract: Glioblastoma, also known as glioblastoma multiforme (GBM), is an aggressive type of glioma. GBMs are not developed from a single cell type and generally contain a mixture of various cell types, determining the difficulty of treatment and impossibility of curing. The best treatment scenario is to slow down its progression. So far, most of the researches on GBM focus on the cells only. However, during GBM development, cells interact with their surrounding extracellular matrix (ECM) and modify it accordingly, for example, regulation of the cystic mineral formation, calcium deposition and angiogenesis, which contribute the invasiveness of GBM. In this regard, it becomes highly desirable to unfold the essential role of ECM in glioblastoma development and identify which ECM components are more relevant. Electrospinning, a high voltage-driven spinning technology, offers potential and robust tools to dissect the complex cell-matrix interactions for its ability to generate nanofibrous scaffolds that can recapitulate the morphologic, compositional and dimensional features of native ECM. G55 cell line (Human Glioblastoma) cultured on nanofibrous scaffolds containing native ECM extract from rat brain tissue provides a suitable substrate to encode the interaction between GBM cells and ECM. After culture for 14 days, significant morphology differences were observed for G55 cells cultured on tissue-derived fibrous scaffolds and gelatin-containing fibrous scaffolds. G55 cells grown on tissue-derived nanofibrous scaffolds exhibited vigorous growth capacity and formed multilayers web-like structure. In contrast, G55 cells grown on gelatin-containing nanofibrous scaffolds did not show such cell organization. Expression for marker genes and proteins was also evaluated to confirm the primary hypothesis that GBM cells are not alone, and they interact with surrounding ECM to regulate GBM development and invasiveness.

Disclosures: B. Luo: None. **C. Jia:** None. **J. Wang:** None. **F. Parnian:** None. **H. Wang:** None.

Poster

128. Neuro-Oncology

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Program #/Poster #: 128.13/M10

Topic: B.13. Neuro-Oncology

Support: SNF P300PA_177804
SNF P2SKP3_164948

DFG GEPRIS: 267716524
DFG FOR2325

Title: PRG5 over-expression induces a pro-apoptotic phenotype with dysfunctional vasculature in a murine glioma model

Authors: *T. BROGGINI[1,2], L. STANGE[2,3], K. E. LUCIA[2], P. VAJKOCZY[2], M. CZABANKA[2]
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Abstract: PRG5, an integral membrane protein linked to neuronal plasticity, impedes NogoA- and LPA-mediated RhoA signaling (Broggini et al., 2010). RhoGTPases regulate i.a. cell motility and are instrumental in tumorigenesis, progression and adaptive response to targeted therapies. In silico analysis showed high PRG5 expression in brain tissue and variable expression in diffuse glioma, the subgroup of tumors with low PRG5 expression increases with grade. This study investigates the role of PRG5 in glioma growth, vascularisation and resistance to anti-angiogenetic therapy. Gl261 murine glioma cells transfected with PRG5 over-expression or control vector were orthotopically implanted in Bl6/J mice. After reaching a size-based threshold determined by weekly MRI scans, high-dose anti-angiogenic drug (sunitinib) or placebo was administered for 6 days (n=5 animals per group), followed by a MRI scan and histological evaluation. Tumor cells were implanted in chronic cranial window model to quantify neovasculature using intravital fluorescence microscopy. In these murine models, PRG5 harboring tumor cells grew significantly slower and were smaller than control tumors. Possibly contributing to this phenotype are the significantly increased apoptotic activity and altered micro-angioarchitecture as determined by intravital microscopy we found significantly more microvessels associated with PRG5 OE tumors (<10μm in diameter: CT 3±4%, OE 22±11%) in over-expressing tumors, vessels had a lower average diameter and the vessels were overall less likely to be perfused. qPCR analysis showed a significant transcriptional upregulation of Mmp9, Fas and Fasl and downregulation of VEGF in placebo-treated OE vs CT. Anti-angiogenic therapy, while not halting the significantly decelerated macroscopic tumor growth in PRG5 OE tumors, induced an increase in apoptotic activity and decreased vascular density in PRG5 OE and control tumors (Δ pre/post-sunitinib: CT -13%; OE -26%). In this syngeneic glioma model, tumors over-expressing PRG5 grow slower than control tumors and are highly susceptible to anti-angiogenic therapy on a microscopic level. The induction of a pro-apoptotic phenotype with insufficient vascular morphology via Mmp9, Fas and Fasl may correspond to their comparatively benign behavior.

Disclosures: T. Broggini: None. **L. Stange:** None. **K.E. Lucia:** None. **P. Vajkoczy:** None. **M. Czabanka:** None.

Poster

128. Neuro-Oncology

Location: SDCC Halls B-H

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Program #/Poster #: 128.14/M11

Topic: B.13. Neuro-Oncology

Support: Progetto IBERNAT-NBL finanziato sul POR Sardegna 2014-2020

Title: Histone deacetylase inhibitors down-regulate anaplastic lymphoma kinase (ALK) and induce apoptosis in human neuroblastoma cells

Authors: *S. DEDONI, M. C. OLIANAS, P. ONALI
Univ. of Cagliari, Dept Biomed. Sci., Cagliari, Italy

Abstract: Anaplastic lymphoma kinase (ALK) is a tyrosine kinase receptor that has been identified as one of the relevant predisposition genes in neuroblastoma. Full-length ALK point mutations in the kinase domain, most frequently occurring at positions F1174 and R1275, are associated with constitutive kinase activity and oncogenic potential. Moreover, when combined with MYCN amplification, the F1174L mutation has been found to confer higher tumor aggressiveness. Histone deacetylase inhibitors (HDACi) are key regulators of epigenetic mechanisms and are emerging as a new class of anti-cancer agents causing cell cycle arrest and death in tumoral cells. In the present study we examined the effects of different HDACi on the expression of ALK and cell survival in different neuroblastoma cell lines. We found that in human neuroblastoma SH-SY5Y cells, a cell line containing the F1174L ALK mutation, prolonged exposure (24-72hrs) to MS-275, a class I HDACi, caused a concentration dependent suppression of ALK protein levels. Exposure to MS-275 also induced a marked impairment of cell survival causing apoptosis as demonstrated by the increase of caspase activation and of poly-(ADP ribose) polymerase cleavage. Time course experiments indicated that the ALK down-regulation preceded the onset of apoptosis. MS-275 induced similar effects on ALK expression and cell survival in the neuroblastoma cell lines LAN-1 and Kelly, which contain the F1174L ALK mutation and MYCN-amplification. Other HDACi, such as romidepsin, trichostatin A, MC1568 and tubacin were also effective in inhibiting ALK expression and causing apoptotic cell death in the neuroblastoma cell lines investigated, thus supporting the involvement of an epigenetic mechanism. The present study suggests that down-regulation of ALK may constitute a critical event which contributes to the pro-apoptotic action of HDACi in neuroblastoma cells bearing or not MYCN amplification.

Disclosures: S. Dedoni: None. **M.C. Olianas:** None. **P. Onali:** None.

Poster

128. Neuro-Oncology

Location: SDCC Halls B-H

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Program #/Poster #: 128.15/M12

Topic: B.13. Neuro-Oncology

Support: Univ. of DE Dept. of Biological Sciences
Univ. of DE Undergraduate Research Program

Title: Paracrine L1CAM ectodomain modulates invasive behavior of human glioblastoma stem cells *in vivo* and stimulates glioblastoma cell motility *in vitro* when immobilized on a substratum or when presented as a soluble chemotactic gradient from nearby cells

Authors: *D. S. GALILEO[1,2], K. PLUSCH[1], A. STUBBOLO[1], C. BERNHEIMER[1]
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Abstract: Our lab has shown previously that the L1 Cell Adhesion Molecule (L1CAM; L1) increases overall glioblastoma (GBM) cell motility and proliferation through autocrine/paracrine stimulation *in vitro* and invasiveness *in vivo* via release of its soluble ectodomain (L1ecto). The objectives here were to determine whether L1 plays a similar role in GBM stem cells (GSCs) derived from surgical specimens and to test whether L1ecto influences GBM cell behavior when immobilized on a substratum or when released from nearby cells as a soluble gradient. Individual GSC lines were isolated from GBM tumors in adherent culture and expressed Sox-2 and nestin, and L1 to various degrees. Their invasive potential in our *in vivo* chick embryo brain tumor model varied by GSC line, as did their behavior when mixed with GBM cells secreting L1ecto. For instance, one GSC line labeled with GFP extended long processes into brain parenchyma, but when mixed before injection with U-118 MG GBM cells expressing L1ecto and mCherry, they formed a cortical layer in larger tumors without long processes. Even in small tumors, these GSCs were rounded without long processes when contacting, or in close proximity to, U-118/L1ecto cells. However, when injected with U-118 control cells lacking L1ecto, these GSCs occupied the interior of tumors and did not extend long processes. The ability of L1ecto to influence GBM cell motility *in vitro* when presented by different means also was tested by time-lapse microscopy and cell tracking. U-118 cells exhibited significantly stimulated velocity on immobilized stripes of L1-Fc compared to control Ig-Fc. Additionally, when U-118 control cells were plated near U-118 cells secreting L1ecto, they preferentially migrated toward the L1ecto secreting cells with increased velocity. This chemotactic migratory behavior did not occur when plated near U-118 control cells. When injected as mixtures *in vivo*, U-118/GFP control cells appeared to invade brain tissue while closely associated with U-118/L1ecto/mCherry cells but not with U-118/control/mCherry cells. These studies show that L1ecto released by nearby cells can have profound effects on human GSC invasiveness *in vivo* and that potential stimulatory

mechanisms could include not only short-range autocrine/paracrine stimulation, but also L1ecto deposited onto a substratum or longer-range effects of released L1ecto acting in a chemotactic manner. Furthermore, our chick embryo brain tumor model provides a suitable microenvironment in which human GSCs can exhibit their individual inherent cell behaviors as well as the influence on GSCs by GBM tumor non-stem cells.

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Poster

128. Neuro-Oncology

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 128.16/M13

Topic: B.13. Neuro-Oncology

Title: Non-invasive focused ultrasound-mediated delivery of oncolytic viruses to the mammalian brain

Authors: *M. A. STAVARACHE[1], A. ZANELLO[2], E. JURGENS[2], M. YUAN[2], J. MARKERT[3], M. G. KAPLITT[4]
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Abstract: The standard treatment of malignant gliomas (GBM), the most common subtype of primary brain tumors, consists of surgery, followed by radiotherapy and chemotherapy. However, the GBM's highly invasive, infiltrative nature, relative resistance to radiation and chemotherapy over time, and physiological isolation due to the blood-brain-barrier (BBB), contribute to a poor prognosis. The limited permeability of BBB also threatens to compromise the oncolytic virus therapy, a major breakthrough in the treatment of GBM, based on the properties of genetically engineered viruses to selectively replicate and kill cancer cells without harming normal tissue. To overcome this challenge, we decided to use magnetic resonance-guided focused ultrasound (MRgFUS), a novel technique that temporarily and safely disrupts the BBB integrity, to deliver Oncolytic Herpes Simplex Virus-1 (oHSV-1) to specific areas in the brain. The challenges of this study consisted in the large size of the oSHV-1 (150nm), and potential adverse effects related to the intravenous administration of the virus. Sprague-Dawley rats (200-250gr) underwent unilateral sonication at the level of striatum under MRI control simultaneously with intravenous administration of an dsRed tagged-oHSV-1 and albumin-coated gas-filled microbubbles cocktail. The virus was administered at three different titers, 5*10^8, 10^8 and 5*10^7 pfu. Intravenously administered Gd-DTPA contrast agent confirmed the BBB disruption in T1-weighed images collected post-sonication. The animals were sacrificed at 2 and 8 days post-sonication, and the brain, liver, heart, lungs and gonads were processed for

histological analysis. Immunostaining of dsRed, our gene reporter, showed a strong expression in the brain strictly limited to the sonicated striatum and the cortex on the trajectory of sonication. The extent of expression was dependent on the amount of virus administered and the time period from the sonication to the tissue harvest. In addition, immunostaining for neuronal marker NeuN showed no neuronal death in the sonicated area, while GFAP (astrocytic marker) and Iba-1 (microglial marker) staining revealed a low local inflammatory response. In the rest of the analyzed organs, dsRed immunostained positive only in the heart and gonads. Regular gross observation revealed no abnormal behavior in the days post-sonication. These results suggest that MRgFUS can be successfully used for non-invasive delivery of larger size viruses such as oHSV-1 to areas that have limited access due to the restricted permeability of BBB, potentially increasing the efficiency of oncolytic virus therapy post-surgery in GBM with poor prognosis.

Disclosures: M.A. Stavarache: None. **A. Zanello:** None. **E. Jurgens:** None. **M. Yuan:** None. **J. Markert:** None. **M.G. Kaplitt:** None.

Poster

128. Neuro-Oncology

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

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Topic: B.13. Neuro-Oncology

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Title: Oncogenic state and cell identity jointly dictate the susceptibility of a glioma incipient cell towards IGF1R targeting

Authors: *A. TIAN, R. LIU, C. LIU
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Abstract: Glioblastoma is the most aggressive cancer of the central nervous system in adults. Despite many years of efforts, little progress has been made in GBM treatment, highlighting the urgent needs of highly effective therapeutic targets for this devastating disease. An ideal target-directed therapy should selectively impair cancerous cells but leave their normal cell-of-origin intact. Due to the limited knowledge of the cellular properties of tumor cells in their native environment and the elusive identity of the cancer cell-of-origin, however, the existence of such target has not been rigorously validated in glioblastoma. Here, by using high-resolution genetic

mouse models with oligodendrocyte precursor cells (OPCs) as the defined cell-of-origin, we show that Insulin-like Growth Factor-I Receptor (IGF1R), despite being broadly expressed, emerges as a highly efficacious and selective therapeutic target for glioblastoma. Deactivating IGF1R selectively disrupts the self-renewal of cancerous but not normal OPCs, likely through controlling cell homeostasis and competition. Moreover, fate-mapping experiments reveal that the desirable outcome of IGF1R targeting requires the incipient cell to commit to the OPC identity regardless of its hierarchical status during tumorigenesis. We further identified a novel OPC-like tumor cell subpopulation in all human glioblastomas that absolutely requires IGF1R for its self-renewal, therefore is highly susceptible to IGF1R-directed targeting. Finally, we developed a next-generation brain-penetrable IGF1R inhibitor that may have immediate clinical impacts on glioblastoma therapy. Our findings elucidate the action mode of IGF1R-directed targeting in gliomagenesis, and provide a referential example for targeted therapy paradigm that may also apply to other cancers.

Disclosures: A. Tian: None. **R. Liu:** None. **C. Liu:** None.

Poster

128. Neuro-Oncology

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Program #/Poster #: 128.18/M15

Topic: B.13. Neuro-Oncology

Support: CSIR, Govt.of India
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Title: Recapitulating developmental cues of neural stem cells into cancer stem cell maintenance: Is pleiotropic Hes-1 responsible?

Authors: *R. A. PAUL[1], S. PARVATHY[1], L. SOUNDARARAJAN[1], T. THOMAS MALIEKAL[2], S. NELSON SATI[3], J. JAMES[1]
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Abstract: In developing neocortex, we have identified that Notch Independent Hes-1 (NIHes-1) expression maintains neural stem cells that later attain Notch Dependency (NDHes-1) while progressing to a progenitor and then to a differentiated state that is unidirectional. These developmental cues prompted us to ask a similar question in the scenario of neuroblastoma where we hypothesize that NIHes-1 expression could maintain cancer stem-like cells and their transition to NDHes-1 expressing state which in turn would maintain the bulk of tumor cells. Assuming if this scenario is true, then targeting canonical Notch pathway will only eliminate the

bulk tumour cells and will retain the cancer stem cells. To understand whether such a scenario exists in neuroblastoma, we transfected IMR32 cells with a reporter system that would report NIHes-1 expressing cancer stem-like cells as green and the bulk NDHes-1 expressing tumor cells as red. Our results showed that consistent expression of NIHes-1 maintains cancer stem-like cells and a shift in mode of Hes-1 expression (NDHes-1) transforms the cancer stem-like cells into cancer cells that form the bulk of the tumour. We further FACS sorted the two populations and analyzed their transition potential which showed a unidirectional transition from NIHes-1 expression to a NDHes-1 expressing state recapitulating the developmental scenario. Characterization of NIHes-1 expressing cancer stem-like cells showed a mesenchymal morphology with high migratory potential and slow proliferation rate and undergo mesenchymal to epithelial transition (MET) along with transition to NDHes-1 expressing state. We also carried out transcriptome analysis in FACS sorted NIHes-1 and NDHes-1 expressing IMR32 cells which revealed very interesting signaling pathways involved in MET and maintenance of cancer stem cells. Further, we generated xenograft tumors in SCID mice with different ratios of NIHes-1/NDHes-1 expressing IMR32 cells which confirmed the stem cell characteristics and tumour forming potential of NIHes-1 expressing cells. Overall, our novel findings show that cancer stem-like cells with NIHes-1 expression transit into cancer cells with NDHes-1 expression recapitulating the developmental process and also emphasizing the need to target NIHes-1 activating pathways to eliminate cancer stem-like cells in neural origin tumors.

Disclosures: R.A. Paul: None. **S. Parvathy:** None. **L. Soundararajan:** None. **T. Thomas Maliekal:** None. **S. Nelson Sati:** None. **J. James:** None.

Poster

128. Neuro-Oncology

Location: SDCC Halls B-H

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Program #/Poster #: 128.19/M16

Topic: B.13. Neuro-Oncology

Support: Conacyt Fronteras 2015-2 1256

Title: Human derived glioblastoma cell line expresses three key enzymes involved in sex steroid synthesis

Authors: J. A. MONDRAGON[1], Y. SERRANO[1], A. TORRES[1], M. OROZCO[1], J. A. HERNANDEZ[1], G. MANJARREZ[3], *J. V. SEGOVIA-VILA[1], M. C. ROMANO[2]
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Abstract: Glioblastoma multiforme (GBM) is the most frequent and aggressive primary brain tumor in human adults. Therapeutic resistance and tumor recurrence after surgical removal

results in poor prognosis. Active metabolites of steroid hormones are locally synthesized in the nervous system and play important roles in neural development and function. The expression of aromatase, the enzyme that converts androgens to estrogens, had been reported in glial cells and GBM cell lines, therefore sex steroids may have a role in tumor pathogenesis. Thus, it is important to have a deeper insight of the tumor steroidogenic capacity. We decided to investigate the expression of three key enzymes involved in androgen synthesis in the U87 human derived GBM cell line. Cells were cultured in DMEM plus FBS and antibiotics on slides for immunocytochemistry or immunonofluorescence, or in multiwells to obtain proteins for western analysis. Primary antibodies against: 3β-hydroxisteroid dehydrogenase (3β-HSD), 17α-hydroxilase/17,20-lyase (P450c17) and 17β-hydroxysteroid dehydrogenase (17β-HSD) were used .We found that the three steroidogenic enzymes are present in the cytoplasm of the U87 cells. Western blot analysis showed bands corresponding to the MW of the three enzymes. The present results indicate that U87 cells contain the key enzymatic machinery necessary to synthesize androgens that besides being estrogen precursors, may have a role in the development and function of GBM.
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Poster

128. Neuro-Oncology

Location: SDCC Halls B-H

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Program #/Poster #: 128.20/M17

Topic: B.13. Neuro-Oncology

Title: Screening targeting agents and their cell surface biomarkers for high specificity and rapid internalization via cell death and fluorescence

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Abstract: Some of the most recent successes in the treatment of cancers or research into passive immunotherapies for neurodegenerative diseases, employ the use of antibodies. These treatments utilize antibodies that either: 1) interfere with cell surface proteins responsible for tumor cell proliferation, 2) act as immune checkpoint inhibitors, or 3) are re-engineered to allow transport of other molecules across the blood-brain barrier (BBB). There are a growing number of antibody and small molecule therapeutic candidates and this demands a quick and efficient technique to screen for biomarkers that internalize effectively upon binding. The method

described provides for the efficient determination of internalization of cell surface biomarkers upon binding of antibodies or peptides. This one-step, robust method uses a targeting agent combined with both a fluorescent reporter and a cytotoxic payload. The construct that makes this method effective was formed by cross-linking a fluorescent reporter, in this case fluorescein (FITC) and streptavidin to the ribosome-inactivating protein, Saporin. The conjugate used in screening potential therapeutics is a mixture of a biotinylated targeting agent mixed in a 1:1 molar ratio with FITC-labeled Streptavidinylated-Saporin. The method provides a definitive assay readout: fluorescence within 1 hour and cell death in 72 hours. This method is designed for rapid screening, in a quick and reproducible manner, for specificity and internalization in various cell types to explore suitability of candidates as therapeutics.

Disclosures: L. Ancheta: A. Employment/Salary (full or part-time):; Advanced Targeting Systems. **R. Bouajram:** A. Employment/Salary (full or part-time):; Advanced Targeting Systems. **D.A. Lappi:** F. Consulting Fees (e.g., advisory boards); Advanced Targeting Systems.

Poster

128. Neuro-Oncology

Location: SDCC Halls B-H

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Program #/Poster #: 128.21/M18

Topic: B.13. Neuro-Oncology

Support: Conacyt 239516

Title: Soluble GAS1 reduces perineural invasion of pancreatic cancer cells

Authors: L. DANIEL-GARCIA[1], L. SANCHEZ-HERNANDEZ[2], P. VERGARA[1], *R. O. GONZALEZ[3], J. V. SEGOVIA-VILA[1]
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Abstract: Perineural invasion (PNI) is the process by which cancer cells invade the perineural spaces of the surrounding nerves, and frequently occurs in pancreatic cancer. The pancreas is innervated by the celiac plexus, surrounding the superior mesenteric artery. The plexus derived from the celiac ganglion is considered to be the major route of invasion of pancreatic cancer. PNI is a targeted process involving many signaling molecules; these signaling molecules are produced by both cancer cells and nerves. We previously demonstrated the capacity of GAS1 (Growth Arrest Specific 1) to inhibit the growth of gliomas by blocking the GDNF-RET signaling pathway. We decided to determine the effect of soluble forms GAS1 and PTEN (tGAS1 and PTEN-L) on cell proliferation and induction of cell cycle arrest in the Hpaf-II pancreatic cancer line. We transfected the tgas1/pten-L plasmid and evaluated cell viability of

the Hpaf-II pancreatic cancer line. We observed a decrease in the number of viable cells treated with tgas1/pten-L, these results indicate that tGas/Pten induces cell death in the Hpaf-II line, in addition the Hpaf-II line was characterized and the expression of Gfra3 and artemin was determined. On the other hand, we evaluated whether tGas1 is able to inhibit cancer cell perineural invasion. Cancer cell invasion was studied using HpafII cells on Boyden chambers. We used primary culture of dorsal root ganglia (DRG) from newborn male CD1 mice. Culture inserts were pre-coated with Matrigel then seeded with HpafII cells (100,000 per insert) and placed into wells containing the medium with soluble tGas1. Cells were counted after 48h of incubation. After cleaning and briefly staining inserts with Coomassie blue, invasion was assessed by counting the number of stained cells in 8-10 fields. These data suggest that tGas1 is able to induce cell death and decrease the cellular perineural invasion thus potentially inhibiting metastasis processes.

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Poster

128. Neuro-Oncology

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 128.22/N1

Topic: B.13. Neuro-Oncology

Title: Interaction of elk-1 and mitotic kinases in brain tumor proliferation

Authors: *O. ARI UYAR[1], B. YILMAZ[2], I. AKSAN KURNAZ[3]
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Abstract: Brain tumors can grow rapidly, crowding or destroying nearby brain tissue and they exhibit resistance to therapy. Each year thousands of people are diagnosed with a primary malignant brain tumor worldwide and the most prevalent and deadliest types of these tumors are glioblastoma in adults and neuroblastoma, the most common solid tumor in children. Therefore, elucidating molecular mechanisms of mitotic proliferation in brain tumors will be an important step towards development of new therapies. Ets-like transcription factor 1 (Elk-1) has been implicated in protecting cells from apoptosis and downregulating apoptosis-associated genes thereby mediating cell survival, and Akt-dependent phosphorylation of Elk-1 has been shown to be important for proliferation of glioblastoma cells. In addition to this, through various projects in our laboratory, we have identified a different mitotic role of the mitogenic transcription factor Elk-1 belonging to the ETS domain superfamily in various brain tumor model cell lines such as

glioma, glioblastoma and neuroblastoma. When Elk-1 protein sequence was investigated for potential kinase phosphorylation motifs, several residues were found to be putative sites for phosphorylation by cell cycle–related kinases, such as Cdks, Plks and Aurora kinases. To investigate possible role Elk-1 transcription factor on the proliferation of brain tumors, phospho-specific antibodies have been developed and these sites analyzed by Western blot, immunofluorescence, flow cytometry and XTT cell viability assay. Our results demonstrated that Elk1 is phosphorylated from different sites during mitosis and these phosphorylations affected cell viability of SH-SY5Y (human neuroblastoma) and U-87 (human glioblastoma) cell lines. In the light of our data, it can be concluded that aberrant phosphorylations of Elk1 by main regulatory kinases of cell cycle may give an advantage to brain tumor cells to proliferate and inhibition of these phosphorylations may affect viability of brain tumor cells.

Disclosures: O. Ari Uyar: None. **B. Yilmaz:** None. **I. Aksan Kurnaz:** None.

Poster

128. Neuro-Oncology

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 128.23/N2

Topic: B.13. Neuro-Oncology

Title: Development of a novel glioblastoma model to study the role of NOX4 in disease pathogenesis

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Abstract: Glioblastoma multiforme (GBM), a cancer of the glial cells in the brain, is the most common and aggressive primary brain tumor and even with aggressive and invasive treatment only has a median survival of about a year. The reactive oxygen species producing NADPH Oxidase 4 (NOX4) has been shown to be significantly increased in GBM and patients with high NOX4 expression have shown reduced progression-free survival. However, the molecular mechanism underlying the functional role of NOX4 in this disease is largely unknown. To study this, we have developed a novel *in vivo* model of GBM in WT and GFAP-specific NOX4 KO background by injecting a single *Cre*-inducible lentiviral vector targeting multiple pathways reported in human GBM (classical subtype). This approach allows us to faithfully model the complex contextual signaling events that drive the human pathology, providing a clear picture of relevant pathways and to generate *in vivo* and *in vitro* models that phenocopy the human disease. Our preliminary data indicates that NOX4 blockade prevents glioma stem cell (GSC) differentiation, reduces proliferation, induces cell death and slows disease progression, in part through the PI(3)K/Akt signaling pathway. Elucidation of the NOX4 pathway in GBM will

improve our understanding of the cellular signaling mechanisms driving this disease and may offer new therapeutic avenues.

Disclosures: L. Adams: None. **Y. Kim:** None.

Poster

128. Neuro-Oncology

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Topic: B.13. Neuro-Oncology

Support: COMECYT-COE/UEI/PG/14/2017

Title: Different patterns of ALDH1A1 expression in brain tumor

Authors: *L. SANCHEZ, R. SAAVEDRA, E. GÓMEZ, D. TORRES, D. AGUIRRE, V. FABELA
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Abstract: Brain cancer accounts for approximately 18% of all cancers and 34% of all cancer-related deaths according to the figures reported by GLOBOCAN. The incidence of primary brain neoplasms varies between 4 and 10 per 100,000 in the general population. This incidence tends to increase with age (4 / 100,000 up to the age of 12, 6 / 100,000 up to the age of 35, 18 / 100,000 up to the age of 55, 70 / 100,000 up to the age of 75). The statistical data in Mexico reported by GLOBOCAN in 2012, being the most up-to-date, indicates that cancers of the central nervous system occupy the 9th place in incidence in men; however, the mortality glioblastoma alone is 3.8% with respect to the total of all cancers, and of 50.12% with respect to cancers of the Central Nervous System. Cytosolic aldehyde dehydrogenase (ALDH1A1) contributes mainly to the biosynthesis of retinoic acid (RA) from vitamin A. Retinoic acid participates in various antiapoptotic processes, cell proliferation and tumor growth. This gives great potential to the elucidation of the expression profile of ALDH1A1 in conditions such as Glioblastoma multiforme. In patients with Glioblastoma multiforme of the Centro Oncológico Estatal ISSEMyM, different patterns of ALDH1A1 expression have been found, both at the protein level and at the messenger RNA level, and it is seen that the higher the expression of ALDH1A1, the shorter the patient's overall survival. Novel identification of early-detection molecular biomarkers, such as ALDH1A1 will facilitate a more timely diagnosis of patients with glioblastoma multiforme, leading to a more opportune treatment of the patient. Additional results obtained from biopsies of patients with glioblastoma, obtained by mass spectrometry analysis of protein lysates in this kind of tumors give promise to the use of ALDH1A1 as such a marker.

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Poster

128. Neuro-Oncology

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Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 128.25/N4

Topic: B.13. Neuro-Oncology

Support: Gluck Fund

Title: The development of a porcine model of spinal cord glioma

Authors: *M. S. TORA[1], N. HARDCASTLE[2], P. HANNIKAINEN[3], Y. KIM[3], T. FEDERICI[3], P. D. CANOLL[4], N. M. BOULIS[2]
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Abstract: Spinal Cord Gliomas (SCG) account for 8-10% of all primary spinal cord tumors, with high grade lesions resulting in significant morbidity and mortality, with no current consensus standard of care. Consequently, clinical outcomes are poor with 5-year-survival of 11-23%. This has prompted significant pre-clinical research in classical models (e.g. orthotopic xenografts, syngeneic models), resulting in therapies that have unfortunately failed in phase I, II, and III clinical trials - with over 1100 to date. While there are several plausible reasons for these failures, it is likely secondary to weaknesses in the classic animal models. Classical models are powerful tools for proof of concept studies, but do not adequately recapitulate the features of human tumors needed for pre-clinical testing. The differences in human and classical disease models are thoroughly documented and include entirely different tumor morphology, lack of infiltration, high immunogenicity, and a wealth of genetic and molecular differences. These differences directly impact the assessment of therapeutic efficacy, yield false positive results, and thus contribute to translational failure. Thus, there is a critical need for model systems that can serve as valid platforms for therapeutic and diagnostic testing beyond classical models. We propose the use of the gottingen minipig as it is the pre-clinical large animal surgical model of choice because of its genetic, immunologic, and anatomic similarity to humans as extensively reported elsewhere. Here we report preliminary data (H&E, IHC, IF), on the development of a porcine model of spinal cord glioma, following orthotopic injection of retroviral PDGF-IRES-DsRED as a driver of gliomagenesis.

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Poster

129. Brain Wellness and Aging: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 129.01/N5

Topic: C.01. Brain Wellness and Aging

Support: NIH 2R15GM110651-02

Title: A novel resveratrol analog protects synaptic transmission from acute oxidative stress at the *Drosophila* neuromuscular junction

Authors: *N. SIAL[1], W. L. BOLLINGER[1], E. J. ST.GERMAIN[2], S. L. MAKI[2], S. D. LEPORE[2], K. DAWSON-SCULLY[1]
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Abstract: Resveratrol, a planar stilbene compound, protects animals from the deleterious effects of oxidative stress, cancer, aging, and a high fat diet. Several groups have developed resveratrol analogs that exhibit therapeutic effects against cancer; however, these analogs have not emerged as successful medicinal agents in part due to their planar structures. In this study, we have designed a compound, fly2, whose structure possesses much of the functional group characteristics of resveratrol but in a non-planar molecular arrangement. Importantly, this novel compound was found to protect neurotransmission, an essential cellular process, from acute hydrogen peroxide (H_2O_2) induced oxidative stress. Our findings indicate that fly2 protects synaptic transmission from acute oxidative stress at the *Drosophila* neuromuscular junction (NMJ). Perhaps as validation of our non-planar design, fly2 and related analogs have emerged as more potent neuroprotectants than resveratrol. Further electrophysiological studies reveal that the acetyl groups on the structure of fly2 were found to be essential for its neuroprotective effects. These results suggest that fly2 may be a good starting point in the design of selective and potent neuroprotective agents.

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Poster

129. Brain Wellness and Aging: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 129.02/N6

Topic: C.01. Brain Wellness and Aging

Support: SERB-DST, Govt of India (EMR/2016/006470)

Title: Neuroprotective effects of fisetin as a caloric restriction mimetic: Therapeutic implications during aging through the modulation of autophagy, apoptosis and neurodegeneration

Authors: *S. SINGH[1,2], G. GARG[2], A. K. SINGH[2], S. I. RIZVI[2]
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Abstract: Although caloric restriction (CR) is an effective anti-aging strategy, it is difficult to implement in our lives due to several limitations such as rationale and regimen of its exact length of restriction and associated malnutrition are also not clearly defined. To address the issue, gerontologists are attempting to develop caloric restriction mimetics (CRMs) that exert similar beneficial effects of CR without causing malnutrition. In the present study, attempts have been made to evaluate the potential role of fisetin as a possible CRM for neuroprotection during aging in D-galactose (D-gal) induced and natural aging models of rat. We perceived that administration of fisetin, a powerful antioxidant can exert neuroprotective effect by inhibiting damage caused by the exposure to D-gal. Young Wistar rats, treated with D-gal (500 mg/kg b.w, subcutaneous) to induce aging and simultaneously with fisetin (15 mg/kg b.w., orally) for 45 days. In addition, young control (4 months old) and naturally aged control rats (24 months old) were also supplemented with fisetin. Our data demonstrated that fisetin significantly decreased the level of pro-oxidants and increased the level of antioxidants to maintain the redox status. Furthermore, fisetin also ameliorated D-gal and natural aging-induced mitochondrial membrane depolarization, apoptotic cell death and impairments in the activities of synaptosomal membrane-bound ion transporters in the rat brain. RT-PCR data revealed that the administration of fisetin significantly up-regulated the expression of autophagy genes (ATG-3 and Beclin-1), sirtuin-1 and neuronal markers (NSE and NGB), and down-regulated the expression of inflammatory (IL-1β and TNF-α) and aging marker (Sirt-2) genes respectively in aging brain. Thus, our study suggested that fisetin supplementation provided possible protection against aging-induced oxidative stress, apoptotic cell death, neuro-inflammation, and neurodegeneration in aging rat brain. The use of fisetin as dietary polyphenol could be a beneficial, effective and safe neuroprotective agent to slow down brain aging.

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Poster

129. Brain Wellness and Aging: Metabolism, Oxidative Stress, and Cellular Mechanisms

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Topic: C.01. Brain Wellness and Aging

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Title: Ketogenic diet modulates NAD^+-dependent enzymes and reduces DNA damage in the hippocampus

Authors: *P. SACCHETTI[1], M. ELAMIN[1], D. N. RUSKIN[2], S. A. MASINO[2]
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Abstract: Cures for neurological conditions remain a major unmet need; effective treatments that reverse dysfunction or prevent disease progression have not been identified. Recently, there has been growing interest in metabolism-based therapies as potential new approaches in disorders as diverse as cancer, Alzheimer's disease, pain, and autism spectrum disorder. The ketogenic diet, a metabolic therapy that is high in fat and low in carbohydrates, has been long prescribed to treat refractory epilepsy; its potential as a metabolic treatment of neurodegenerative disorders has been explored more recently. We have determined previously that a ketogenic diet consumed by healthy rats increased rapidly the hippocampal levels of nicotinamide adenine dinucleotide (NAD^+), a fundamental cellular coenzyme associated with beneficial effects such as decreased inflammation, metabolic resilience, and anti-aging. In the current study, we aimed at quantifying changes in downstream molecular mechanisms activated by the ketogenic diet-induced NAD^+ level increases. Here, rats were fed ad libitum regular chow or ketogenic diet for two days or three weeks and the levels of hippocampal sirtuins and PARP-1 enzymes, and the oxidative DNA damage marker 8-hydroxy-2`-deoxyguanosine (8-OHdG) were quantified. As previously reported, ketosis and increased levels of NAD^+ were rapidly induced by a ketogenic diet-based regimen. In addition, sirtuins and PARP-1 enzymes' levels and activities were altered within two days of treatment. Similarly, quantification of hippocampal 8-OhdG showed a decrease after two days of ketogenic diet and a further reduction after three weeks treatment. Our current experiments show that the beneficial NAD^+ changes induced by the ketogenic diet are correlated with activation of key anti-aging downstream effectors and an improvement in DNA integrity in the brain. As NAD^+ levels are used as an indicator of cell health and anti-aging, these results suggest that NAD^+-dependent processes mobilized by a ketogenic diet may be a fundamental mechanism that addresses a variety of health conditions and increases longevity and health span.

Disclosures: P. Sacchetti: None. **M. Elamin:** None. **D.N. Ruskin:** None. **S.A. Masino:** None.

Poster

129. Brain Wellness and Aging: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 129.04/N8

Topic: C.01. Brain Wellness and Aging

Support: We acknowledge financial support from the King Abdullah University of Science and Technology (KAUST) baseline funding from Pierre Magistretti

Title: Forever young - Lactate and pyruvate delay aging related phenotypes in C. elegans

Authors: *A. TAUFFENBERGER, L. MOTTIER, H. FIUMELLI, P. J. MAGISTRETTI
King Abdullah Univ. of Sci. and Technol., Thuwal, Saudi Arabia

Abstract: Aging increases the risk of neurological disorders that can be observed from middle to late adulthood. A common feature of aging and neurological disorders is reduction in energy metabolism efficiency, especially when it comes to the maintenance of intracellular homeostasis, crucial for neurons to maintain intense activity or in the context of diseases. Neuron-astrocyte coupling plays an important role in promoting neuronal plasticity and survival. Released by astrocytes during synaptic activity, L-lactate, has also an important signaling function for neuronal plasticity and protection (Magistretti and Allaman, Nature Reviews Neuroscience, 2018). In our lab, we observed that treatment with L-lactate protects SH-SY5Y cells against oxidative stress (H_2O_2). Using RNAseq, we identified pathways differentially expressed upon L-lactate treatment, such as chaperones involved in Unfolded Protein Response in the ER (UPR^{ER}). We used *Cænorhabditis elegans* to further investigate the role of lactate *in vivo* by supplementing the nematode's diet with L-lactate and pyruvate. We assessed the role of both metabolites in oxidative stress resistance, aging and neuronal protection, using a model of polyglutamine disease (Q67::CFP). L-lactate and pyruvate treatments resulted in increased lifespan and reduced sensitivity to oxidative stress as well as in a delay in neuronal dysfunction. Using mutant lines we established that the protective effect of L-lactate and pyruvate is mediated by detoxifying enzymes involved in oxidative stress and UPR^{ER}. These results suggest that both metabolites slow down aging and protect against cellular stress through a hormetic effect and stimulate cellular activity in both mammalian and nematode biological systems

Disclosures: L. Mottier: None. **H. Fiumelli:** None. **P.J. Magistretti:** None.

Poster

129. Brain Wellness and Aging: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 129.05/N9

Topic: C.01. Brain Wellness and Aging

Support: SERB-DST, Govt of India (EMR/2016/006470)

Title: Synergistic effect of metformin and L-cysteine against aging induced neurodegeneration and oxidative damage in rat brain

Authors: *G. GARG, S. SINGH, A. K. SINGH, S. I. RIZVI
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Abstract: Brain undergoes ultrastructural modifications, biochemical deficits and inflammatory changes during aging, linked to oxidative stress. Metformin a well-known antidiabetic drug has been recently proposed to protect against neurodegeneration. L-cysteine act as free radical scavenger by promoting the synthesis of reduced glutathione. The study is undertaken to examine whether the combination of metformin and L-cysteine would provide benefits in reducing oxidative stress, neurodegeneration and inflammation in rat brain during aging. Male Wistar rats of age 4 (young) and 24 months (old) were co-exposed to metformin (300 mg/kg b.w.) and L-cysteine (300 mg/kg b.w.), and data were compared to the response of rats receiving an independent exposure to these chemicals at similar doses. The exposure of individual supplementation of drugs significantly reversed the age-dependent alterations in the endpoints associated with oxidative stress such as reactive oxygen species (ROS), ferric reducing ability of plasma (FRAP), lipid hydroperoxides (LHPs), reduced glutathione (GSH), protein oxidation products and acetyl cholinesterase (AChE) brain tissue of aging rats. However, the co-treatment of metformin and L-cysteine showed a significant augmented effect as compared to the individual drug interventions on the reversal of these age-dependent biomarkers of oxidative stress, suggesting a synergistic response. Furthermore combination of metformin and L-cysteine downregulated the age dependent induced expression of inflammatory markers such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) whereas upregulated the expression of marker genes associated with neurodegeneration such as neuroglobin (NGB), neuron specific enolase (NSE) and synapsin-1 (Syn-1). Thus, the findings open up further possibilities for the design of new combinatorial therapies to revert oxidative stress and age-associated health problems.

Disclosures: G. Garg: None. **S. Singh:** None. **A.K. Singh:** None. **S.I. Rizvi:** None.

Poster

129. Brain Wellness and Aging: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 129.06/N10

Topic: C.01. Brain Wellness and Aging

Support: JP15H05015
JP16H01340
JP17K17093

Title: Increased expression and altered subcellular distribution of cathepsin B in microglia induces cognitive impairment through oxidative stress and inflammatory response in mice

Authors: *N. JUNJUN[1], Z. WU[1], V. STOKA[2], Y. HAYASHI[1], C. PETERS[3], H. QING[4], V. TURK[2], N. HIROSHI[1]
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Abstract: During normal aging, innate immunity progresses to a chronic state. However, how oxidative stress and chronic neuroinflammation arise during aging remains unclear. In this study, we found that genetic ablation of cathepsin B (CatB) in mice significantly reduced the generation of reactive oxygen species (ROS) and neuroinflammation and improved cognitive impairment during aging. In cultured microglia, pharmacological inhibition of CatB significantly reduced the generation of mitochondria-derived ROS and proinflammatory mediators induced by L-leucyl-L-leucine methyl ester (LLOMe), a lysosome- destabilizing agent. In the CatB over-expressing microglia after treatment with LLOMe, which mimicked the aged microglia, CatB leaked in the cytosol is responsible for the degradation of the mitochondrial transcription factor A (TFAM), resulting in the increased generation of mitochondria-derived ROS and proinflammatory mediators through impaired mtDNA biosynthesis. Furthermore, intra-lateral ventricle injection of LLOMe-treated CatB over-expressing microglia induced cognitive impairment in middle-aged mice. These results suggest that the increase and leakage of CatB in microglia during aging are responsible for the increased generation of mitochondria-derived ROS and proinflammatory mediators, culminating in memory impairment.

Disclosures: N. Junjun: None. **Z. Wu:** None. **V. Stoka:** None. **Y. Hayashi:** None. **C. Peters:** None. **H. Qing:** None. **V. Turk:** None. **N. Hiroshi:** None.

Poster

129. Brain Wellness and Aging: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 129.08/N12

Topic: C.01. Brain Wellness and Aging

Support: NSERC DISCOVERY GRANT
MEMORIAL UNIVERSITY MULTIDISCIPLINARY RESEARCH SEED GRANT

Title: Potential brain health consequences following modulation of brain mitochondria lipid metabolism and cell cycle suppression by enteric microbial metabolites

Authors: T. A. FILLIER[1], K. M. DOODY[1], S. K. B. SHAH[2], S. K. CHEEMA[2], T. H. PHAM[1], *R. H. THOMAS[1]
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Abstract: The enteric microbiota communicates with the central nervous system (CNS) and can modulate brain chemistry, normal brain function, and brain health. How such communication occurs is not fully understood. The ability of neuroactive microbial metabolites such as short chain fatty acids (SCFA) to modulate the gut brain axis has been proposed as one of the mechanisms through which the enteric microbiota communicate with the CNS and exerts its influence on normal brain function. Acute doses of short chain fatty acids (acetate, propionate, and butyrate, 60:20:20 molar ratio respectively) were administered to differentiated SHSY5Y cells (50-1000µM) and adult male and female Long Evans rats (500mg/kg via intraperitoneal (IP) injection) for 7 days. Membrane lipids, acyl carnitines, cell cycle, apoptosis and mitochondrial morphology were assessed. The results indicated that brief exposure of Long Evans rats or cell lines to acute doses of short chain fatty acids modulated brain mitochondrial lipid metabolism in a sex specific manner. Females animals displayed increased accumulation of C18:1 enriched cardiolipin molecular species concomitant with a decrease in CL species enriched with saturated fatty acids. Male animals on the other hand showed a significant reduction in acylcarnitines, while female animals had enhanced levels of odd chained acylcarnitines. Similar and contrasting modulation of cardiolipins and acylcarnitines were observed in the cells. Additionally, brief exposure of short chain fatty acids suppressed cell division at the G2/M phase, and induced apoptosis in cells. These results demonstrated that brief exposure to acute doses of short chain fatty acids produced during gut dysbiosis altered brain cardiolipins and acylcarnitines suggesting impaired brain mitochondria maybe a common feature or response in both animal and neuronal cells. Suppression of neuronal cells at G2/M phase of cell division further suggest that elevated levels of short chain fatty acids observed in systemic circulation during gut dysbiosis could be neurotoxic to brain cells.

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Poster

129. Brain Wellness and Aging: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 129.09/O1

Topic: C.01. Brain Wellness and Aging

Support: NIA-R15-AG050292

Title: The role of ICV pramlintide treatment in modulating metabolism and AD-pathology in high fat diet fed APP/PS1 mice

Authors: *J. GRIZZANTI[1], R. R. CORRIGAN[2], S. SERVIZI[1], G. CASADESUS[3]
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Abstract: Metabolic diseases, such as obesity and type II diabetes, (T2D) remain some of the strongest correlates for the development of Alzheimer's disease (AD). In T2D amylin, an amyloid co-secreted with insulin, is overproduced and aggregates to form plaques similar to AD. Interestingly, pramlintide (PRAM), a non-aggregating form of amylin, is used in conjunction with insulin therapy in diabetes to improve metabolic health and has shown benefit in AD models; however, the mechanism through which it works remains unclear. To address whether pramlintide can slow T2D mediated acceleration of AD pathology we exposed WT and APP/PS1 mice to either rodent chow or high fat diet (HFD) and treated mice with either aCSF or PRAM ICV. Our data suggests that HFD exacerbates both the AD and T2D phenotype in APP/PS1 mice. Preliminary data suggests that PRAM treatment modulates the T2D phenotype in both WT and APP/PS1 mice to affect AD-related markers. Additionally, PRAM treatment appears to differentially effect metabolic heath, Aβ pathology, and cognition in rodent chow and HFD fed WT and APP/PS1 mice.

Disclosures: J. Grizzanti: None. **R.R. Corrigan:** None. **S. Servizi:** None. **G. Casadesus:** None.

Poster

129. Brain Wellness and Aging: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 129.10/O2

Topic: C.01. Brain Wellness and Aging

Support: NIA-R15-AG050292

Title: Use of amylin receptor inhibitor AC187 to determine the therapeutic mechanism of action of pramlintide in Alzheimer's disease

Authors: *R. R. CORRIGAN[1], J. GRIZZANTI[1], G. CASADESUS[2]
[1]Sch. of Biomed. Sci., [2]Biol. Sci., Kent State Univ., Kent, OH

Abstract: Metabolic disease, including complications of obesity such as type II diabetes, (T2D) remains one of the strongest correlates for the development of Alzheimer's disease (AD). In T2D, amylin, an amyloid co-secreted with insulin, is overproduced and aggregates to form plaques similar to AD. Evidence suggests that amylin has a homeostatic function in the brain and removal or blockade of amylin signaling in the brain leads to pathology. Pramlintide (PRAM), a synesthetic non-aggregating form of amylin, has been shown to reduce classic AD hallmark pathology and improve cognition *in vivo*, as well as reduce oxidative stress *in vivo* and *vitro.* However, whether these benefits result from peripheral improvements on metabolic tone or rather direct activation of the amylin receptor (AMYR) within the brain is unknown. To address this, we treated APP/PS1 and WT mice with pramlintide peripherally while congruently blocking the AMYR using the antagonist AC187 via ICV cannulation in order to see whether central blockade of AMYR negates the therapeutic effects of PRAM on AD pathology, cognition, and oxidative stress. Our preliminary data suggest that the AD phenotype may be blocked by central blockade of AMYR suggesting a direct role of pramlintide onto the AMYR.

Disclosures: R.R. Corrigan: None. **J. Grizzanti:** None. **G. Casadesus:** None.

Poster

129. Brain Wellness and Aging: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 129.11/O3

Topic: C.01. Brain Wellness and Aging

Support: NIH 1R01AG036871
NIH 5R01EY010804
NIH 1R01NS079965

Title: Lack of cytochrome c in adult forebrain neurons *in vivo* leads to a decrease in cytochrome c oxidase, increased oxidative stress but no overt cell death

Authors: *M. PINTO, U. D. VEMPATI, F. DIAZ, S. PERALTA, C. T. MORAES
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Abstract: Background: Cytochrome c (Cyt c), a heme-containing protein present in the mitochondria, has a critical function in both respiration and apoptosis. Consistent with these vital functions, somatic Cyt c mouse knockout is embryonically lethal.Methods: In order to investigate the sensitivity of postnatal neurons to Cyt c functions, we developed a conditional neuron-specific knockout model. Neuron-specific Cyt cKO mouse ($nCytc^{KO}$) was created by crossing the floxed Cyt c mouse with a CamKII-a cre transgenic mouse, which deletes the floxed alleles post-natally.Results: $nCytc^{KO}$mice were normal at birth but developed an abnormal phenotype at 2 months of age with weight loss, tremor, decreased sensorimotor coordination and early death between 3-4 months. Histological analysis did not show major neuronal degeneration. Analyses of oxidative phosphorylation showed a specific reduction in complex IV levels. Markers of oxidative stress were also increased suggesting reverse electron transfer involvement in the pathomechanism.Conclusions: This novel model showed that complex IV levels are modulated by Cyt c levels. It also showed that decreased Cyt c in neurons lead to severe behavioral abnormalities and premature death without neuronal loss or inflammation.

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Poster

129. Brain Wellness and Aging: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 129.12/O4

Topic: C.01. Brain Wellness and Aging

Title: Changes in protein expressions in isolated neurite of hydrogen peroxide-treated N1E-115 cells

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Abstract: Reactive oxygen species (ROS) attack several living organs and increase the risks of several diseases, such as Alzheimer's disease (AD), cancer and diabetes via induction of cell death. Previously, we found axonal/dendrite degenerations prior to the cell death in hydrogen peroxide-treated neuroblastoma: N1E-115 cells, primary neurons, and its prevention by treatment with antioxidant vitamin. The reasons of these dysfunctions may be connected with membrane oxidation, microtubule destabilization, autophagy dysfunction and disruption of intracellular calcium homeostasis. However, its detailed mechanisms are not fully understood. Here, we identified neurite alteration-related proteins after treatment with hydrogen peroxide using new neurite isolation method by LC-MALDI-TOF/TOF analysis. Twenty-one proteins were increased

after treatment with a low concentration of hydrogen peroxide. Specifically, 5 proteins which were secretogranin-1, heat shock protein family D (Hsp60) member 1 (HSPD1), Brain acid soluble protein 1, heat shock 70-kDa protein 5 (Hspa5) and superoxide dismutase 1, were identified of all experiments and increased in isolated neurites of hydrogen peroxide-treated cells compared to the controls. Furthermore, secretogranin-1 and HSPD1 protein expressions were significantly increased in normal aged and Alzheimer's transgenic mice brains. These results indicate that secretogranin-1 and HSPD1 might contribute to ROS-induced neurite degeneration. Both proteins have been related to neurodegenerative disorders, so their study may shed light on neurite dysfunction.

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Poster

129. Brain Wellness and Aging: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 129.13/O5

Topic: C.01. Brain Wellness and Aging

Support: T32MH064913
R01AG033679
Glenn Foundation Award
5R01MH098260-03

Title: Characterization of parvalbumin inhibitory interneuron vulnerability in mouse prefrontal cortex and hippocampus in response to ketamine-induced inflammation

Authors: *J. B. RUDEN[1], Q. TANG[2], E. A. SCHNEIDER[1], T. W. SIMON[1], L. L. DUGAN[3]
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Abstract: We and others have previously reported that aged wild-type mice have fewer parvalbumin-positive interneurons in prefrontal cortex and hippocampus than young wild-type mice, and that young ketamine-injected wild-type mice have less parvalbumin fluorescence per cell in prefrontal cortex and fewer parvalbumin-positive interneurons in CA1 of hippocampus than young saline-injected wild-type mice. We linked these changes to activation of inflammatory pathways and found that pretreatment with a superoxide dismutase mimetic with anti-inflammatory properties reverses some of these changes. Here, we asked whether parvalbumin interneurons in the prefrontal cortex and hippocampus of ketamine-injected mice have decreased expression of parvalbumin protein and/or have degenerated. We also asked how ketamine affects parvalbumin interneurons functionally. To distinguish between loss of

parvalbumin protein and degeneration of the interneuron itself, we generated Parvalbumin-tdTomato mice by crossing PV-Cre mice with ROSA-tdTomato mice. In these mice, after development has occurred, parvalbumin interneurons will fluoresce red, irrespective of changes in parvalbumin protein levels. We injected young Parvalbumin-tdTomato mice with ketamine or saline and then performed either hippocampal slice electrophysiology or parvalbumin immunostaining and confocal imaging of prefrontal cortex and hippocampus. We compared the number of tdTomato-positive cells with the level of parvalbumin immunostaining within each tdTomato-positive cell to distinguish neuronal degeneration from loss of parvalbumin protein. We also calculated gamma oscillation power and parvalbumin interneuron spike rate to observe whether these measures change in response to intraperitoneal ketamine injections. These experiments provide insight into the vulnerability of a class of interneurons which are believed to be central to information encoding and memory formation and retrieval.

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Poster

129. Brain Wellness and Aging: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 129.14/O6

Topic: C.01. Brain Wellness and Aging

Support: PR2015-0157

Title: Brain aging and neurovascular coupling

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Abstract: Emerging evidence demonstrates that impaired neurovascular coupling (NVC) precedes impairments in cognitive performance and brain degeneration. Impaired NVC attenuates the hemodynamic response to neural activation, exacerbates neuroinflammatory responses, critically alters neurogenic microenvironment and determines the magnitude of neuronal injury. Great advances have been made in elucidating the cellular processes initiating, transmitting, propagating, and implementing the vascular response, but the precise cellular mechanisms have yet to be elucidated.
We have developed a rodent model of premature brain aging utilizing whole brain irradiation (IR), and demonstrated in two separate series of experiments in mice and rats that even low doses of IR profoundly reduced NVC, as judged by altered blood-oxygen-level-dependent (BOLD) signals in fMRI in rats as well as reduced vessel reactivity and lack of cerebral blood flow (CBF) response to electrical forepaw stimulation using intravital two-photon microscopy.

Though neurons are resistant to IR-induced apoptosis, they display persistent DNA damage response foci. By utilizing magnetic-activated cell sorting to isolate neurons from the brain after IR, we found that neurons entered a state of senescence after IR, as indicated by the elevated expression of multiple senescence-associated genes, including Ets 2, Akt 1, Cdkn1c, etc. This suggests that IR-induced senescence in neurons might play an active, upstream role in NVC dysfunction by dysregulation of vasodilation and homeostasis, thereby causing premature aging and degeneration. Our novel and intriguing results strongly indicate that IR can be used as a model of brain aging and premature degeneration, and that neuron senescence may be the key to unravel this mystery.

Disclosures: W. Han: None. **D. Liang:** None. **K. Blomgren:** None.

Poster

129. Brain Wellness and Aging: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: SDCC Halls B-H

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Topic: C.01. Brain Wellness and Aging

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The 1000 Young Talents Program
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Title: Identification of SAD and its glial functions in maintaining neural integrity in *Drosophila* during aging

Authors: Y. (. FANG[1,2], S. SHU[1,2], X. CAO[1,2], X. DENG[1,2], Y. DENG[1]
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Abstract: An integral nervous system is important for the longevity and healthy aging. However, the mechanisms maintaining neural integrity during aging remain poorly understood. To reveal unknown genes and signaling pathways that control neural integrity during aging, we conducted a genetic screen in *Drosophila* for brain-enriched genes whose loss of function (LOF) led to shortened lifespan.
In the screen, we identified a novel gene, *SAD*, whose downregulation in adult flies dramatically reduced the lifespan and caused age-dependent neurodegeneration. Interestingly, despite that *SAD* was highly expressed in the fly brain, neuronal downregulation of *SAD* showed no deleterious effect. In sharp contrast, glial knockdown of *SAD* resulted in remarkable neurodegeneration and shortened lifespan. Further study revealed that the subtypes of blood-

brain barrier (BBB) glia and cortex glia were specifically involved. Moreover, we found that the fly BBB and the glia matrix were severely damaged in the *Repo*-Gal4>RNAi-*SAD* flies. To further investigate how *SAD* regulates glial functions at the molecular level, we performed a RNA-seq analysis. The data suggested that *SAD* might function as a chromatin repressor in glia that surveilled the innate immunity in the brain. Without *SAD* repression, the expression of immune response genes went unleashed. This, together with other detrimental consequences, might lead to BBB disruption. In addition, our recent metabolomics data uncovered abnormal lipid metabolism in the brain of aged RNAi-*SAD* flies. Ongoing experiments are to establish the casual link of activated immune response with BBB disruption and the deficits in lipid metabolism. In summary, by investigating the function of *SAD* in glia, we hope to cast new insights to the glial role in regulating innate immunity and how it is involved in the process of aging.

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Poster

129. Brain Wellness and Aging: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 129.16/O8

Topic: C.01. Brain Wellness and Aging

Support: EASTBIO BBSRC (DTP) Studentship

Title: Hippocampal proteomics and primary cell cultures demonstrate proteins key in neuronal plasticity are rapidly changed in rodents on a high-fat diet

Authors: *F. H. MCLEAN[1], F. M. CAMPBELL[2], R. F. LANGSTON[1], L. M. WILLIAMS[2]
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Abstract: The rise in global obesity and related diseases, such as age-related cognitive decline and Alzheimer's disease, underlines the importance of understanding how nutrition can negatively impact human health, particularly cognition. Thus, the aim of this study was to identify changes in the hippocampus in a well-defined model of diet-induced obesity, the C57Bl/6 mouse on a high-fat diet (HFD). This follows on from our earlier study where hippocampal-dependent episodic memory was shown to be rapidly compromised by a HFD. To this end we fed mice a HFD for either 3 days, 1 week, 2 weeks, or 1 week HFD followed by 1 week low-fat diet (LFD). Proteomic analysis of mouse hippocampi identified altered expression patterns of proteins involved in metabolism, inflammation, cell stress, cell signalling, and the cytoskeleton which changed after only 3 days on a HFD and recovered rapidly on the LFD. The largest number of proteins changed were associated with the cytoskeleton including microtubule-

associated protein 2 (MAP2). Further experiments were carried out in hippocampal primary cell cultures immuno-stained for MAP2. Incubation of neurons with physiological levels of palmitic acid (PA), the most common long-chain saturated fatty acid in the diet, caused reduced dendritic length and arborisation. However the n-3 polyunsaturated fat, docosahexaenoic acid (DHA), had no effect and DHA in combination with PA negated the effects caused by PA. Hippocampal neurons treated with PA recovered rapidly when PA was removed. Dendrites are essential for cell communication, with dendritic spines forming the majority of post-synaptic sites, and are essential for neuronal plasticity. This study links diets high in saturated fats to indices of hippocampal neuronal damage and has implications for the link between diet, obesity and cognitive decline.

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Poster

129. Brain Wellness and Aging: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 129.17/O9

Topic: C.01. Brain Wellness and Aging

Support: USPHS Grant ES 022614

Title: The impact of genetics and dietary iron on regulation and co-regulation of iron, copper and zinc in mouse ventral midbrain

Authors: E. ADAMOVA[1], P. JIMENEZ[1], W. ZHAO[1], R. W. WILLIAMS[1], L. LU[3], *B. C. JONES[2]
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Abstract: Keywords: *(3 keywords)*
Ventral midbrain, metal regulation, GeneNetwork

Abstract:
Iron, copper and zinc are trace elements essential for the normal growth and development in mammals. The imbalance of these trace elements can cause various dysfunctions, e.g. low levels can produce some developmental disorders; high levels are connected to neurodegeneration. For example, iron overload in the substantia nigra is a risk factor for Parkinson's disease. Parkinson's disease is considered to be a combination of genetic background and environmental factors. Our research interest is in dietary iron and its impact on regulation/co-regulation of iron, copper and zinc in the brain. We studied genetic based individual differences in iron, copper and zinc concentrations their regulation and co-regulation under two iron diets. We studied metal

regulation in 28 BXD recombinant inbred mouse strains plus the parental C56BL/6J and DBA/2J inbred strains, The mice were fed two different iron-containing diets, 40 ppm and 240 ppm from weaning to 6 months. Thereafter, metal concentrations in the ventral midbrain were analyzed using X-ray fluorescence spectroscopy. To detect candidate genes, we performed QTL (quantitative trait loci) analysis using the GeneNetwork database followed by correlational analysis among the metals and with gene expression in the ventral midbrain to nominate possible candidate genes. Based on our analyses, we observed one suggestive QTL on chromosome 4, candidate gene: myosin head-like domain related to co-regulation of the three metals and one significant QTL for copper concentration on chromosome 4containing Slc24a2 as a possible candidate gene. Slc24a2 codes the potassium-dependent Na/Ca exchanger and is highly expressed in neurons. Our results show the impact of the interactions between genetics and dietary iron on iron, copper and zinc regulation/co-regulation in the brain. Implications for the co-association is likely to be relevant to neurological function in health and disease.
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Disclosures: E. Adamova: None. **P. Jimenez:** None. **W. Zhao:** None. **R.W. Williams:** None. **L. Lu:** None. **B.C. Jones:** None.

Poster

129. Brain Wellness and Aging: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 129.18/O10

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant AG054523
NIH Grant AG049638
NIH Grant HL087103

Title: Bioenergetic profiling of mitochondria isolated from three distinct brain regions in non-human primates

Authors: *A. AMICK[1], G. MAHAPATRA[2], J. L. GONZALEZ-ARMENTA[2], J. STONE[2], S. CRAFT[2], T. C. REGISTER[3], C. SHIVELY[3], A. J. A. MOLINA[2]
[1]Section on Gerontology, [2]Sticht Ctr. for Healthy Aging and Alzheimer's Prevention & Dept of Int Med., [3]Dept. of Pathology/Comparative Med., Wake Forest Sch. of Med., Winston-Salem, NC

Abstract: Different brain regions have unique metabolic demands based on activity and function. Previous studies have explored how various parameters such as mitochondrial content, density, and enzyme activity are different between specific brain regions. While these studies are vital toward the understanding of brain metabolism, they do not address potential differences in

core mitochondrial properties, such as the electron transport chain function, between regions. Our goal for this study was to examine intrinsic mitochondrial function, by utilizing respirometric analyses of mitochondria isolated from three brain regions: prefrontal cortex (PFC), entorhinal cortex (ERC), and cerebellum (CB), of middle aged female cynomolgus macaques (*Macaca fasicularis*; n=21; 12.2± 0.6 years; 3.41 ± 1.24 kg). The CB was used as the control when comparing the PFC and ERC. We used two protocols combining strategic additions/titrations of substrates, uncouplers, and inhibitors to measure nine parameters of mitochondrial function. By using a non-human primate model we were able to acquire adequate amounts of tissue for mitochondrial isolation and to consider the implications for human health within the context of a highly translatable model. We found that PFC mitochondria had the highest respiration across all measured parameters when compared to the CB. And we found that ERC mitochondria have variable respiration when compared to the CB; however, the most striking difference between the ERC and the CB was that the ERC variability depends on fatty acid oxidation and complex II activity. Additionally, protein quantification of the electron transport chain (ETC) components shows that the PFC has the highest concentration of ETC proteins, followed by the ERC, and then the CB. To our knowledge, this study provides the first characterization of differences in regional mitochondrial function from a non-human primate model.

Disclosures: A. Amick: None. **G. Mahapatra:** None. **J.L. Gonzalez-Armenta:** None. **J. Stone:** None. **S. Craft:** None. **T.C. Register:** None. **C. Shively:** None. **A.J.A. Molina:** None.

Poster

129. Brain Wellness and Aging: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 129.19/O11

Topic: C.01. Brain Wellness and Aging

Support: NIA Grant AG013854
NIA Grant AG014706
NIA Grant AG027141

Title: Calbindin-D_{28K} and parvalbumin immunoreactivities are virtually absent from human locus ceruleus noradrenergic neurons

Authors: S. LAMERAND[1], K. KIEFFER[1], G. KIM[1], R. SHAHIDEHPOUR[1], *M.-M. MESULAM[2], C. GEULA[1]
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Abstract: We have shown that the cholinergic neurons of the basal forebrain (BFCN), which are vulnerable to degeneration early in the course of aging and Alzheimer's disease (AD), are rich in the calcium binding protein calbindin-D28K (CB) in the human and non-human primates. The human BFCN display a substantial loss of CB in the course of normal aging, which is associated with accumulation of abnormally phosphorylated tau, tangle formation and degeneration of these neurons in AD. The purpose of the current study was to determine whether the noradrenergic neurons of the human locus ceruleus (LC), which are also vulnerable to tangle formation and degeneration early in the course of AD, also contain calcium binding proteins which are lost in the course of normal aging. Blocks of brainstem containing the LC from two young (20 and 57 years old) and two aged (73 and 77 years old) human brains were fixed in 4% paraformaldehyde for 30-36 hours at 4º C and taken through sucrose gradients for cryoprotection. Blocks were sectioned at a thickness of 40 µm on a freezing microtome and 1 in 24 series of sections were stored in 0.1 M phosphate buffer until use. One series of sections were each immunohistochemically stained for the monoaminergic synthetic enzyme tyrosine hydroxylase (TH), and the calcium binding proteins CB and parvalbumin (PV). Microscopic examination revealed robust TH immunoreactivity in the LC neurons and their processes in both young and aged participants. However, CB and PV immunoreactivity were virtually absent from LC neurons. Occasionally, sparse LC neurons displayed CB immunoreactivity which was slightly above background staining. These findings indicate that, unlike the human BFCN, LC neurons are virtually devoid of CB and also of PV. They are consistent with previously reported virtual absence of CB and PV from LC neurons in the rhesus monkey. It remains to be determined whether human LC neurons contain appreciable concentrations of other ubiquitous calcium binding proteins, such as calretinin.

Disclosures: S. Lamerand: None. **K. Kieffer:** None. **G. Kim:** None. **R. Shahidehpour:** None. **M. Mesulam:** None. **C. Geula:** None.

Poster

129. Brain Wellness and Aging: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 129.20/O12

Topic: C.01. Brain Wellness and Aging

Support: NRF-2016R1A2B4014707

Title: Tenovin-1-induced senescence in astrocytes downregulate wound healing

Authors: *M. BANG, O. RYU, D. KIM, D. MABUNGA, K. CHO, S. JOO, E. GONZALES, R. KIM, R. KANG, K. KWON, C. SHIN
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Abstract: Astrocytes have various functions in the brain and occupy a large portion in the human brain. Blood-brain barrier (BBB) permeability is regulated by astrocytes. BBB of the aging brain shows increased permeability, leading to cognitive decline and dementia. Nevertheless, astrocytes received less attention than neurons in aging-related studies. Aged brain shows features of accumulated senescent cells.. Tenovin-1 has been known as inhibitor of SIRT1 and SIRT2 and as a histone deacetylase inhibitor. Both SIRT1 and SIRT2 inhibitors delay tumor growth in vivo without significant general toxicity, while HDAC inhibitors are known to trigger senescence. In this study, we induced astrocyte senescence through tenovin-1 treatment. Cellular senescence usually is characterized by irreversible cell cycle arrest and induced senescence-associated β-galactosidase (SA-β-gal) activity. Tenovin-1 treated astrocytes show increased SA-β-gal positive cell number, SASP including IL-6 and IL-1β, cell cycle-related proteins like phosphor-histone H3 and CDK2, and impaired wound healing activity. These data suggest that tenovin-1 can induce cellular senescence in astrocytes and senescent astrocytes may induce changes in immune response and impaired wound healing function, which may play some role in normal aging and neurodegenerative conditions...

Disclosures: M. Bang: None. **O. Ryu:** None. **D. Kim:** None. **D. Mabunga:** None. **K. Cho:** None. **S. Joo:** None. **E. Gonzales:** None. **R. Kim:** None. **R. Kang:** None. **K. Kwon:** None. **C. Shin:** None.

Poster

129. Brain Wellness and Aging: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 129.21/O13

Topic: C.01. Brain Wellness and Aging

Title: Increased mitochondrial respiration without parallel changes in ATP production and sexually dimorphic responses to indoleamine 2,3 dioxygenase inhibition in a murine, two hit model of schizophrenia

Authors: *O. HUBERT[1,2], C. MAURICE-GÉLINAS[2], C. MONPAYS[2], J. DESLAURIERS[3], P. SARRET[2], S. GRIGNON[2,4]
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Abstract: Schizophrenia pathophysiology involves, among others, disturbances of oxidative/inflammatory status and energy metabolism. In a juvenile, murine two-hit model of schizophrenia (THMS), we previously demonstrated increased oxidative stress[1] and enhanced complex I- and complex II-induced respiratory activity (IRA) in the prefrontal cortex (PFC) and striatum in both sexes, but an increase in complex II IRA only in males, without obvious

modulations in respiratory chain complexes and mitochondrial fusion/fission proteins. The antioxidant and mitochondrial modulator, lipoic acid (LA), partially reversed these effects[2]. This pattern was partly reminiscent of the effect of the psychotomimetic ketamine, an NMDA antagonist[3]. The kynurenine metabolite, kynurenic acid (KA) provides an attractive link between schizophrenia pathophysiology, oxidative/inflammatory status and NMDA inhibition. As a preliminary test of its potential involvement, we report on the effect of the indoleamine 2,3-dioxygenase inhibitor 1-methyl tryptophan (1MT) on mitochondrial O_2 consumption and ATP production, in the THMS.

Methods

Mitochondrial O_2 consumption and ATP production were measured from mitochondria isolated from the PFC and striatum with MitoXpress and Invitrogen kits, respectively. Control or THMS mice were treated with vehicle, lipoic acid or 1-MT. Leak respiration (glutamate-malate (G-M) + succinate (S) + ADP + oligomycin), complex I (G-M + ADP) and complex II (G-M+ S + rotenone + ADP) IRA and ATP production were measured.

Results

Striatal complex I, complex II, and complex I+II IRA, as well as leak respiration, were increased in THMS males ($p < 0.05$) but not in females.

Complex I+II IRA was also increased, at trend level ($p=0.0737$), in male PFC.

ATP production was essentially unchanged.

Of note, 1MT elicited sexually dimorphic responses in THMS mice only (2 way ANOVA (sex X 1MT) interaction $p<0.05$), with a decrease in male striatal respiration (% change: I: -6; I+II -33) and an increase in females (+184 and 186).

Conclusion

1- The THMS elicits increases in striatal mitochondrial respiration, notably in males.

2- The lack of parallel effect on ATP production points to a pattern of “mild uncoupling” and warrants the assessment of UCP2 and UCP4 levels.

3- The dimorphic response to 1MT suggests that the mechanisms underlying increased respiration might differ in females and males, in line with recent results demonstrating a direct inhibitory effect of estrogens on KA production[4].

1 Deslauriers et al Neurosci 2014 272:261-70
2 Monpays et al J Mol Neurosci 2016 59(4):440-51
3 de Oliveira et al Metab Brain Dis 2011 26(1):69-77
4 Jayawickrama et al Sci Rep. 2017 7(1):17559

Disclosures: O. Hubert: None. **C. Maurice-Gélinas:** None. **C. Monpays:** None. **J. Deslauriers:** None. **P. Sarret:** None. **S. Grignon:** None.

Poster

129. Brain Wellness and Aging: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 129.22/O14

Topic: C.01. Brain Wellness and Aging

Support: PAPIIT/UNAM IN206015
CONACyT CB2013-220515
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Title: Autophagy dysfunction during neuronal senescence

Authors: *D. MORENO BLAS, E. GOROSTIETA SALAS, G. MUCIÑO HERNÁNDEZ, A. POMMER ALBA, S. CASTRO OBREGÓN
Dept. of Neurodevelopment and Physiol., Natl. Autonomous Univ. of Mexico-UNAM, Mexico City, Mexico

Abstract: Cellular senescence is an irreversible state of cell cycle arrest induced by different stimuli, including telomere shortening, reactive oxygen species, DNA damage, oncogene activation and developmental cues. In aging, senescent cells are not efficiently cleared by the immune system leading to their accumulation in old tissues, where they can modify the microenvironment, promote local inflammation and induce paracrine senescence, spreading the senescent phenotype along old tissues. At least in mice, persistent senescent cells contribute to aging and age-related diseases; therefore, it is fundamental to understand the molecular mechanisms of cellular senescence establishment and maintenance. To date, most of the knowledge related to cellular senescence has been collected through the study of mitotic cells, and it has been theorized that post-mitotic cells are incapable of entering into a senescent state. Nevertheless, neurons with several senescent features have been observed in old mice brain, although the molecular mechanisms to induce neuronal senescence are unknown. Since alterations in autophagic flux have been observed during brain aging and neurodegenerative disorders, we hypothesized that autophagy dysfunction could contribute to neuronal senescence establishment. In this study, we developed an *in vitro* model of neuronal senescence in long-term primary culture of rat cortical cells, to study the role of autophagy during the acquisition of neuronal senescence. We observed neurons with several senescence-related features, together with accumulation of autophagosomes and p62/SQTSM, which is indicative of autophagic flux impairment. In addition, we observed that activation of autophagy decreased the number of

senescent neurons, whereas autophagy inhibition increased them, indicating autophagy dysfunction as a possible inductor of neuronal senescence.

Disclosures: D. Moreno Blas: None. **E. Gorostieta Salas:** None. **G. Muciño Hernández:** None. **A. Pommer Alba:** None. **S. Castro Obregón:** None.

Poster

129. Brain Wellness and Aging: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 129.23/O15

Topic: C.01. Brain Wellness and Aging

Support: Murray Family Foundation Grant

Title: Hyperglycemia and thrombin induce cellular injury, activation of matrix metalloproteinase-2 and mitochondrial fission protein Drp1 in cultured primary human brain microvascular endothelial cells

Authors: *H. VITTAL RAO, J. IANNUCCI, W. RENEHAN, P. GRAMMAS
Ryan Inst. of Neurosci., Univ. of Rhode Island, Kingston, RI

Abstract: Diabetes is a hyper-coagulable state and a risk factor for Alzheimer's Disease (AD). Our lab has shown earlier that the microvasculature in the AD brain is activated and secretes pro-inflammatory and pro-angiogenic factors, including thrombin and matrix metalloproteinases (MMPs) which are also pro-apoptotic to some cell types. In this study, we tested whether cultured primary human brain microvascular endothelial cells (HBMVECs) exposed to hyperglycemic conditions undergo cellular injury, MMP activation and changes in mitochondrial fission. Taking together the observations that thrombin is secreted by AD microvessels and is also elevated in diabetics, we first tested whether glucose induced thrombin in cultured HBMVECs. Our data showed that these cells did secrete elevated levels of thrombin under prolonged (7d) hyperglycemic conditions. Acute (6h) hyperglycemia and thrombin induced cellular injury measured by LDH release independently and to a greater extent in combination. High-glucose and thrombin both induced an increase in the activity of matrix-metalloproteinase-2 (MMP-2), individually as well as together. Activated MMP-2 has been shown to damage mitochondria and accelerate apoptosis in diabetic retinal endothelial cells. We show here that both high glucose and thrombin induce expression of mitochondrial fission protein Drp1 and together their effect is exacerbated. Excessive Drp1-induced mitochondrial fission is known to induce apoptosis through excessive mROS generation. Therefore, under hyperglycemic conditions, the combined presence of glucose and glucose-induced thrombin within the brain microvasculature can create a highly deleterious environment that can trigger a vicious cascade of events leading to cell death. Taken together, our data show that thrombin and glucose act

independently and synergistically in inducing injury to primary human brain microvascular endothelial cells in culture.

Disclosures: H. Vittal Rao: None. **J. Iannucci:** None. **W. Renehan:** None. **P. Grammas:** None.

Poster

129. Brain Wellness and Aging: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 129.24/O16

Topic: C.01. Brain Wellness and Aging

Support: ICMR

Title: Establishing WNIN/Ob obese rat as a novel animal model to study the neurobiology of premature aging

Authors: *J. K. SINHA[1,2,3], S. GHOSH[2], M. RAGHUNATH[2]
[1]Neurobio., Ctr. for Cell. and Mol. Biol. (CCMB), Hyderabad, India; [2]Natl. Insitute of Nutr., Hyderabad, India; [3]Amity Inst. of Neuropsychology & Neurosciences (AINN), Noida, India

Abstract: Purpose: Proportion of aged individuals is on the rise in general population and most of the older people die due to non-communicable diseases like obesity. Wistar of National Institute of Nutrition obese (WNIN/Ob) rat is a novel strain developed at NIN, Hyderabad, India. These rats have significantly reduced the average lifespan of 15-18 months in contrast to 36 months in normal WNIN rats. Elucidation of various molecular and biochemical characteristics in these rats would help to establish it as an appropriate model to study the neurobiology of aging and obesity. **Methods**: Different growth characteristics were studied and the lifespan analysis was performed using OASIS software. The neuronal and glial changes were studied using Nissl staining and immunohistochemistry. Levels of oxidative stress, antioxidant enzyme activity, and extent DNA damage were studied in various brain parts. **Results**: The brain weights were significantly decreased and there was a 60% decrease in the total lifespan in the WNIN/Ob obese rats as compared to the lean littermates as well as WNIN normal rats. Various neuronal and glial changes were observed that are seen in the aging brain. In addition, oxidative stress levels and extent of DNA damage were observed to be significantly high in the brain of young WNIN/Ob obese rats as compared to age-matched rats and it was as high as compared to that observed in 15 months old WNIN normal rats. The levels of antioxidants enzyme activity were also significantly low in the WNIN/Ob obese rats. **Conclusion**: Onset of various degenerative features like increased oxidative stress, astrogliosis, DNA damage and decreased antioxidant levels in different brain regions of WNIN/Ob obese rats at a much younger age is a plausible cause of

reduced longevity observed in this novel obese rat model. This model may be used to study the connecting link between obesity and aging.

Disclosures: J.K. Sinha: None. **S. Ghosh:** None. **M. Raghunath:** None.

Poster

129. Brain Wellness and Aging: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 129.25/P1

Topic: C.01. Brain Wellness and Aging

Support: FONDECYT 11170546, CONICYT
PAI77170091, CONICYT

Title: Phosphorylated tau at Ser396/404 sites accumulates in the synaptic mitochondria affecting its bioenergetics function during aging

Authors: *C. TAPIA-ROJAS[1], C. HERRERA-CID[1], C. JARA[2], R. A. QUINTANILLA[2]
[1]Univ. San Sebastián, Santiago, Chile; [2]Univ. Autónoma de Chile, Santiago, Chile

Abstract: Aging is a natural process in which protein aggregates are regularly accumulated in the brain. Commonly, the aggregates are formed by phosphorylated tau protein, which dissociates from the microtubules and accumulates in the neurons; leading to mitochondrial dysfunction, synaptic failure and eventually to neurodegenerative disease during aging. Synaptic mitochondria are crucial to maintain the bioenergetics balance at synapses, providing the ATP to the synaptic function. In fact, synaptic mitochondria are more susceptible to damage and they are the first to be altered during the aging compared to mitochondria from non-synaptic sites. Tau has been detected in mitochondrial extracts from AD and could interact with mitochondrial proteins; however, if the accumulation of phosphorylated tau occurs in synaptic mitochondria during aging, before the development of neurodegenerative diseases, is unknown. To determine whether phosphorylated tau at Ser396/404 is present during normal aging, we dissected the hippocampus and the cortex from the brain of 2, 12 and 18 month-old wild-type (WT) C57 mice. Additionally, we isolated non-synaptic mitochondria, synaptosomes and synaptic mitochondria from the hippocampus and the cerebral cortex from these WT animals. Our results showed that during the aging, phosphorylated tau at Ser396/404 sites is increased in the hippocampus and the cortex of 18 month-old (18mo) mice, at same time that oxidative stress is observed in this brain regions. Also, increased levels of phosphorylated tau were detected in synaptosomes and in synaptic mitochondria from aged mice (18mo) compared with young mice. In contrast, this difference was not observed in non-synaptic mitochondria. More importantly, we reported that synaptic mitochondria have negative alterations in its structure and bioenergetics function when phosphorylated tau protein is present in this mitochondrial population, in samples

of aged WT mice.
Altogether, these results suggest that phosphorylated tau at Ser396/404 sites could be associated mainly with synaptic mitochondria, affecting its function during normal aging.

Disclosures: C. Tapia-Rojas: None. **C. Herrera-Cid:** None. **C. Jara:** None. **R.A. Quintanilla:** None.

Poster

129. Brain Wellness and Aging: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 129.26/P2

Topic: C.01. Brain Wellness and Aging

Support: ICMR

Title: Altered levels of neurotrophic factors and neurochemical profile in the brain as the probable causes of the decreased longevity of WNIN obese rats

Authors: *S. GHOSH[1], J. K. SINHA[1,2], M. RAGHUNATH[1]
[1]Natl. Inst. of Nutr., Hyderabad, India; [2]Amity Inst. of Neuropsychology & Neurosciences (AINN), Noida, India

Abstract: Purpose: Wistar NIN obese (WNIN/Ob) rats developed at the National Institute of Nutrition are the heaviest inbred rat strain in the world. These rats are hyperphagic, hyperinsulinemic, hyperleptinemic and have reduced longevity (an average lifespan of 15-18 months in contrast to 36 months in normal Wistar rats). In the WNIN/Ob rats, we intend to delineate the factors responsible for reduced longevity. **Methods:** Neurotrophic factors are responsible for the survival of developing neurons and the maintenance of mature neurons. We have estimated levels of key neurotrophic factors using BioPlex assay and done neuro-glial profiling using Immunohistochemistry in different brain regions of these rats (n=6). As Glutamate (Glu) and γ-aminobutyric acid (GABA) are the major excitatory and inhibitory neurotransmitters in mammalian CNS, we have looked at the levels of these neurometabolites in different brain regions of WNIN/Ob rats (n=4) and their age-matched normal rats (n=4) using Magnetic Resonance Spectroscopy (MRS). We have evaluated if there are any volumetric differences in the brain of WNIN/Ob rats in contrast to the age-matched controls using Magnetic Resonance Imaging (MRI). **Results:** Our findings show that the levels of key neurotrophic factors like BDNF and IGF-1 are altered in the WNIN/Ob rats. MRS data indicates hypo-metabolism in the brain of WNIN/Ob rats. But there are no significant volumetric changes in the brain of the WNIN/Ob rats when compared to controls. **Conclusion:** Altered levels of neurotrophic factors and neurochemical profile in the brain are one of the many factors of the decreased longevity of WNIN obese rats.

Disclosures: S. Ghosh: None. **J.K. Sinha:** None. **M. Raghunath:** None.

Poster

129. Brain Wellness and Aging: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 129.27/P3

Topic: C.01. Brain Wellness and Aging

Support: NIGMS 5P20GM109025

Title: Examination of Alzheimer's disease-related pathology as a result of hyperglycemia in aged versus young mice

Authors: *A. A. ORTIZ[1], A. M. SALAZAR[2], A. LEISGANG[2], J. W. KINNEY[2]
[1]Psychology, [2]Univ. of Nevada Las Vegas, Las Vegas, NV

Abstract: Alzheimer's Disease (AD) is a neurodegenerative disorder that is characterized by progressive learning and memory deficits, neuronal loss, and ultimately death. The two core pathological hallmarks of AD are the accumulation of amyloid beta (Aβ) plaques and neurofibrillary tangles composed of hyperphosphoralated tau (ptau). A third characteristic that has emerged is a chronic inflammatory response within the brain. Several studies have demonstrated that chronic neuroinflammation exacerbates Aβ and ptau pathology. The exact cause of AD remains unknown; however, several risk factors exist that greatly increase the likelihood of AD.

Genetic risk factors such as ApoE and a mis-sense mutation in TREM2 have been linked to increasing the likelihood of developing AD. Non-genetic risk factors include age, cardiovascular disease, obesity, and diabetes mellitus (DM). DM confers up to a 4-fold increase in risk that arises based on hyperglycemia, insulin receptor resistance, and changes in the vasculature. As DM is far more common in the aging population there exists a likely link between DM risk and age associated risk for AD. Further, approximately 30% of the population over the age of 65 exhibits pre-diabetic symptomatology, including hyperglycemia. Given the overlap between two risk factors for AD, an investigation of age associated hyperglycemia as well as the effects of inducing hyperglycemia in older animals produces AD related pathology.

We have previously demonstrated that intermittent administration of streptozotocin (STZ), induces sustained hyperglycemia in an otherwise healthy animal. STZ mice exhibit learning and memory deficits, increased ptau, and inflammation in the brain. In the present study we investigated these same measures in aged (10 months) versus young (3 months) wild-type mice (WT), as well as the effects of STZ in aged vs. young mice. We also investigated if any differences in these measures was observed based on sex.

Our data indicate altered blood glucose in older mice as well as differences between old and

young mice in response to the STZ administration. Additional differences exist between males and females in several measures between groups.

Disclosures: A.A. Ortiz: None. **A.M. Salazar:** None. **A. Leisgang:** None. **J.W. Kinney:** None.

Poster

129. Brain Wellness and Aging: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 129.28/P4

Topic: C.01. Brain Wellness and Aging

Support: NSFC Grant 81400956
NSFC Grant 81722017

Title: De novo fatty acid synthesis in $CD4^+$ T cells after cerebral ischemic stroke - A new target of post-stroke immune modulation

Authors: *X. WANG, Y. ZHOU, Z. ZHU, Y. LI, P. LI, W. YU
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Abstract: Cerebral ischemic stroke results in profound activation of the peripheral T cells, which in turn significantly affect post-stroke neuroinflammation and secondary brain injury. $CD4^+$ T cells are a major subset of T cells that have been suggested to play important roles in ischemic brain injury, however the underlying mechanisms of their activation and differentiation after stroke are yet to be explored. Here we report that cerebral ischemic stroke increased the expression of ACC1, a key enzyme of *de novo* fatty acid synthesis, in peripheral $CD4^+$ T cells. Pharmacologic inhibition of ACC1 by soraphen-A reduced the infarct volume and neuroinflammation after stroke. The balance of peripheral Treg/Th17 was broken after ischemic stroke and was rescued to the normal percentage after conditional knockout of *ACC1* in $CD4^+$ T cells. We further identified that caloric restriction down-regulated the expression of ACC1 in $CD4^+$ T cells, which might underlie the neuroprotection of caloric restriction against cerebral ischemic stroke. These data suggest that ACC1 might be a novel immune metabolic modulation target for neuroprotection of stroke.

Disclosures: Y. Zhou: None. **Z. Zhu:** None. **Y. Li:** None. **P. Li:** None. **W. Yu:** None.

Poster

129. Brain Wellness and Aging: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 129.29/P5

Topic: C.01. Brain Wellness and Aging

Title: Exploring the effects of age, diet, and motor performance on cognitive assays in fruit flies

Authors: *C. B. BARCENAS[1], A. M. BRISENO[2], L. S. VILLALPANDO[2], W. L. HARDEMAN[2], J. M. NAPAN[2], A. D. TROFIMOVA[2], B. TOLAN[2], R. E. HARTMAN[2]
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Abstract: Because validated behavioral assays for measuring cognitive ability in Drosophila melanogaster (fruit fly) generally rely on locomotor activity, changes in motor performance may confound interpretation of data from studies designed to assess learning and/or memory. Data from another poster at this conference (Briseno et al), for example demonstrate a significant decrease in fly locomotor activity with age, such that data from assays in which a fly must move toward or away from some stimulus may become increasingly skewed as age increases. Understanding the conditions in which locomotor activity is either impaired or significantly decreased is an important factor for assessing the cognitive abilities. In this study, flies of varying ages were subjected to multiple assays of motor performance and learning ability. Half of the flies were raised on a standard fly diet, and half were raised on media supplemented with polyphenols. Preliminary data suggest that the sharp decline in locomotor activity was associated with worse performance on learning and memory tests, demonstrating the importance of statistically controlling for this parameter when assessing learning ability in Drosophila melanogaster.

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Poster

129. Brain Wellness and Aging: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 129.30/P6

Topic: C.01. Brain Wellness and Aging

Support: NIA-R15-AG050292

Title: Regulation of AMYR and RAMP expression by high fat diet in wild type & APP/PS1 mouse model mice

Authors: *S. SERVIZI[1], J. GRIZZANTI[1], G. CASADESUS[2]
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Abstract: Amylin, a satiety hormone co-secreted from the pancreas with insulin, is overproduced in Type II Diabetes (T2D) and aggregates in a similar fashion to amyloid-beta aggregates in Alzheimer's disease (AD). In fact, amyloid-beta has been shown to signal through the amylin receptor (AMYR). AMYR is comprised of the calcitonin receptor (CT) complexed to a receptor activity modifying protein (RAMP). Expression of the AMYR has been shown in the brain; however, it is not known if receptor expression and complex formation changes during high amyloid/amylin states such as those seen in AD and/or T2D brains. To address this, we have identified transcriptional expression and complex differences between the APP/PS1 transgenic AD mouse model and wild-type mice under regular diet and high fat conditions. Furthermore, we sought to identify the impact of pramlintide, a non-aggregating form of amylin, currently used to treat T2D and shown to be effective in AD mouse models. Our preliminary data suggest differential expression of RAMPs and/or CT in different brain regions and changes associated with transgene and/or diet and potential modulation of pramlintide. The results of this analysis seek to elucidate the involvement of the amylin receptor in AD development and mechanistic underpinnings of pramlintide as a therapy.

Disclosures: S. Servizi: None. **J. Grizzanti:** None. **G. Casadesus:** None.

Poster

130. Brain Wellness and Aging

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 130.01/P7

Topic: C.01. Brain Wellness and Aging

Support: UK MRC: G1001354
HDH Wills 1965 Charitable Trust: 1117747

Title: Associations between socio-intellectual activities and the ageing brain: An analysis of data from the Whitehall II imaging sub-study

Authors: *M. ANATÜRK[1], S. SURI[1], E. ZSOLDOS[1], A. SINGH-MANOUX[2,3], M. KIVIMÄKI[2], K. EBMEIER[1], C. SEXTON[1,4]
[1]Dept. of Psychiatry, Univ. of Oxford, Oxford, United Kingdom; [2]Dept. of Epidemiology and Publ. Health, Univ. Col. London, London, United Kingdom; [3]INSERM U1018, Ctr. for Res. in

Epidemiology and Population Hlth., Villejuif, France; [4]Global Brain Hlth. Institute, Univ. of California, San Francisco, San Francisco, CA

Abstract: Introduction: Engaging in socio-intellectual activity in older ages has been linked to a lower risk of Alzheimer's disease (AD), better cognitive outcomes and structural brain integrity. The extent to which social (SA) and cognitive activities (CA) independently relate to underlying brain structure and functional connectivity in old age is, however, not fully understood. The ε4 variant of the Apolipoprotein E (APOE) gene is a known risk factor for AD, related to poorer cognitive outcomes and neural abnormalities in old age. This genetic variant may act as a potential moderator of the associations between leisure activities and the ageing brain. **Aims:** The main aim of this study was to examine SA and CA levels in relation to grey matter (GM) volume, white matter (WM) microstructure, resting-state functional connectivity and cognition in older adults. APOE ε4 status (i.e. ε4-carriers versus non-carriers) was examined as a moderator of these associations. **Methods:** The sample consisted of 617 healthy older participants (mean age ± SD = 69.6 years ± 5, 18.8% females) from the Whitehall II Imaging Sub-Study (2012-2016). All participants completed the Community Healthy Activities Model Program for Seniors (CHAMPS) questionnaire, cognitive assessments and underwent a 3T MRI scan. APOE genotype information was available for a sub-sample of participants (n = 424; 119 ε4-carriers). Voxel-Based Morphometry, Tract Based Spatial Statistics and Dual Regression were used to investigate GM volume, WM microstructure and resting-state functional connectivity at a voxel-wise level, respectively. Permutation Analysis of Linear Models was used to examine activity-cognition associations. **Results:** CA were positively correlated with performance on executive function, memory and processing speed tests, whereas SA levels correlated solely with processing speed. Brain structure analyses revealed a significant interaction between SA levels and APOE ε4 status on GM volume in the left supracalcarine cortex and axial diffusivity in several white matter tracts. Visualizations of the interactions indicated a positive association between SA levels and these imaging metrics, for APOE ε4-carriers. **Conclusions:** These results are consistent with previous studies, which find a positive link between leisure activities and cognitive outcomes in older adults. This study also suggests that socializing in later-life may be related to better grey and white matter brain integrity, in APOE ε4-carriers.

Disclosures: S. Suri: None. **E. Zsoldos:** None. **A. Singh-Manoux:** None. **M. Kivimäki:** None. **K. Ebmeier:** None. **C. Sexton:** None.

Poster

130. Brain Wellness and Aging

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 130.02/P8

Topic: C.01. Brain Wellness and Aging

Title: The use of EEG neurofeedback, a non-pharmacological approach towards brain wellness and function

Authors: *F. FRANCO[1], S. DRUMMOND[1], W. O. NEESE[1], A. CAYCE[1], J. P. ABARA[1], D. MCCLENDON[2]
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Abstract: Neurofeedback provides a real time mirror of brain electrical activity. The feedback is presented in multiple modalities (visual, auditory, tactile) that allow the brain to self-regulate. A significant up-regulation of functional connectivity was observed by Ros and colleagues (2013) in their fMRI study with a EEG NFB session during an auditory attention task. Hutton, Joseph, Weiskopf, and Rees (2012) and Sinotte and Coelho (2007) documented the use of neurofeedback that enhanced perceptual processing, performance in working memory, and attention tasks. NFB has also been shown to have a favorable effect on PTSD symptoms (Gapen et al., 2016). These previous studies provide empirical evidence that EEG NFB has a favorable impact on function. The present study is part of a broader project on brain wellness and engagement in the workplace during a transitional phase. The aim of this particular study was to evaluate the efficacy of EEG NFB training on subjective self-reported stress. A pre- and post-subjective self-report stress questionnaire was administered to an experimental group (n = 34) and a comparison group (n = 30) before and after one session of EEG NFB training. A 2 x 2 Mixed-ANOVA on subjective self-reported stress scores yielded a significant interaction effect, $F(1, 62) = 9.06$, $p = .004$, partial $\eta 2 = 0.128$. Subjective self-reported stress decreased only within the experimental group. The subjective self-reported stress for the comparison group remained the same across time. The findings support previous report on the favorable effect of EEG NFB training. The present study demonstrated that brain wellness can be fostered with a non-pharmacological approach using EEG NFB training. EEG NFB is a promising option in promoting wellness, especially during a transitional phase in a work environment.

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Poster

130. Brain Wellness and Aging

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 130.03/P9

Topic: C.01. Brain Wellness and Aging

Support: CIHR MOP-125915

Title: Integrated cognitive-motor training improves motor skill and movement consistency in adults at risk for dementia

Authors: *H. V. ECHLIN[1], D. J. GORBET[2], C. DE BOER[4], L. E. SERGIO[3]
[1]Kinesiology and Hlth. Sci., York Univ., North York, ON, Canada; [2]Sch. of Kinesiology and Hlth. Sci., [3]Ctr. for Vision Res., York Univ., Toronto, ON, Canada; [4]Alzheimer Res. Ctr., VUmc, Amsterdam, Netherlands

Abstract: With the prevalence of dementia increasing each year, preclinically implemented therapeutic interventions are critical for halting pathophysiological changes associated with neurodegeneration. It has been suggested that cascading neural network failures may bring on behavioural deficits associated with AD[1]. Previously we have shown correlations between impaired movement performance in tasks requiring the integration of rules (cognitive-motor integration, CMI) and both functional[2] and structural[3] fronto-parietal brain network deficits. We have also shown recently that CMI training in the early stages of dementia generalized to improved global cognitive and activities of daily living scores[4]. Since neurodegenerative damage to the brain can go on for decades before behavioural deficits become evident, engaging in CMI tasks may assist with strengthening these networks. Here we employ a novel movement-control based training approach involving CMI rather than traditional cognition-only brain training. We hypothesized that such training would stimulate widespread neural networks and enhance rule-based visuomotor ability in at-risk individuals. Participants (M_{age}=61.7, SD=6.3) with either maternal/multiple family history of dementia (n=10) or no dementia history (n=13, age- & sex-matched) were assigned to either a 16-week training (30 min, 2X/week) or a no-training group (i.e. 4 groups total). The 'stages' of the game required either direct interception of moving targets on a touchscreen while avoiding no-go targets, or more complex target interception where the visual stimuli were on a separate display, the visual feedback was reversed, or both. Pre- and post-tests included neuropsychological measures, a bimanual coordination task, and CMI assessment. We observed a significant improvement in bimanual coordination and in speed on the CMI task in the at-risk training group. We also observed significant decreases in movement variability for the most complex CMI condition in the at-risk and healthy training groups, while variability in at-risk and healthy no-training groups increased over time. These data suggest that integrating cognition into action in a training intervention may be effective at strengthening vulnerable brain networks in asymptomatic adults at risk for developing dementia. Such an approach may be a useful tool for functional decline prevention in individuals facing neurodegenerative disease.1.Jones et al. Brain, 139(2), 547-562.2.Hawkins KM, Sergio LE. 2014. J Alz. Dis. 42:607-621. 3. Hawkins et al. 2015, J Alz. Dis.44:867-878.4.deBoer et al.2018. Dem.&Ger.Cog.DisordEx(in press).

Disclosures: H.V. Echlin: None. **D.J. Gorbet:** None. **C. De Boer:** None. **L.E. Sergio:** None.

Poster

130. Brain Wellness and Aging

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 130.04/P10

Topic: C.01. Brain Wellness and Aging

Support: CONICET
CONICYT/FONDECYT Regular (1170010)
INECO Foundation
FONDAP 15150012

Title: The senescent encephalon: A morphometric and functional study of normal brain aging

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Abstract: As humans age, their neurological systems normally manifest structural and functional alterations, accompanied by cognitive decline. In order to characterize such mechanisms, we aimed to identify those structures and networks which feature major changes as individuals grow old. We obtained structural and functional magnetic resonance images from 136 healthy subjects, ranging from age 17 to 84. Gray matter (GM) atrophy was evaluated via voxel-based morphometry (VBM). First, we assessed the association between GM volume and aging through non-parametric correlations. Then, we compared GM differences between three samples separated by age: young adults (YAs, 17-35), adults (As, 31-59), and seniors (Ss, 60-84). Functional connectivity (FC) changes were evaluated with the network-based statistic (NBS) toolbox. A whole-group analysis at the lobule level revealed that aging was associated with reduced GM in the frontal, temporal, thalamic, and occipital areas. At the voxel-level, this relationship was observed specifically in the caudate, hippocampus, supramarginal, middle cingulate, thalamus, lingual and cerebellum regions.
The group comparison revealed differences between YAs and As in the same areas as the whole-group correlation between GM and age, but only frontal differences were found between As and Ss. Regarding FC, the whole-group analysis yielded a negative association between age and frontal connectivity. However, in the between-group analyses, As presented lesser connectivity than YAs only between parietal and cerebellar areas, whereas connectivity alterations for Ss relative to As were confined to posterior areas. Taken together, our findings suggest that age-related structural and functional brain changes are maximal in the transition from young adulthood into adulthood, and less marked towards seniority, when structural loss is more anterior, but connectivity differences are mainly posterior. In this way, our study provides new insights on the neurobiological correlates of healthy aging.

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Poster

130. Brain Wellness and Aging

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 130.05/P11

Topic: C.01. Brain Wellness and Aging

Title: The impact of age on subareas of the amygdala

Authors: *E. LUDERS[1], N. CHERBUIN[3], F. KURTH[2]
[1]Psychology, [2]Univ. of Auckland, Auckland, New Zealand; [3]Australian Natl. Univ., Canberra, Australia

Abstract: The amygdala has been reported to decrease in size with increasing age and also to differ in size between men and women. However, findings are inconsistent, and studies assessing sex and age effects on different subareas of the amygdala are largely missing. Thus, here we set out to investigate effects of age and sex on the amygdala and its centromedian (CM), laterobasal (LB), and superficial (SF) subareas. Our study sample included 100 healthy subjects (50 men / 50 women) aged between 18 and 69 years. T1-weighted images were preprocessed accordingly, and the resulting gray matter segments were multiplied with cytoarchitectonic probabilities. Ultimately, this yielded a probability-weighted measure of gray matter within each subarea as well as the amygdala (AMG) as a whole. The statistical analysis employed a mass-univariate general linear model with the left / right volumes for CM, LB, SF, and AMG as independent variables; age and sex as dependent variables; and total intracranial volume as nuisance variable. Bonferroni corrections for multiple comparisons were applied. There were significant negative correlations between age and all subareas of the amygdala indicating decreases over time, but with area-specific trajectories. Such regional information may serve as a frame of reference in future studies, not only for normative samples but potentially also for clinical populations known to present with an atypical decline of the amygdala over time. No significant effects of sex were evident, even when omitting the correction for multiple comparisons. This suggests that the size of the amygdala is similar in male and female brains, at least when properly accounting for individual brain volumes.

Disclosures: E. Luders: None. **N. Cherbuin:** None. **F. Kurth:** None.

Poster

130. Brain Wellness and Aging

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 130.06/P12

Topic: C.01. Brain Wellness and Aging

Support: P41EB015922
U54EB020406
R01MH094343

Title: Effects of smoking and alcohol intake on brain ageing

Authors: *K. NING, L. ZHAO, W. MATLOFF, F. SUN, A. W. TOGA
USC, Los Angeles, CA

Abstract: Heavy smoking and heavy alcohol intake have been shown to be associated with morphology of specific brain regions. However, to what extent these factors impact brain aging on a whole-brain level remains unclear. To investigate this, we analyzed smoking, alcohol intake and brain magnetic resonance imaging (MRI) data from around 9,000 cognitively healthy UK Biobank subjects. We first built a statistical model to predict a subject's brain age based on MRI measurements. For each subject, we then computed relative brain age (RBA), a metric that described the degree to which a subject's predicted brain age was older or younger than that of subjects of same chronological age and gender. We further carried out association analyses between RBA and smoking intensity, and between RBA and alcohol intake. We found that both factors were significantly associated with RBA (ANOVA p-value = 9E-8 and 2E-4, respectively). Subjects who had smoked on most or all days had higher brain ageing level (with an average RBA of 0.52 years), while subjects who smoked occasionally had lower brain ageing level (average RBA = -0.23 years). Further, subjects who drank daily or almost daily had higher brain ageing level (average RBA = 0.32 years), while subjects who drank at special occasions only had lower brain ageing level (average RBA = -0.34 years). Our analyses indicated that heavy smoking and heavy drinking were associated with accelerated brain ageing while small amount of smoking and drinking were associated with decelerated brain ageing.

Disclosures: K. Ning: None. **L. Zhao:** None. **W. Matloff:** None. **F. Sun:** None. **A.W. Toga:** None.

Poster

130. Brain Wellness and Aging

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 130.07/P13

Topic: C.01. Brain Wellness and Aging

Title: Effect of aging on the neuronal morphology of the C57BL/6 male mouse

Authors: *E. MONROY HERNÁNDEZ, ESQ[1], F. DE LA CRUZ[1], G. FLORES[2]
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Abstract: Aging leads to a progressive deterioration of cellular functions and organs in general. At the cerebral level, it is associated with a decrease in attention and speed in information processing, with deficits in spatial memory and motor function, that correlate with changes in neuronal morphology, which translate into a reduced dendritic arborization and in the density of dendritic spines. The main objective of this work was to investigate changes in neuronal morphology in the hippocampus, nucleus accumbens (NAcc) and the prefrontal cortex (PFC) in different ages in C57BL/6 male mice. For this, we formed groups of mice of 3, 6, 12 and 18 months mice and we evaluated neuronal morphology by Golgi Cox stain. The preliminary data showed that 18 months mice had neuronal atrophy in the hippocampus and NAcc compared to young mice and there are also differences in the type of spines in the groups studied. Supported by CONACYT grants (280276 to EMH and 252808TO GF).

Disclosures: E. Monroy Hernández: None. **F. De la Cruz:** None. **G. Flores:** None.

Poster

130. Brain Wellness and Aging

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 130.08/P14

Topic: C.01. Brain Wellness and Aging

Title: Extracellular potassium concentration is disturbed in murine models of neurodegenerative disease

Authors: *F. DING[1,2], Q. SUN[1], S. PENG[1], Q. XU[1], J. O'DONNELL[1], M. NEDERGAARD[1]
[1]Univ. of Rochester, Rochester, NY; [2]Tongji Hospital, Tongji Med. Col., Huazhong Univ. of Sci. and Technol., Wuhan, China

Abstract: Neurodegenerative diseases, such as, Huntington disease, Amyotrophic lateral sclerosis, Alzheimer's disease, etc, involved chronic progressive pathological changes in neurons and glial cells. Extracellular $[K^+]$ ($[K^+]_e$) is crucial for maintaining brain physiological functions, and is precisely regulated by $[K^+]$ production and clearance mechanisms that remain incompletely understood. Previously we and other have shown that $[K^+]_e$ is elevated in the striatum of Huntington disease mice brain (R6/2 heterozygotes) versus littermate controls. In current study, we compared the cortical extracellular $[K^+]$ baseline level in vivo in Huntington disease (R6/2) and Alzheimer's disease (APP/PS1), Amyotrophic lateral sclerosis (SOD1) mice models in quiet awake states. The analysis showed that motor cortical $[K^+]_e$ measured at a depth of 300 um was 4.64 ± 0.15 mM in male SOD1 heterozygotes (n=8), versus 4.05 ± 0.08 mM in male littermate controls (n=6) (t test, $P < 0.05$). Using the same protocol, somatosensory cortical $[K^+]_e$ was 4.49 ± 0.08 mM in APP/PS1 heterozygotes (n=5), versus 4.12 ± 0.12 mM in littermate controls (n = 7) (t test, $P < 0.05$). In Huntington disease model, cortical $[K^+]_e$ was 4.01 ± 0.06 mM in R6/2 heterozygotes (n = 17), versus 3.26 ± 0.05 mM in littermate controls (n = 14) (t test, $P < 0.05$) at 600 um below the pial surface. By startling mice with high frequency facial airpuffs, our preliminary data show that significantly higher peak $[K^+]_e$ was induced in 1st startle stimulus in SOD1 heterozygotes and APP/PS1 heterozygotes versus littermate controls respectively. Data on R6/2 mice was not collected due to that R6/2 heterozygotes were easily irritable to startle stimulus. In summary, our data indicates a disturbance in maintaining extracellular $[K^+]$ both in quiet awake and startled-induced states in neurodegenerative disease models.

Disclosures: F. Ding: None. **Q. Sun:** None. **S. Peng:** None. **Q. Xu:** None. **J. O'Donnell:** None. **M. Nedergaard:** None.

Poster

130. Brain Wellness and Aging

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 130.09/P15

Topic: C.01. Brain Wellness and Aging

Title: Effects of moderate prenatal alcohol exposure in rats on GABAergic interneuron expression in the dorsal hippocampus

Authors: *J. T. MADDEN[1,2], S. M. THOMPSON[2], D. A. HAMILTON[2], D. D. SAVAGE[3], B. J. CLARK[2], N. S. PENTKOWSKI[2]
[2]Psychology, [3]Neurosciences, [1]Univ. of New Mexico, Albuquerque, NM

Abstract: Prenatal alcohol exposure (PAE) has been shown to detrimentally impact hippocampal development and impair performance in learning and memory tasks. However, there has been a lack of research describing the neural adaptations in the hippocampus in response to moderate PAE. This study used a rat model to examine the effects of moderate PAE on GABAergic interneuron expression in fields CA1, CA3 and the dentate gyrus of the dorsal hippocampus. Long-Evans dams were given daily access to 5% (vol/vol) ethanol or saccharine control solutions throughout the course of gestation. The offspring were divided into four separate groups: PAE males (n=7), saccharine males (n=7), PAE females (n=8) and saccharine females (n=8). Offspring were aged to adulthood and perfused with 0.9% saline followed by 4% paraformaldehyde. Following cryoprotection brains were sliced to collect 40 μm coronal sections across the regions of interest. Next, slices were stained using the nickel-enhanced 3,3'-diaminobenzidine technique to stain for the calcium binding protein parvalbumin, which is selectively expressed in GABAergic hippocampal interneurons. To determine the GABAergic neuron counts, images of fields CA1, CA3 and the dentate gyrus were taken at 40x magnification using a BX51 Olympus microscope. Using an ImageJ plugin, images from each region were divided into 9x11 grids and diaminobenzidine-positive GABAergic neurons were manually counted across each grid. PAE animals exhibited lower GABAergic interneuronal counts across all regions regardless of sex; however, the only significant differences detected were in CA3 in males, and the DG in females. These results suggest that one mechanism underlying the detrimental effects of moderate PAE on hippocampal dependent tasks involves the loss of dorsal hippocampal GABAergic interneurons.

Disclosures: J.T. Madden: A. Employment/Salary (full or part-time):; Graduate Researcher, University of New Mexico. **S.M. Thompson:** None. **D.A. Hamilton:** A. Employment/Salary (full or part-time):; University of New Mexico. **D.D. Savage:** A. Employment/Salary (full or part-time):; Department of Neurosciences, University of New Mexico. **B.J. Clark:** A. Employment/Salary (full or part-time):; University of New Mexico. **N.S. Pentkowski:** A. Employment/Salary (full or part-time):; University of New Mexico.

Poster

130. Brain Wellness and Aging

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 130.10/Q1

Topic: C.01. Brain Wellness and Aging

Title: The relationship between obesity and brain dysfunction via acceleration of oxidative stress on mice; its prevention by tocotrienols

Authors: *Y. KATO, M. SHIRAI, K. FUKUI
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Abstract: Obesity is a serious public health issue in developed countries, and is known to increase the risk of several diseases such as diabetes, cardiovascular events and arteriosclerosis. These phenomena are closely correlated that neurodegenerative diseases such as Alzheimer's and Parkinson's are also related to oxidative damage. To clarify the relationship between obesity and oxidative brain injury, we investigated brain antioxidant networks in high-fat (HF) diet-treated mice in the presence or absence of tocotrienols (T3s).
In this study, we fed C57BL/6 mice with HF diet (5.24kcal/g) and in order to effect of T3s, we mixed T3-mix in diets for 2 (short-term) or 5 (long-term) months. Food intake and body weight were measured on a weekly basis. After the breeding, we assessed cognitive function of HF diet-treated mice in the presence or absence of T3s by the Morris water maze and rotor rod test. Furthermore, we measured amount of vitamin E, thiobarbituric acid reactive substances (TBARS) level and antioxidant enzyme activities in those mice liver, serum and brain. In addition, we measured antioxidant protein expressions and oxidative stress-related protein expressions by western blotting. We will report the comparison of these results of short- and long-term HF diet-treatment and possibility of anti-obesity effects of T3s.

Disclosures: Y. Kato: None. **M. Shirai:** None. **K. Fukui:** None.

Poster

130. Brain Wellness and Aging

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 130.11/Q2

Topic: C.01. Brain Wellness and Aging

Title: Age-related dysfunction of the cholinergic synapse may cause a decline of the food intake with aging in *Aplysia* kurodai

Authors: *T. NAGAHAMA[1], M. MURAMATSU[2]
[1]Teikyo Heisei Univ/ Fac Hlth. Med. Sci., Tokyo, Japan; [2]Biophysics, Toho Univ. / Fac Phar Sci., Funabashi, Japan

Abstract: In the wild animals of *Aplysia kurodai*, we previously reported that the amount of food intake significantly decreased with age and this may cause weight loss in old animals. We also previously explored the effects of aging on the synaptic function in the feeding neural circuit and showed that the inhibitory post-synaptic response in the jaw-closing (JC) motor neurons induced by the cholinergic buccal multiaction (MA) neurons significantly decreased with age. In the present experiments, we further explored whether the age-related dysfunction of this synapse may affect the patterned jaw movements in the feeding behavior. During ingestion of the food in the mature animals the MA and JC neurons show almost simultaneous rhythmic bursts, but the MA firing delay the firing onset of the JC neurons at each depolarizing phase by inhibiting the JC firing. As previously reported, repetitive electrical stimulation of the esophageal nerve can

drive the basic central pattern generator for the feeding patterned movements of the buccal muscles. In the present experiments, therefore, the rhythmic bursts were induced in the MA and JC neurons by the esophageal nerve stimulation in a given condition and their firing patterns were compared in mature and old animals. In a few old animals, the rhythmic bursts could not be induced by the stimulation, and then we analyzed the data for the old animals representing rhythmic bursts in both neurons. As compared with the mature animals, the period of the rhythmic bursts in both neurons was significantly longer in old animals. In the rhythmic bursts of the JC neurons, there was no statistical difference in the length of the JC depolarization between two ages but the delay of firing onset of the JC at each depolarization was significantly shorter in old animals compared with mature animals. In addition, there was no statistical difference in the frequency and length of the MA firing at each MA depolarization between two ages. These results suggest that the decline in the cholinergic synaptic response with aging may be a major factor changing the firing pattern of the JC neurons with aging, although the aging can be expected to affect many types of synapses. As previously reported, the onset of the JC firing at each depolarizing phase advanced during rejection of aversive seaweed. The present results suggest that the age-related dysfunction of the cholinergic synapse may largely contribute to a decline of the food intake with aging.

Disclosures: M. Muramatsu: None.

Poster

130. Brain Wellness and Aging

Location: SDCC Halls B-H

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Program #/Poster #: 130.12/Q3

Topic: C.01. Brain Wellness and Aging

Support: DVAMC VISN 10 Reseach Initiative Program

Title: The human brain is exposed to aromatic amino acid metabolites linked to the intestinal microbiome

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Abstract: Elevated urinary levels of several phenolic acids, believed to be co-metabolites of the host and intestinal microbiome, have been reported in patients with schizophrenia and autism (Shaw, 2010; Xiong et al, 2016). The kinetics of 3-(3-Hydroxyphenyl)-3-hydroxypropionic acid

(3,3-HPHPA) and related metabolites, as well as their possible bioactivity and relevance to health and disease remain uncharacterized. This is in large part due to the technical challenges of measuring low levels of structurally similar chemicals. For instance, without considering the possible contribution of chirality, there are 10 known structural isomers of 3,3-HPHPA. We have developed a high-performance liquid chromatography - tandem mass spectroscopy (LC-MS) approach that separates and allows quantification of over 70 metabolites, including commercially available structural isomers of 3,3-HPHPA. The method was applied to deidentified samples of human urine and cerebrospinal fluid (CSF). 3,3-HPHPA as well as the structural isomers 2,3-dihydroxyhydrocinnamic acid (2,3-DHHCA), 2,4-DHHCA, 3,4-DHHCA, 3,5-DHHCA were quantified in CSF (means 50 - 600 nM). The identity of 3,3-HPHPA was supported by COSY-NMR, photodiode array UV detection and HPLC-fluorescence studies. The related metabolites 3-(2-Hydroxyphenyl) propionic acid (3,2-HPPA) and 3,3-HPPA were also measured in CSF. In urine, we quantified 2,3-DHHCA, 2,4-DHHCA, 3,4-DHHCA, 3,5-DHHCA, 3,3-HPHPA, 3,2-HPPA and 3,4-HPPA (means 0.4 - 34 nmol/mole creatinine). Since several structural isomers of 3,3-HPHPA are not currently commercially available, unequivocal structural confirmation and the possible presence of additional isomers await further studies. Our data demonstrate that the human brain is exposed to numerous structurally similar, phenolic acid metabolites at concentrations that could be neuroactive. Several studies implicate the human intestinal microbiome, particularly *C. difficile*, in generating some of these metabolites. Our findings provide a potential mechanism through which the gut microbiome could affect brain health and contribute to the pathogenesis and pathophysiology of disorders such as autism and schizophrenia.

Disclosures: G.E. Jaskiw: None. **M.E. Obrenovich:** None. **I.T. Schiefer:** None. **R. Bongiovanni:** None. **L. Li:** None. **C.R. Donskey:** None.

Poster

130. Brain Wellness and Aging

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 130.13/Q4

Topic: C.01. Brain Wellness and Aging

Support: NINDS 1R01NS099595-01A1
NINDS 5K01NS079461
AARGD-16-440893

Title: Reduced levels of brain insulin alters synaptic genes and impairs vibrissae-dependent cognition in a mouse model of hyperinsulinemia

Authors: L. S. WATSON[1], D. WILLIAMS[2], J. BOGGS[2], *C. S. ROBINSON[2,1]
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Abstract: High-fat diet (HFD), the most commonly used experimental model of metabolic disorders, negatively impacts cognitive function. HFD leads to both spatial and object recognition deficits; however, the underlying mechanisms are not known. Our previous studies demonstrated that HFD leads to hyperinsulinemia and impaired hippocampal insulin signaling. Given that insulin is involved in memory and synaptic plasticity, it is imperative to understand the impact of HFD-induced hyperinsulinemia on brain insulin levels. Hence, the purpose of the following studies are to determine HFD-induced alterations in brain insulin signaling and levels and the subsequent synaptic and behavioral deficits. In the current study, at four weeks of age, male B6 mice were placed on either a standard diet (STD) or a HFD ($n \geq 8$ per group). Hippocampal insulin signaling was evaluated following *in vivo* insulin stimulation studies using hyperinsulinemic-euglycemic clamps after 6 weeks of diet. To determine whether impaired insulin signaling was due to brain insulin deficiency, brain insulin levels were measured in the cerebrospinal fluid and hippocampal tissue lysate after 6 and 24 weeks of diet. Furthermore, changes in synaptic plasticity and subsequent behavior were evaluated in STD and HFD mice after 6 and 24 weeks of diet. We observe impairments in downstream insulin signaling in the HFD mice but not in the STD mice compared with naïve mice (non-clamped). HFD mice have reduced levels of insulin in the hippocampus and in the cerebrospinal fluid. This correlates with changes in synaptic genes involved in phosphorylation and glutamatergic neurotransmission. In addition, the HFD mice exhibit deficits in the novel tactile recognition task, analyzed by a blind observer. Our data confirms that chronic hyperinsulinemia, induced by a HFD, leads to impaired insulin signaling and a deficiency of insulin in the brain. This deficiency of insulin correlates with changes in synaptic plasticity that is conducive to facilitating excitotoxicity. Furthermore, given that mice heavily rely on the whiskers for spatial recognition and exploration, we demonstrate that a HFD impairs tactile recognition. Future studies will evaluate the mechanisms involved in brain insulin transport that contribute to HFD-induced brain insulin deficiency.

Disclosures: L.S. Watson: None. **D. Williams:** None. **J. Boggs:** None. **C.S. Robinson:** None.

Poster

130. Brain Wellness and Aging

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 130.14/Q5

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant DP5-OD012178
NIA Grant R01-AG053382
NIA Grant R01-AG055797
ARCs Foundation

Title: Regulation of hippocampal aging by the hematopoietic system

Authors: *L. K. SMITH[1,2], E. VEROVSKAYA[5], K. LIN[1,3], E. PASSEGUE[5], S. VILLEDA[1,2,3,4]
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Abstract: Hippocampal aging is characterized by molecular, structural, and functional changes that lead to decreased regenerative capacity, increased neuroinflammation, cognitive decline, and ultimately increased risk for neurodegenerative diseases. Understanding what drives these changes is therefore of paramount importance in developing therapeutics to slow or even reverse aging in the brain, and thereby extend human healthspan. It is now appreciated that exposure to old blood, and the immune-related blood-borne factors contained therein, drive age-related neuroinflammation, decreased adult neurogenesis, and impaired spatial and episodic memory in young mice. Despite recent evidence suggesting that neuroinflammation, neurogenesis and cognition are all modulated by peripheral immune cells, a gap in knowledge exists as to whether cellular changes in the aged hematopoietic system contribute to the pro-aging effects of old blood. Indeed, the hematopoietic system undergoes complex changes during aging, leading to immunosenescence. These changes are, at least partially, due to cell intrinsic changes in the hematopoietic stem cell (HSC) compartment. Here we report that cellular aging of the hematopoietic system promotes hallmarks of hippocampal aging. Employing the use of isochronic- and heterochronic-HSC transplantation paradigms, in which the hematopoietic systems of young mice are reconstituted by young or old HSCs, respectively, we have observed that exposure to an aged hematopoietic system leads to impaired neurogenesis, increased microglial activation, and decreased synaptic plasticity. Moreover, young heterochronic-HSC transplanted mice exhibited impairments in the contextual fear conditioning paradigm, indicating that the aged hematopoietic system contributes to age-related decline in hippocampal-dependent associative memory. Collectively this work identifies age-related changes in HSCs and corresponding changes in the hematopoietic system as drivers of aging in the brain, identifying a novel therapeutic target for brain rejuvenation.

Disclosures: L.K. Smith: None. **E. Verovskaya:** None. **K. Lin:** None. **E. Passegue:** None. **S. Villeda:** None.

Poster

130. Brain Wellness and Aging

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 130.15/Q6

Topic: C.01. Brain Wellness and Aging

Support: QB3-Calico Longevity Fellowship
NIH grant R01MH104227

NIH grant R01MH109475
NIH grant R01NS078791
NIH grant R01NS091144

Title: Natural aging contributes to synaptic defects and perceptual abnormalities through dysfunction of cortical parvalbumin interneurons

Authors: *C.-C. CHEN, J. LU, Y. ZUO
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Abstract: Aging is a major risk factor for many common neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and vascular dementia. Although much research has emphasized on diseases related to aging, very few studies have focused on how natural aging may influence the behavior and/or neurobiology in the absence of neurodegenerative diseases. Here, we investigated how naturally aged mice perform in a variety of cognitive tasks, including whisker discrimination task, novel object recognition task, and attentional set shifting task. Naturally aged mice exhibit significant behavioral difference compared with young mice, including slowed motoric movements, decreased discriminability, and defects in memory components. Posthoc immunohistochemistry analyses revealed that the inhibitory neuronal networks, specifically the expression of parvalbumin positive interneurons, are considerably decreased in the cerebral cortical areas of naturally aged animals. The overall cortical activities, as measured by immediate early genes c-Fos, were also reduced in the brains of naturally aged mice. Elucidating the underlying circuitry mechanisms of the aged brain provides the first step towards pinpointing the potential therapeutic strategies in reversing the impact of natural aging.

Disclosures: C. Chen: None. **J. Lu:** None. **Y. Zuo:** None.

Poster

130. Brain Wellness and Aging

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 130.16/Q7

Topic: C.01. Brain Wellness and Aging

Support: Alzheimer's Society and BRACE Research Fellowship (208)

Title: Fornix myelin mediates hippocampal aging

Authors: *C. METZLER-BADDELEY[1], J. MOLE[2], R. SIMS[1], F. FASANO[3], J. EVANS[1], R. BADDELEY[4]
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Abstract: Given the global rise of late-onset Alzheimer's disease (AD) it is pivotal to understand the impact of healthy and pathological aging on the hippocampus (HC) and its fornix white matter pathway as they are amongst the first regions to develop AD pathology. At present, the causal relationship between HC and fornix changes remains unclear but may hold important clues about the biophysical mechanisms underpinning healthy and pathological aging. The aim of this study was to investigate age, genetic and lifestyle risk-related changes in the HC and fornix and their relationship with mediation analysis. Individual differences in HC and fornix microstructure were assessed in 166 cognitive healthy individuals at midlife (M_{age} = 55.8, SD = 8.2; 38-71 years of age; 56% female) enriched with genetic risk [38.1% APOE4 carriers, 35% positive family history (FH)] and lifestyle risk factors of AD (related to the metabolic syndrome eg obesity, hypertension, physical activity). Complementary biophysical tissue properties of white and gray matter including apparent axon myelin and density were measured with quantitative multi-modal indices from high angular resolution diffusion (HARDI) and quantitative magnetization transfer (qMT) MRI. Left and right HC were segmented from high resolution T_1- and T_2- weighted images with FreeSurfer 6.0 (https://surfer.nmr.mgh.harvard.edu/). The fornix and the parahippocampal cingulum (PHC) as comparison tract were reconstructed with deterministic tractography based on the dRL spherical deconvolution algorithm in ExploreDTI 4.8.3. Multi-parametric maps derived from diffusion tensor, neurite orientation dispersion and density (NODDI) and qMT fittings were aligned with affine registration to the T_1-weighted images and to all tract and HC masks and average indices were extracted for HC, fornix and the PHC. Aging was associated with significant reductions in myelin sensitive metrics in the HC and the fornix but not with any changes in fornix axon density. No differences were observed in the PHC. Importantly, mediation analysis revealed that age-related differences in fornix myelin mediated age differences in the hippocampus but not *vice versa*. Lifestyle related risk factors of central obesity and alcohol consumption were related to reduced fornix myelin whilst genetic risk factors of FH and APOE genotype affected hippocampal structure. These results provide novel insights into the relationship of age and risk-related changes in the HC and the fornix and indicate a key role of tissue myelination in mediating pathological aging that may contribute to the development of AD.

Disclosures: C. Metzler-Baddeley: None. **J. Mole:** None. **R. Sims:** None. **F. Fasano:** None. **J. Evans:** None. **R. Baddeley:** None.

Poster

130. Brain Wellness and Aging

Location: SDCC Halls B-H

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Program #/Poster #: 130.17/Q8

Topic: C.01. Brain Wellness and Aging

Support: FAPERGS – PQG 02/2017 - 27971.414.15498.22062017

CNPq – Universal - 403029/2016-3

Title: 18FDG-PET Prefrontal hypometabolic activity observed in older adults compared with middle-aged subjects

Authors: *W. V. BORELLI[1], M. A. ANDRADE[2], P. K. FELTES[2], R. B. SODER[2], L. M. HARTMANN[2], C. S. MATUSHITA[2], L. P. SCHILLING[2], A. M. M. DA SILVA[2], C. M. MORIGUCHI-JECKEL[2], M. W. PORTUGUEZ[2], J. C. DACOSTA[1]
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Abstract: INTRODUCTION: Aging is associated with several clinical and imaging alterations, not always representing pathological changes. Herein, we aim to describe regional cortical metabolism with PET-FDG on cognitively healthy older adults compared with middle-aged controls. METHODS: Nine healthy, community-dwelling older adults (7 females, mean age = 83.81; SD = 3.68; range = 80-91)and eleven middle-aged controls (7 females, mean age = 58.50±5.81; range = 51-65) underwent a cognitive examination followed by MRI and PET imaging with ^{18}F-FDG. Medical history of neurologic or psychiatric condition was considered an exclusion criterion and all patients gave a written consent for this study. Cognitive evaluation included the Rey Auditory-Verbal Learning Test (RAVLT), the Mini-mental State Examination (MMSE), and the Reduced Geriatric Depression Scale (GDS). FDG-PET imaging was performed after 4 hours of fasting and capillary glucose levels between 70-90 mg/dL. Mean injected activity was 336.5±77.3 MBq. Static PET images were acquired at 30-60 min interval post-injection, coregistered with volumetric MRI and VOI analysis was performed using PMOD (v3.5). Regional tracer uptake normalized to whole brain tracer uptake (SUVR) was calculated. Groups were compared with independent t-tests using the software RStudio (v1.0.136). Statistical significance was considered when $p<0.001$, after Bonferroni's correction. RESULTS: Older adults showed a significant decrease in delayed-recall score of the RAVLT (6.9±1.6 vs. 10.7±3.3, $p=0.006$) and in MMSE when compared with Controls (28.1±1.3 vs. 29.5±0.7, $p=0.006$), but normal GDS scores (<5 points). Older adults showed similar whole-brain SUV when compared with Controls ($p>0.1$). Some brain areas showed a significant decrease in SUVR in older adults when compared with controls, notably the right inferior frontal gyrus (1.06±0.06 vs. 1.21±0.07, $p<0.001$) and the left presubgenual anterior cingulate (0.91±0.09 vs. 1.08±0.09, $p>0.001$), areas related with selective attention and executive abilities. Some areas presented a small difference between groups, namely orbitofrontal gyrus bilateral, anterior temporal lobe bilateral, left middle and inferior temporal gyrus, left parahippocampal gyrus, left fusiform gyrus and occipital lobe (except cuneus) bilateral ($p>0.1$). CONCLUSION: In the present study, cognitively healthy older adults presented age-related hypometabolic patterns in comparison with middle-aged individuals. Furthermore, medial and lateral frontal areas were particularly vulnerable to the aging process.

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Poster

130. Brain Wellness and Aging

Location: SDCC Halls B-H

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Program #/Poster #: 130.18/Q9

Topic: C.01. Brain Wellness and Aging

Support: F32AG055292
R01AG053382
R01AG055797

Title: The aging hippocampal microenvironment drives microglial activation

Authors: *J. SHEA[1], J. BOUCHARD[2], P. B. VENTURA[2], S. A. VILLEDA[3]
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Abstract: Inflammation is one of the hallmarks of brain aging, linked to cognitive decline and dementia-related neurodegenerative disorders. It is essential to elucidate mechanisms underlying inflammation in the aged brain, as a means to develop strategies to combat its deleterious effects on cognitive function. Microglia, the resident innate immune cells of the central nervous system, mediate neuroinflammation and play essential roles in the establishment and refinement of neuronal circuits. In particular, microglia incorporate signals from the microenvironment, derived from both the brain parenchyma and systemic environment, to initiate appropriate responses in support of neuronal function. On the other hand, microglial dysfunction adversely affects neuronal and cognitive functions, including processes susceptible to aging such as hippocampal learning and memory. With the importance of microglia to brain homeostasis in mind, we set out to determine how the microenvironment influences microglial aging. Using RNA-sequencing of bulk hippocampus, we find that genes specifically expressed in microglia increase in expression during aging, with many of these genes integral to inflammatory processes. At a cellular level, we find that the inflammatory activation of microglia originates in the hilus of the dentate gyrus, and spreads to other regions of the hippocampus with advancing age. In search of the factors leading to microglial activation, we used a heterochronic parabiosis model to find that systemic factors from aged mice lead to the activation of young hippocampal microglia. Additionally, we developed a heterochronic microglia transplantation model, whereby we stereotactically inject microglia from newborn UBC-GFP pups into the hippocampi of young and old recipients. The results from transplanting microglia corroborated the parabiosis findings, indicating that young microglia are responsive to the old hippocampal microenvironment. Transcriptional profiling of the transplanted microglia showed that the aged microenvironment led to increased expression of several disease-related genes, while subsets of immune-related genes were either controlled by the microenvironment or cell-autonomously. Lastly, we

demonstrate that systemic exposure to young blood resolves the inflammatory state of microglia in the aged brain at both the transcriptional and cellular level. These results underscore the influence of the aging microenvironment on microglia, and will provideinsights into what drives the microglial aging that contributes to the vulnerability of the aged brain to functional decline.

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Poster

131. Alzheimer's Disease and Other Dementias: Abeta, Tau, and Neurodegeneration

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 131.01/Q10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: BK21 PLUS
NRF-2016R1C1B2010206

Title: TRIMosome: The platform for key components regulating core stages of autophagy

Authors: *M. S. LEE[1], S.-Y. JUNG[2], H. HEO[1], J. CHANG[1,2]
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Abstract: Autophagy is a fundamental intracellular process for clearance of unwanted or damaged materials accumulated in the cell. Autophagy has been also tightly linked to the pathogenesis of various neurodegenerative diseases, including Alzheimer's disease (AD) and Parkinson's disease. The autophagic process is initiated by formation of phagophore mediated by sequential recruitment of specific protein complexes. The phagophore matures into the autophagosome which engulfs autophagic cargos such as cytoplasmic proteins and organelles. Autophagy is finally completed by the degradation of the materials at the autolysosome which is formed by the fusion of the autophagosome and the lysosome. Each step of autophagy is spatially and temporally regulated by various groups of proteins. Tripartite motif (TRIM) proteins are a subfamily of the RING-type E3 ubiquitin ligase family. Recent studies have suggested that several TRIM proteins can act as scaffolds of a functional complex, called TRIMosome, composed of key autophagic regulators implicated in multiple stages of the autophagic process. Here, we aimed to find the detailed mechanism of TRIM-mediated autophagic regulation especially focused on one of TRIM proteins which is specifically related to the genetic risk of AD. Our results suggest that autophagic role of TRIMosome could be linked to the onset or progression of neurodegenerative diseases.

Disclosures: S. Jung: None. **H. Heo:** None. **J. Chang:** None.

Poster

131. Alzheimer's Disease and Other Dementias: Abeta, Tau, and Neurodegeneration

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 131.02/Q11

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: City University of Hong Kong grant 7200484
Research Grants Council grant 904807

Title: The role of aminoacyl-tRNA synthetases in neurological diseases: More than translation

Authors: *S. HUANG[1], D.-J. HAN[2], J. LEE[2], S. KIM[2], J. KIM[1]
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Abstract: For the past decade, aminoacyl-tRNA synthetases (ARSs), enzymes catalyzing the ligation of amino acids to their cognate tRNAs, have been addressed as the pathophysiological implications in human diseases such as cancers and neurological diseases. Although accumulated studies have provided mechanisms of how ARSs are involved in tumorigenesis, it is still not clear how these enzymes are linked to neurological diseases. Here, using systematic analysis approaches, we provide new insight into ARSs as the potential pathophysiological factors of neurological diseases. In diverse neurological diseases including Alzheimer’s disease (AD), we found that gene expression patterns of ARSs and their associated proteins were changed. In addition, neurological disease-associated interaction network revealed that ARSs were directly or indirectly involved in both disease-specific and shared pathological pathways. For example, in AD, this analysis suggests amyloid precursor protein catabolic process and learning/memory as the disease-specific pathways and response to oxidative stress and autophagy as the shared pathological pathways with other neurological diseases. Next, we validated the analysis results in animal models of neurological diseases by qRT-PCR and confirmed that the analysis results were highly consistent with the experimental results. Therefore, we believe that this unexpected regulatory network of ARSs provides researchers with new direction to better understand the pathogenic processes of neurological diseases and presents a potential therapeutic opportunity in the near future.

Disclosures: D. Han: None. **J. Lee:** None. **S. Kim:** None. **J. Kim:** None.

Poster

131. Alzheimer's Disease and Other Dementias: Abeta, Tau, and Neurodegeneration

Location: SDCC Halls B-H

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Program #/Poster #: 131.03/Q12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: FP7 GA ERC-2012-SyG_318987–ToPAG

Title: Studying toxic protein aggregation with amyloid-like proteins in primary neurons and a novel mouse model

Authors: *I. RIERA TUR[1], D. HORNBURG[2], L. GARRET[3], S. HOELTER-KOCH[3], M. MANN[4], F. MEISSNER[4], R. KLEIN[1], I. DUDANOVA[1]
[1]Max Planck Inst. of Neurobio., Martinsried, Germany; [2]Stanford Univ., Stanford, CA; [3]German mouse clinic, Munich, Germany; [4]Max Planck Inst. of Biochem., Martinsried, Germany

Abstract: Unraveling common underlying mechanisms of aggregation toxicity in neurodegenerative diseases may enable the therapeutic targeting of a whole range of disorders. In this work, we use artificial proteins (termed β4, β17, and β23) rationally designed to form amyloid-like aggregates as a model to investigate the toxicity mechanisms of protein misfolding *in vitro* and *in vivo* in the absence of potential loss-of-function effects. Expression of mCherry-tagged artificial amyloid-like proteins in primary neurons lead to the appearance of cytoplasmic aggregates, impairment of neuronal morphology, and neuronal cell death. To identify molecular candidates involved in the underlying mechanisms of β-sheet induced cell death, we performed interactome analysis. Interestingly, β-sheet proteins interact with multiple proteins that have essential cellular functions, as showed by Crispr/Cas9 knockout and viability analysis. Moreover, overexpression of selected candidates from the interactome screen partially rescued β-sheet effects on neuronal viability. To study protein aggregation *in vivo*, we have generated a novel reversible β23 transgenic mouse model of neurodegeneration, based on the Tet-off system. After crossing the β23 mice to the activator CamKIIα:tTa mouse line, adult double transgenic mice express β23 specifically in the forebrain, including regions especially interesting for neurodegenerative diseases, such as cortex, hippocampus, and striatum. Importantly, we observed progressive brain atrophy in adult β23 expressing mice, as well as moderate learning and memory defects. Altogether these results suggest that expression of an artificial aggregating protein is sufficient to induce a neurodegenerative phenotype. Therefore, the β23 transgenic mouse model represents a useful tool for deciphering gain-of-function effects of protein aggregation.

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Poster

131. Alzheimer's Disease and Other Dementias: Abeta, Tau, and Neurodegeneration

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 131.04/Q13

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA PO1AG14449
RO1AG043375
P30AG010161

Title: HDAC2 nuclear protein reduction within cholinergic basal forebrain neurons is associated with NFT formation during the progression of Alzheimer's disease

Authors: *L. MAHADY[1], S. PEREZ[1], M. NADEEM[1], M. MALEK-AHMADI[2], K. CHEN[2], J. MIGUEL[1], E. J. MUFSON[1]
[1]Barrow Neurolog. Inst., Phoenix, AZ; [2]Banner Alzheimer's Inst., Phoenix, AZ

Abstract: Cholinergic basal forebrain (CBF) neurons within the nucleus basalis of Meynert (nbM), which provide the major source of acetylcholine to the entire cortical mantle, degenerate and display neurofibrillary tangles (NFTs) early in the onset of AD. The balance between histone acetyltransferases and histone deacetylases (HDACs) regulates choline acetyltransferase (ChAT) expression, the synthesizing enzyme for acetylcholine, and HDAC2 accumulates in neurons in preclinical AD [Braak stages (I/II)]. Thus HDAC dysregulation may be an early event related to the selective vulnerability of CBF neurons. Whether alterations in HDAC2 nuclear levels in CBF neurons occur during the early or late stages of NFT formation remains unknown. Here we quantified changes in HDAC2 immunoreactivity within CBF neurons by triple immunohistochemistry using antibodies directed against $p75^{NTR}$ (an excellent marker for CBF neurons), AT8 (pretangle), TauC3 (late stage), and dual staining for AT8/Thioflavin-S, and TauC3/Thioflavin-S from tissue obtained from subjects who died with a premortem clinical diagnosis of NCI (n=5), MCI (n=5), mild/moderate AD (n=5) and severe AD (n=5) from the Rush Religious Orders Study (RROS) and the Rush RADC, respectively. Groups were matched by age and postmortem interval (PMI=5 hr) and underwent detailed postmortem neuropathologic evaluations. HDAC2-ir was significantly decreased in single labeled CBF neurons across disease progression ($p<0.001$, NCI>mAD, sAD; MCI>mAD, sAD; mAD>sAD). However, in each clinical group $p75^{NTR}$ neurons displayed higher HDAC2 compared to AT8, TauC3, AT8/Thioflavin, or TauC3/Thioflavin positive neurons ($p<0.05$). CBF AT8 or TauC3 positive neurons displayed a decrease in nuclear HDAC2-ir across clinical groups ($p<0.001$, NCI, MCI, mAD>sAD). HDAC2-ir was also decreased in AT8/Thioflavin-S and TauC3/Thioflavin-S stained neurons ($p<0.001$, NCI>sAD, MCI>mAD>sAD; NCI, MCI, mAD>sAD, respectively). In MCI, HDAC2-ir was greater in CBF neurons that were AT8, TauC3 or AT8/Thioflavin-S

positive compared to TauC3/Thioflavin-S perikarya ($p<0.05$). In mAD, HDAC2-ir was higher in $p75^{NTR}$ neurons dual stained for AT8 or TauC3 compared with $p75^{NTR}$ neurons triple stained for AT8/Thioflavin-S or TauC3/Thioflavin-S NFTs ($p<0.05$) These data indicate that CBF neurons display reduced HDAC2 nuclear immunoreactivity during AD progression. The reduction in CBF HDAC2-ir in neurons containing AT8, TauC3, and Thioflavin-S in prodromal AD suggests that drugs which enhance HDAC2 and reduce pathological tau may prevent CBF neuronal degeneration during the onset of AD.

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Poster

131. Alzheimer's Disease and Other Dementias: Abeta, Tau, and Neurodegeneration

Location: SDCC Halls B-H

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Program #/Poster #: 131.05/Q14

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: MINECO grant # SAF2016-75768-R

Title: Stereological assessment of neuronal and glial changes in the human hippocampus in Alzheimer’s disease

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Abstract: Alzheimer’s disease is the most prevalent neurodegenerative disease worldwide. Once cognitive deficits appear and patients are diagnosed, the brain has suffered irreversible damage decades before. Therefore, early diagnosis is a challenge at the present. The hippocampus is one of preferentially involved brain areas and it is deeply involved in memory formation. In the last decade, several studies using image techniques have described a reduction of the hippocampus volume related with disease progression and focused on grey matter. However, whether neurons, astroglia or neuroglia are involved in this volume decline is still unclear. Moreover, the role of glia in promoting or stopping disease progression is nowadays a controversial issue. To shed some light on this issue, we have measured the hippocampal volume using Nissl stain and Cavalieri method and we have stereologically quantified neurons, microglia and astroglia through immunohistochemical stain for Neu-N, Iba-1 and GFAP, respectively. We used 10 postmortem brains from Alzheimer’s disease diagnosed patients and 10 age-matched controls provided by three different brain banks: IDIBAPS (Barcelona), BTCIEN (Madrid), and BIOBANC-MUR (Murcia) Biobanks. Experimental procedures were approved by the Ethical

Committee of Clinical Research at Ciudad Real University Hospital (SAF2016-75768-R). Results confirm a volume reduction in the hippocampus and point to a reduction in astroglial cell population in contrast with increased number of microglial cells in Alzheimer's cases. In addition, no changes in the number of neurons was reported. These results indicate a preferential vulnerability of astrocytes in the hippocampus in Alzheimer's disease. This work is supported by the Spanish Ministry of Economy and Competitiveness-FEDER (grant # SAF2016-75768-R).

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Poster

131. Alzheimer's Disease and Other Dementias: Abeta, Tau, and Neurodegeneration

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 131.06/R1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH RO1 AG054025
NIH RO1 AG055771
NIH RO1 NS094557
George & Cynthia Mitchell Center for Neurodegenerative Diseases

Title: Nuclear tau, p53 aggregation, and impaired DNA damage response in Alzheimer's disease

Authors: *K. FARMER[1], M. MONTALBANO[2], G. GHAG[2], N. PUANGMALAI[2], N. BHATT[2], S. A. MCALLEN[2], R. KAYED[2]
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Abstract: DNA damage is one of the earliest pathological changes in Alzheimer's disease (AD) and a reduction in the DNA damage response significantly exacerbates neurodegeneration. Data from our laboratory showed that both ataxia telangiectasia mutated (ATM) and functional phospho-ATM levels were significantly decreased in AD brain tissues compared to control tissues. Other downstream markers including CHK2 and liver kinase B1 (LKB1) were also impaired, implicating DNA damage response disruption in AD. The homotetrameric tumor suppressor, p53, another important target of ATM, acts as a master regulator of cell cycle control, apoptosis, and DNA repair. While previous research has repeatedly shown an increase in total p53 protein levels in AD cases, an investigation into the functional levels and structural form of p53 have not been well investigated. Moreover, we found aggregated p53 in AD human brain tissue and Tg2576 mouse tissue. It is known that aggregated p53 can undergo seeding and cross-seeding similar to other amyloidogenic proteins, like tau, α-synuclein, etc. This led us to speculate whether there may be an interaction between aggregated p53 and/or aggregated tau, specifically nuclear tau, as it has been shown to be one of the important players in DNA damage

response. Preliminary studies of human AD tissue shows colocalization between tau oligomers and p53. Our findings suggest that tau oligomers may be interacting with p53 and causing a down regulation of functional p53 in AD. This may suggest that tau may be playing a role in DNA damage response signaling and therefore affecting cell death. These results may have implications for a number of other neurodegenerative disorders. Therefore, further research is needed to understand the underlying signaling pathways of tau and p53 interaction.

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Poster

131. Alzheimer's Disease and Other Dementias: Abeta, Tau, and Neurodegeneration

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 131.07/R2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01 NS085070
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Gerstner Family Career Development Award
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Title: Increase of the mitophagy marker phospho-ubiquitin in Alzheimer’s disease

Authors: *X. HOU[1], F. C. FIESEL[1,2], M. E. MURRAY[1,2], D. W. DICKSON[1,2], W. SPRINGER[1,2]
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Abstract: Dysfunctional mitochondria are common and among the earliest events observed in the progression of Alzheimer’s disease (AD). Mitophagy, a cargo-specific autophagy-lysosomal pathway for removal of damaged mitochondria, constitutes a key cellular pathway in mitochondrial quality control. Failure of mitophagy is a feature of both familial and sporadic AD and appears to play an early key role in AD pathophysiology. Both Aβ/APP and tau have been shown to induce mitochondrial stress directly or indirectly through alterations of the flux through the autophagy-lysosome system. Upon mitochondrial stress, PINK1 and Parkin cooperatively label damaged mitochondria with phosphorylated ubiquitin (pS65-Ub) to tag them for

destruction. The selective clearance of damaged organelles ensures the integrity and function of the mitochondrial network thereby preventing cell death. We have generated antibodies against pS65-Ub as specific and sensitive tools to monitor and quantify mitophagy in neurons and in brain tissue. Here, we set out to quantify pS65-Ub in human post-mortem brain sections from age-matched neurologically normal and pathological aging controls as well as from early and late stage AD patients. On the whole tissue level, we observed significant increases of pS65-Ub levels in both early and late stage AD across selectively vulnerable regions. The increase of the mitophagy tag was closely associated with tau pathology and granulovacuolar degeneration bodies (GVBs) that are thought to be autophagic remnants of incomplete degradation. On the single cell level, and similar to GVBs, pS65-Ub levels appeared to increase early on with tangle development, but disappeared in cells with fully mature tau pathology. Altogether, our study provides novel hints to potential mechanisms underlying the mitophagy deficits in AD and suggests that pS65-Ub may serve as an early marker and quantitative trait to identify modifiers of disease pathogenesis.

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Poster

131. Alzheimer's Disease and Other Dementias: Abeta, Tau, and Neurodegeneration

Location: SDCC Halls B-H

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Program #/Poster #: 131.08/R3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH grant AG054719
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Alzheimer’s Association NIRG-339422

Title: Dendritic spine pathology links tauopathy mouse models to Alzheimer’s disease

Authors: *C. K. WALKER[1], B. D. BOROS[1], K. M. GREATHOUSE[1], K. A. CURTIS[1], R. RAMDAS[1], J. H. HERSKOWITZ[2]
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Abstract: Alzheimer’s disease (AD) is the most common cause of dementia and by 2050, approximately 14 million people will be living with AD in the United States. AD is classified as a tauopathy, a disease where the primary pathology is aggregation of the microtubule-associated protein tau. The Tau P301S (Line PS19) transgenic mouse model is commonly used to study tauopathies, including AD. Neuronal synapse or dendritic spine loss correlates more strongly with cognitive impairment than classical pathologic hallmarks of AD, and a recent study by our

group indicated that detrimental changes in dendritic spine density and morphology among AD patients correlated strongly with neurofibrillary tangle pathology, but not Aβ plaques. Therefore, we sought to explore the contribution of tau pathology to dendritic spine changes in AD by determining whether the PS19 mouse line recapitulates the spine alterations that occur in dementia patients. Tau P301S and non-transgenic (NTG) littermates aged 6-9 months underwent behavioral testing, including elevated plus maze, Y maze, open field, and passive avoidance test. Individual pyramidal neurons in the prefrontal cortex, hippocampus, and entorhinal cortex were targeted for iontophoretic microinjection of Lucifer yellow fluorescent dye, followed by high-resolution confocal microscopy and neuronal three-dimensional reconstructions for morphometry analysis. Tau P301S mice exhibited memory deficits in the passive avoidance test as well as abnormalities in open field, including increased ambulatory distance, in comparison to age and sex-matched NTGs. Behavioral deficits in the Tau P301S mice correlated with alterations in dendritic spine density and morphology in the prefrontal cortex, hippocampus, and entorhinal cortex. Additional statistical analyses showed similarities and differences in dendritic spine morphologic profiles among Tau P301S mice and AD patients.

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Poster

131. Alzheimer's Disease and Other Dementias: Abeta, Tau, and Neurodegeneration

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 131.09/R4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R43-OD023025

Title: Advancing Alzheimer's disease animal modeling: Intrathecal administration of amyloid-beta oligomers (ABOs) in the African green monkey

Authors: *J. D. ELSWORTH, M. R. WEED[1], M. S. LAWRENCE[1], E. N. CLINE[2], K. L. VIOLA[2], W. L. KLEIN[2], S. E. PEREZ[3], E. J. MUFSON[3], K. C. WILCOX[4], P. B. JACOBSON[4], D. R. WAKEMAN[1]
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Abstract: No effective remedies exist to halt the inexorable progression of Alzheimer’s disease (AD). A principal obstacle to developing new treatment strategies is the inadequacy of available preclinical models to predict clinical outcomes. While transgenic mice are the mainstay of AD animal research, their shortcomings have likely contributed to the lack of clinical efficacy of promising candidate interventions identified in such models. As nonhuman primate (NHP)

neurobiology shares unparalleled homology to humans in virtually all respects relevant to AD modeling, we decided to refine an inducible NHP model of AD based on administration of soluble amyloid-beta oligomers (ABOs) (Forny-Germano et al., 2014). We chose to use the St Kitts African green monkey which are free of major primate pathogens and naturally develop pathological features of AD. ABOs play a major role in provoking an AD-like pathological cascade that includes induction of phosphorylated tau (p-tau), leading to neurofibrillary tangles, amyloid plaques, loss of synapses and neurons, inflammation and cognitive decline. To produce a reliable NHP model for AD, it is necessary to evaluate in statistically meaningful group sizes the influence of critical experimental parameters, such as the effect of ABO dose, frequency and route of administration, and to define the expression, spread and persistence of induced pathology, together with confirming changes in cognition and documenting alterations in CSF and plasma biomarkers. We determined that repeated intracerebroventricular injection of ABO to sedated animals or intrathecal delivery of ABOs at the spinal lumbar level of awake animals effectively induces an AD-like pathology in brain regions implicated in AD. The latter procedure has the benefit of involving a less invasive and more reproducible surgery. Intrathecal administration of 200 micrograms of standardized ABO, but not vehicle, 3-times a week for 4 weeks to young adult NHPs induced elevated expression of p-tau in the medial temporal cortical memory circuit (e.g., hippocampus and entorhinal cortex), which persists for at least a month after the last injection, detected by different tau antibodies. Immunostaining also revealed the emergence of neuropil amyloid precursor protein aggregations with an increase in microgliosis in hippocampus and entorhinal cortex following ABO administration. In this NHP model, ABOs induce pathological features similar to those seen in sporadic AD and should be a valuable resource to advance our understanding of AD pathogenesis and enable testing of novel diagnostic and therapeutic strategies in a manner that will significantly de-risk clinical trials.

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Poster

131. Alzheimer's Disease and Other Dementias: Abeta, Tau, and Neurodegeneration

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 131.10/R5

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: PO1AG14449
P30AG010161

Title: Braak stage, cerebral amyloid angiopathy, and cognitive decline in early AD

Authors: *M. MALEK-AHMADI[1], K. CHEN[1], S. E. PEREZ[2], E. J. MUFSON[2]
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Abstract: Braak stages I to VI delineate the progression of neurofibrillary tangle (NFT) pathology from least to greatest within the medial temporal lobe memory circuit and it relation to cognitive impairment in Alzheimer's disease (AD). Interestingly, Braak stage III is associated with a spectrum of clinical diagnoses including no cognitive impairment (NCI), mild cognitive impairment (MCI), and AD. Although the factors underlying this heterogeneity are unclear, cerebrovascular pathology may have a moderating effect in the development and severity of cognitive decline in early Braak stage cases. The aim of this study was to determine the interaction between cerebral amyloid angiopathy (CAA) and Braak stage on cognition. We evaluated a total of 141 (72 NCI, 33 MCI, 36 AD) cases from the Rush Religious Order Study cohort, divided into two Braak groups (0-II vs III), and compared on several domains of cognition. CAA ($p = 0.31$) and Braak Stage groups ($p = 0.12$) were evenly distributed among the clinical groups. Braak stage III was significantly more likely to have CAA [OR = 2.47, 95% CI (1.12, 5.44), $p = 0.02$] after adjusting for clinical diagnosis, age at death, sex, education, and APOE ε4 status. The interaction between Braak stage and CAA status on cognition was tested in a series of linear regression models adjusted for age at death, sex, education, APOE ε4 status, and CERAD neuropathological diagnosis. The interaction between Braak stages and CAA status was statistically significant for global cognition ($\beta = -0.58$, SE = 0.25, $p = 0.02$). Groupwise comparisons of this interaction found that among those with CAA, the Braak III group had significantly lower performance than the Braak 0-II group ($p = 0.04$). The episodic memory domain also showed a significant effect for the Braak by CAA interaction ($\beta = -0.75$, SE = 0.35, $p = 0.03$). However, semantic memory ($p = 0.13$), working memory ($p = 0.12$), visuospatial function ($p = 0.13$), and perceptual speed ($p = 0.33$) were not affected by the Braak and CAA interaction. Braak stage III cases had significantly higher neuritic plaque load ($p<0.001$), even after adjusting for age at death, sex, education, clinical diagnosis, APOE ε4 status, and CAA ($\beta = 0.77$, SE = 0.38, $p = 0.04$). Our data lend support to the suggestion that Braak stage III represents a transition phase and highlight a putative modulatory role for CAA upon the neuropathological and clinical progression of AD.

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Poster

131. Alzheimer's Disease and Other Dementias: Abeta, Tau, and Neurodegeneration

Location: SDCC Halls B-H

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: RO1 AG043375
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Brightfocus Foundation
P50 AG16573
R01 AG21912
R01 HD065160

Title: Neurofibrillary tangle evolution in the frontal cortex of demented and non-demented subjects with down syndrome

Authors: *S. E. PEREZ[1], J. C. MIGUEL[1], M. NADEEM[1], M. N. SABBAGH[1], I. T. LOTT[2], E. DORAN[2], E. MUFSON[1]
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Abstract: Although all individuals with Down syndrome (DS) exhibit an age-related increase in Aβ plaque and tau neurofibrillary tangle (NFT) pathology, not every case develops dementia. We found that phosphorylated NFT pathology in the frontal cortex was greater in demented compared to non-demented DS, while Aβ pathology was similar in both groups, suggesting that tau pathology plays a greater role in the dementia seen in DS. The present study examined the appearance of phosphorylation, truncation and conformational posttranslational tau epitopes in frontal cortex pyramidal layers V-VI neurons in age-matched DS without dementia (n=6) and DS with dementia (DS-D) (n=10) using immunofluorescence combined with quantitative analysis. Triple immunofluorescence was performed using antibodies against early and late tau markers: phosphorylated AT8 (1:50), early phosphorylated pre-tangle pS422 (1:50), conformational Alz50 (1:50) or truncated TauC3 (1:50) epitopes. Quantitation revealed that the number of AT8+pS422+Alz50, TauC3+pS422+Alz50, pS422+Alz50 and TauC3+pS422 positive NFTs were significantly higher in DS-D compared to DS, suggesting a differential evolution of frontal cortex NFT formation in DS-D. A within group analysis revealed that cortical AT8+pS422+Alz50 positive NFT numbers were significantly greater than pS422+Alz50 NFTs in DS with and without dementia, while AT8+pS422+Alz50 NFTs were greater than AT8+Alz50 NFTs in DS-D, but not in DS. Numbers of NFTs reactive for TauC3+pS422+Alz50 were significantly greater than those displaying pS422+Alz50 and TauC3+Alz50 in DS-D. TauC3+pS422 positive NFTs were significantly greater than pS422+Alz50 NFTs in DS-D. Conversely no differences were found between double or triple labeled NFTs containing TauC3 in DS. In conclusion, the large number of pS422, Alz50, TauC3 as well as pS422+TauC3 NFTs in DS-D compared to DS, suggest that truncation at glutamic 421 (TauC3) and phosphorylation at serine 422 (pS422) are differentially altered between DS groups. In addition, the observation that NFT numbers containing Alz50+pS422 and Alz50+TauC3 are lower compared to AT8+pS422+Alz50 and TauC3+pS422+Alz50 NFTs respectively, in DS and DS-D, indicates that phosphorylation and truncation changes precede conformational tau events in DS.

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Poster

131. Alzheimer's Disease and Other Dementias: Abeta, Tau, and Neurodegeneration

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 131.12/R7

Topic: C.05. Tauopathies, Tau-dementias, and Prion diseases

Support: NINDS
Dept. of Veterans Affairs
FQRS

Title: Subgenual anterior cingulate white matter and depressive etiology in chronic traumatic encephalopathy

Authors: *I. MAHAR[1], S. E. RIND[1], R. MATHIAS[1,2], J. D. CHERRY[1], A. C. MCKEE[1,2]
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Abstract: BACKGROUND
Chronic traumatic encephalopathy (CTE) is a neurodegenerative disease involving cognitive, behavioral, and psychiatric symptoms. CTE involves accumulation and progressive spread of hyperphosphorylated tau (pTau), which has been associated with neuroinflammation. However, the etiology of depressive CTE symptoms is still unclear. Among brain regions of interest, Brodmann area (BA) 25 (subgenual cingulate) white matter has been implicated in depression and suicide, yet has not been examined in relation to CTE.
METHODS
Fixed BA25 samples were obtained from the VA-BU-CLF Brain Bank (N=43), including controls and CTE cases with and without depressive symptoms. Sections were stained for glial, neuronal, inflammatory, and pathological markers. Stained slides were digitally scanned and traced, and staining was quantified using a Leica Aperio system.
RESULTS
Preliminary results indicated that numbers of IBA1-expressing cells in BA25 white matter were highly increased in CTE cases relative to controls. For the first time in this region, AT8 (indicating pTau) and AB4G8 (indicating amyloid beta) staining were identified in CTE cases, and pathological marker staining was significantly increased for depressed suicides in particular.
CONCLUSIONS
These are the first findings indicating microgliosis, inflammation, and pathological hallmarks in BA25 white matter for CTE cases, suggesting that localized changes in these markers may underlie depressive phenotypes in CTE. We are currently increasing sample size to strengthen these preliminary findings, as well as assessing white matter integrity in this region.

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Poster

131. Alzheimer's Disease and Other Dementias: Abeta, Tau, and Neurodegeneration

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 131.13/R8

Topic: C.05. Tauopathies, Tau-dementias, and Prion diseases

Title: Juvenile rats as a model to screen tau kinase inhibitors with sub-optimal brain exposure characteristics

Authors: *M. CALHOUN[1], K. KING[2], M. ROONEY[2], R. GRATER[2], O. GOLONZHKA[2], C. ROWBOTTOM[2], G. M. DILLON[1]
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Abstract: Tau hyper-phosphorylation and the formation of neurofibrillary tangles are established as hallmarks in the post-mortem diagnosis of both Alzheimer's disease (AD) and Frontotemporal Dementia (FTD). Therefore, inhibition of tau kinases could prove to be a potential therapy for AD and other tauopathies. Prior research has demonstrated that phosphorylated tau is significantly elevated in the developing rat brain and, as a disease model, juvenile rats have advantages over transgenic AD models in that both tau and endogenous tau kinases are not overexpressed. In addition, we demonstrate that brain exposure characteristics for compounds with less than ideal exposure is more favorable in young rats than in adults. This suggests that juvenile rats may be a useful tool for evaluating small molecule tau kinase inhibitors.In the first series of experiments we wanted to confirm that postnatal day 10 (P10) rats would serve as a useful model of tau hyper-phosphorylation. The rats were treated with the small molecule inhibitor of tau phosphorylation, AZD1080, and western blot analysis of brain lysates obtained after 1, 3, 6, and 24 hours of treatment revealed moderate reductions in tau phosphorylation at several disease-relevant sites. Interestingly this study demonstrated significantly higher brain exposure of AZD1080 than expected based on a) in vitro MDR1-MDCK substrate assays and b) in vivo PK studies performed on adult rats. To follow up, we designed an additional series of experiments to characterize the pharmacokinetic profile of a Biogen tau kinase inhibitor (here called BG1) in rats at different developmental stages. Rats at P10, P20, and P60 (adult) were treated with BG1, as well as loperamide (a known PGP substrate) and dantrolene (a known BCRP substrate). Plasma and brains were harvested 0.25, 0.5, 1, 3, 6, and 24 hours following administration and drug concentrations were determined by reverse phase HPLC-MS/MS. In addition, equilibrium dialysis experiments were performed to investigate developmental changes in unbound Br:Pl partitioning, Kp,u,u. Our results show that plasma and brain AUC of all three compounds was highest in P10 rats with exposures in P10 rats ranging from approximately 2.4-

to >10-fold higher. A trend towards increased Br:Pl partitioning in P10 rats was observed for the two molecules known to be PgP substrates, BG1 and loperamide, with Br:Pl ratios 2- to 3 fold higher in P10 rats. These results suggest that juvenile rats present an opportunity to achieve unbound brain levels suitable for evaluating proof-of-biology or PK/PD relationships with tool compounds lacking optimal drug-like characteristics.

Disclosures: M. Calhoun: A. Employment/Salary (full or part-time):; Biogen. **K. King:** A. Employment/Salary (full or part-time):; Biogen. **M. Rooney:** A. Employment/Salary (full or part-time):; Biogen. **R. Grater:** A. Employment/Salary (full or part-time):; Biogen. **O. Golonzhka:** A. Employment/Salary (full or part-time):; Biogen. **C. Rowbottom:** A. Employment/Salary (full or part-time):; Biogen. **G.M. Dillon:** A. Employment/Salary (full or part-time):; Biogen.

Poster

131. Alzheimer's Disease and Other Dementias: Abeta, Tau, and Neurodegeneration

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 131.14/DP04/R9

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH National Institute on Aging (AG005134)
NIH National Institute on Aging (AG036694)

Title: Pathologic correlations of *in vivo* [18F]-AV-1451 imaging in autopsy-confirmed Alzheimer's disease and control cases

Authors: *C. AGUERO[1], M. DHAYNAUT[2], M. D. NORMANDIN[2], N. GUEHL[2], K. NGUYEN[3], V. FLEMING[3], A. C. AMARAL[3], D. H. OAKLEY[4], S. N. GOMPERTS[3], K. A. JOHNSON[3], M. P. FROSCH[4], T. GOMEZ-ISLA[3]
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Abstract: Background: The development of novel PET tracers tailored to detect tau in the brain has opened the opportunity of using them to improve diagnostic accuracy in Alzheimer’s disease (AD) and related tauopathies and to quantify tau pathology burden in the human living brain. Emerging data from early studies -including our own- on legacy postmortem material with the most validated thus far, [18F]-AV-1451 (T807, Flortaucipir), have shown that this ligand binds with strong affinity to paired helical filament (PHF)-tau aggregates in AD brains, and those that form as a function of age. But our data also suggest that AV-1451 is not exempt of pitfalls. AV-1451 has relatively low affinity for tau aggregates in non-AD tauopathies and exhibits strong off-target binding to neuromelanin- and melanin-containing cells, and some weaker binding to

hemorrhages. Goal:To examine the correlation between *in vivo* [18F]-AV-1451 retention and postmortem *in vitro* binding and tau lesion load in multiple regions of interest (ROIs) in brains from three individuals scanned while alive who have come to autopsy (one mixed AD/Lewy body disease, one primary progressive aphasia due to AD and one control free of AD pathology). Methods: Comparison of in vivo measures of [18F]-AV-1451 relative standardized uptake values (SUVR) as measured by PET in multiple ROIs with autoradiographic signal and quantitative tau measurements in matching ROIs at postmortem (burden of tau aggregates in immunostained tissue and measurements of different soluble tau species in whole tissue homogenates and synaptic/cytosolic enriched fractions as detected by Western Blot and ELISA). Results: Autoradiography experiments confirmed strong binding of AV-1451 to brain slices containing neurofibrillary tangles in the two AD cases. Slices from the control brain free of neurofibrillary pathology showed no AV-1451 signal with the exception of incidental binding to layer II entorhinal cortex (age-related tangles) and the substantia nigra (off-target binding). Detailed comparisons of *in vivo* [18F]-AV-1451 retention in multiple ROIs and quantitative pathological and biochemical tau measurements in matching ROIs at postmortem in the three cases are ongoing. Conclusion: Neuroimaging-pathologic correlation studies conducted on postmortem material from individuals imaged while alive are the gold standard to accurately interpret tau neuroimaging in clinical settings, and to unequivocally confirm the potential utility of tau PET tracers for the reliable detection/quantification of tau aggregates and disease-progression tracking.

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Poster

131. Alzheimer's Disease and Other Dementias: Abeta, Tau, and Neurodegeneration

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 131.15/R10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R21-AG058859
K01-AG051718

Title: Tau PET imaging with ^{18}F-PI2620 in aging and Alzheimer’s disease

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Abstract: Background: Measurement of the spatial distribution of Tau pathology is critical for early diagnosis and disease monitoring. We sought to investigate a novel Tau PET ligand, ^{18}F-PI2620 throughout the spectrum of Alzheimer's disease (AD).
Methods: Seventeen participants within known Amyloid status (via CSF or Amyloid PET) underwent Tau PET scanning with ^{18}F-PI2620 on a GE PET-MRI scanner: ten older clinically normal (CN) individuals (five Amyloid- CN, mean age=72.6±4.0; and five Amyloid+ CN, mean age=72.2±6.8), and six clinically impaired patients on the AD trajectory (mean age=65.0±8.2; three Amyloid+ Mild Cognitive Impairment and three Amyloid+ AD dementia). Standardized uptake value ratios were computed 60-90 minutes post-injection and normalized to the inferior cerebellum. We examined target regions known to show high Tau uptake in AD (entorhinal cortex, hippocampus, amygdala, inferior temporal cortex, precuneus, and lateral parietal cortex). Group differences (Amyloid- CN vs. Amyloid+ CN vs. Amyloid+ Impaired) in regional Tau were assessed with Wilcoxon signed-rank tests, whereas associations between continuous CSF measures (Aβ42 and pTau) with regional Tau within the CN group were assessed with Spearman's Rank correlation coefficients.
Results: Compared to Amyloid- CN, Amyloid+ CN showed greater PI2620 uptake in entorhinal cortex, hippocampus, and amygdala (p-values<0.032). The Amyloid+ Impaired group showed elevated Tau in all regions compared to Amyloid- CN (p-values<0.008), as well as elevated Tau in inferior temporal cortex (p= 0.016), precuneus (p<0.001), and lateral parietal cortex (p<0.001) compared to the Amyloid+ CN group (Figure 1). Within the CN group, continuous levels of CSF Aβ42 were negatively associated with elevated Tau PET in entorhinal cortext (p=0.026), hippocampus (p=0.004), and amygdala (p=0.007). CSF pTau was not related to any regional Tau PET value (Figure 2).
Conclusions: Preliminary results suggest strong differences in cortical uptake of ^{18}F-PI2620 in Amyloid+ impaired patients compared to older CN. Amyloid related differences among the CN were detected in medial temporal lobe regions. This work suggests promise for ^{18}F-PI2620 in detecting Tau aggregation throughout the course of AD.

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Poster

131. Alzheimer's Disease and Other Dementias: Abeta, Tau, and Neurodegeneration

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 131.16/R11

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Belfer Foundation
TARCC
Jeffry M. and Barbara Picower Foundation
HHMI

Title: Identifying therapeutic targets for Alzheimer's disease: A cross-species genetic screen for molecules that lower tau levels

Authors: *J. KIM[1], I. AL-RAMAH[1,2], M. DE HARO[1,2], L. GARAICOECHEA[2], H.-H. JEONG[1,2], J. REVELLI[1,2], M. ROUSSEAUX[1,3], Q. XU[4], S. ELLEDGE[4], J. BOTAS[1,2], H. ZOGHBI[1,2]
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Abstract: Tau is a central causative agent in the pathogenesis of Alzheimer's Disease (AD) and various dementias. Data from several studies indicate that the brain is particularly vulnerable to toxicity resulting from increased expression of aggregation-prone proteins. Conversely, reduction of tau levels has proven effective in AD mouse models. Thus, lowering tau levels might mitigate disease progression and provide an attractive target for therapy in AD and other dementias. However, to date, very few suppressors of tau levels have been identified. To identify novel regulators of tau, we employed a cross-species high-throughput screen. We used a pooled shRNA-retrovirus library targeting 7,532 "druggable" human genes in genetically engineered Daoy cells overexpressing a tau: EGFP. Cells with shRNAs that decreased tau levels were separated by flow cytometry and the bar-coded shRNAs identified by deep sequencing. In parallel, we conducted a siRNA screen in *Drosophila* targeting 4,000 fly homologs of the human genes tested. Our combined primary screens and *in vitro* validation in HEK293T cells led to the discovery of 91 potential tau modifiers. For *in vivo* validation in the mouse, we performed postnatal day zero intracerebroventricular (ICV) injection of AAV-shRNA to knockdown 19 prioritized targets and measured tau protein levels. Among these, gene suppression of 12 hits, including an ubiquitin specific protease (USP7), reduced tau protein levels by more than 20 percent. Interestingly, Usp7 interacts with tau and promote its deubiquitination, presenting a potential mechanism by which tau is stabilized. These results establish our cross-species high-throughput screen as a powerful approach to identify new targets involved in the regulation and processing of tau. Understanding the molecular mechanisms that underlie tau regulation will provide a foundation for identifying new potential therapeutics for AD as well as candidate risk genes.

Disclosures: I. Al-Ramah: None. **M. de Haro:** None. **L. Garaicoechea:** None. **H. Jeong:** None. **J. Revelli:** None. **M. Rousseaux:** None. **Q. Xu:** None. **S. Elledge:** None. **J. Botas:** None. **H. Zoghbi:** None.

Poster

131. Alzheimer's Disease and Other Dementias: Abeta, Tau, and Neurodegeneration

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 131.17/R12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH grant 5R01NS095922-02

Title: Post-transcriptional regulation of tau proteostasis by miR-219

Authors: *I. SANTA-MARIA
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Abstract: Intracellular accumulation of hyperphosphorylated misfolded tau proteins is one of the main hallmarks in many neurodegenerative diseases but what mechanism causes this accumulation is not completely understood. Alterations of microRNA-219-5p (miR-219) levels in human brain tissue have been recently linked to Alzheimer's disease (AD) and primary age-related tauopathy. Thus, abnormal accumulation of toxic tau species and neurodegeneration could be exacerbated by dysregulation of miR-219. However, the extent to which dysregulation of miR-219 directly alters tau proteostasis by modifying tau expression, phosphorylation or both is currently unknown. We have previously found in a *Drosophila* model of neurodegeneration caused by human tau gain of toxic function that reduction of miR-219 exacerbated tau toxicity, while overexpression of miR-219 partially abrogated toxic effects. Moreover, bioinformatic analysis indicates miR-219 is also predicted to target Glycogen synthase kinase 3 beta (GSK3β), Calcium/calmodulin-dependent protein kinase 2 gamma subunit (CAMKIIγ) and tau tubulin kinase 1 (TTBK1), which are all implicated in the generation of abnormal hyperphosphorylated tau. In human neuronal cells, miR-219 directly binds to the tau and tau kinases mRNA 3' untranslated region and silences its expression at the post-transcriptional level. We further show that suppression of miR-219 by tough decoys causes an upregulation of tau, GSK3β, CAMKIIγ, and TTBK1 protein synthesis and tau hyperphosphorylation. Conversely, overexpression of miR-219 reduce synthesis of Tau protein and Tau kinases. Accordingly, in rat primary neurons, suppression of miR-219 increases tau synthesis and tau phosphorylation at specific sites. Finally, we have also observed miR-219 overexpression prevents neurotoxicity. Our work expands the current knowledge regarding microRNA's role in neurodegeneration. Together, our findings identify a major role for miR-219 in the regulation of tau expression and phosphorylation contributing to the pathogenesis of AD and related tauopathy.

Disclosures: I. Santa-Maria: None.

Poster

131. Alzheimer's Disease and Other Dementias: Abeta, Tau, and Neurodegeneration

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 131.18/R13

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NRF-2015M3A9E2028884
HR14C0002

Title: miR-200c deficiency promotes hyperphosphorylation of tau through up-regulation of 14-3-3γ in 5xFAD mice model of Alzheimer's disease

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Abstract: Alzheimer’s disease (AD) is a neurodegenerative disease characterized by impaired cognitive function and the deposition of extracellular amyloid plaques and tau phosphorylation. Micro-RNAs (miRNAs) are non-coding RNA molecules with a length of 18-25 nucleotides, which serve as post-transcriptional regulators of gene expression. More recently, a number of dysregulated miRNAs have been identified in AD patients and more studies are being conducted to correlate these pathological change. Here, the purpose of this study is to identify miRNAs that are abnormally expressed and to investigate whether they affect AD pathological changes in 5xFAD AD mice model. Cognitive impairment and pathological changes were confirmed in 4-month-old 5xFAD mice. We found that miR-200c was reduced in the hippocampus of 4-month-old 5xFAD mice using microarray and real-time PCR. Expression of miRNAs in the hippocampus of 4-month-old 5xFAD mice were analyzed using microarray and real-time PCR. The target molecules of miR-200c was knowns to target 14-3-3γ through the miRbase online site. 14-3-3 family is known to phosphorylate microtubule-associated tau protein. MiR-200c has been shown to regulate 14-3-3γ using the dual-luciferase reporter gene assay in HEK-293 cells. Also, 14-3-3γ protein and phosphorylated Tau was increased in the hippocampus of the 5xFAD mouse. We confirmed that 14-3-3γ protein increased by inhibiting miR-200c in primary hippocampal neurons. Taken together, our results suggests that dysregulation of miR-200c expression contributes to the pathogenesis AD by cognitive impairment and hyperphosphorylated tau.

Disclosures: H. Park: None. **E. Nam:** None. **S. Kang:** None. **Y. Suh:** None. **K. Chang:** None.

Poster

132. Alzheimer's Disease and Other Dementias: APP/Abeta: Animal and Cellular Models II

Location: SDCC Halls B-H

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Program #/Poster #: 132.01/R14

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant P30 NS045776
NIH Grant AG048080
NIH Grant AG15379
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Cure Alzheimer's Fund
Bright Focus Foundation

Title: Exploring Aβ-regulated protein kinase signaling cascades in a 3D human cell culture model of Alzheimer's disease

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Abstract: The pathological hallmarks of Alzheimer's disease (AD) are extracellular amyloid- β (Aβ) deposition and intracellular neurofibrillary tangles (NFTs). According to the Aβ cascade hypothesis, excess accumulation of toxic Aβ peptides triggers pathological features such as synaptic/neuronal dysfunction and hyper-phosphorylation of tau proteins. However, the underlying signaling cascades which connect Aβ accumulation to tau hyper-phosphorylation has not been fully elucidated. In this study, we used a three-dimensional (3D) human neural cell culture model of AD to explore the signaling pathways that regulate Aβ-driven tau pathology. Previously, we have shown that a 3D human cell culture model of AD can recapitulate robust Aβ deposition (Aβ plaques) and Aβ-induced NFTs pathology without overexpressing tau protein with Fronto Temporal Lumbar Degeneration (FDLD) mutations (Choi *et al.* 2014; Nature 515, 274). In an effort to explore the underlying mechanisms, we performed an unbiased phospho-kinase array assay to identify kinases that are selectively regulated by Aβ accumulation in the 3D culture model of AD. We found four kinases, p38, p70S6K, RSK and WNK1, are significantly altered in AD cells as compared to control. The alterations of phosphorylation were reversed by BACE1 inhibitor, LY2886712 (1μM), or γ-secretase modulator, SGSM36 (500 nM), treatment. We focused more on WNK1 (**W**ith-**N**o-Lysine (**K**) 1) kinase, as WNK1 has not been previously studied for mediating Aβ pathology. We observed that the phosphorylation/activation levels of WNK1 were increased by 3.7-fold in 3D-differentiated AD cells compared to the controls. Consistent with this finding, phosphorylated WNK1 kinase levels were increased by 1.75-fold in brains of AD patients as compared to age-matched controls. WNK1 plays important roles on

regulating neuronal Cl^- ion homeostasis and thereby regulates GABAergic inhibitory circuits, and excitotoxicity driven by excessive Ca^{2+} influx. Indeed, treatments with WNK1 inhibitor, WNK463 (20 μM), reversed early Ca^{2+} influx in our 3D cell culture model of AD, which supports a role of WNK1 kinase on AD pathogenesis, by contributing to the disturbance of Ca^{2+} homeostasis. In summary, our study showed that 1) human 3D culture model of AD can be useful for exploring pathogenic cascade of AD in human brain-like environment and 2) also suggests WNK1 activation as a potential pathogenic cascade leading to neuronal deficits and possibly tau pathology.

Disclosures: S. Kwak: None. **K.J. Washicosky:** None. **J. Aronson:** None. **R.E. Tanzi:** None. **D. Kim:** None.

Poster

132. Alzheimer's Disease and Other Dementias: APP/Abeta: Animal and Cellular Models II

Location: SDCC Halls B-H

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BrightFocus Foundation

Title: A 3D model of Alzheimer's disease using clonal human neural progenitor cells

Authors: *K. J. WASHICOSKY, J. L. ARONSON, S. KWAK, J. PARK, D. VON MAYDELL, K. BRENNER, S. CHOI, R. E. TANZI, D. KIM
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Abstract: Previously, we developed a three-dimensional (3D) human neural cell culture model of Alzheimer’s disease (AD) using immortalized human neural progenitor cells (hNPCs) expressing amyloid β precursor protein (APP) and presenilin 1 (PS1) with multiple familial AD (FAD) mutations. However, these engineered hNPC populations are heterogeneous and have variable familial AD gene expression that limit their use for drug discovery and basic mechanistic studies. Here we describe an improved 3D culture model using clonal hNPCs expressing uniform levels of AD genes with multiple FAD mutations. Clonal AD cells were selected by FACS-assisted 96-well single-cell sorting, expanded/differentiated and screened for

pathogenic Aβ secretion using Western blot analysis and ELISA. Expression levels of APP and pathogenic Aβ levels are stable across multiple passages (>20) in these clonal AD lines and differentiate into neurons and glial cells under 2D and 3D conditions. We also found clonal AD cells express increased levels of pathogenic Aβ42 as compared to parental heterogeneous AD hNPCs. 3D-differentiated clonal AD cells exhibit accelerated accumulation of insoluble Aβ and phospho-tau species as compared to previous 3D AD models using heterogeneous AD cells (4-7 weeks vs 6-14 weeks). More importantly, we found that insoluble phospho- and total-tau accumulations correlate with Aβ42/40 ratio, not total Aβ levels. Finally, we performed unbiased RNA-seq analysis in control and 3D AD cell cultures with different Aβ42/40 ratios. Canonical pathway analysis of differentially expressed genes between 3D-differentiated control and AD cells showed significantly enriched pathways including glutamate receptor signaling, synaptic long-term potentiation/depression, cAMP/CREB signaling, LPS/IL1 and RXR, which overlap with previously reported pathogenic cascades of AD. These results demonstrate that our 3D AD cell culture system using clonal hNPCs can provide a valid model for AD drug screening and basic mechanical studies.

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Poster

132. Alzheimer's Disease and Other Dementias: APP/Abeta: Animal and Cellular Models II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

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Topic: C.02. Alzheimer's Disease and Other Dementias

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NIH P01 AG015379
NIH RF1 AG048080
NIH R01 AG014713

Title: The impact of APP TMD mutations on AB42/40 ratio and AB/tau pathology in 3D human neural cell culture model on Alzheimer's disease

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Abstract: Amyloid-β42 (Aβ42) is thought to be a key mediator of AD pathogenesis. However, the impact of Aβ42, Aβ42/40 ratio and other Aβ42 species on Aβ/tau pathology has not been

fully studied in human neuronal cells. To modulate either Aβ42/40 ratio and other Aβ species, we overexpressed amyloid β precursor protein (APP) with transmembrane domain (TMD) mutations that can modulate Aβ48-45-42 or Aβ49-46-43-40 sequential cleavage cascade in human neural stem cells and then differentiated these cells in 3D Matrigel culture conditions for 4-6 weeks. Previously, we showed that 3D-differentiated human neural stem cells harboring familial mutations in APP and presenilin 1 (PS1) display robust aggregation of Aβ (Aβ-plaque like) and phospho/total tau (neurofibrillary tangle (NFT) -like. We analyzed soluble and insoluble Aβ38/40/42 species, phospho-tau and total tau levels using ELISA and immunofluorescence staining. We found that I45F APP TMD mutations robustly increased Aβ42/40 ratio, insoluble Aβ accumulation and more importantly, phospho-tau accumulation in 4 weeks. Interestingly, cells harboring I47F, which blocks Aβ45-42 generations, do not show insoluble Aβ accumulation nor phospho tau accumulation. We are currently exploring the impact of additional APP TMD mutations on Aβ and tau pathology. Our study shows that the 3D human neural cell culture models with various APP TMD mutations can provide a system to explore pathogenic contribution of different Aβ species.

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Poster

132. Alzheimer's Disease and Other Dementias: APP/Abeta: Animal and Cellular Models II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 132.04/R17

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: the Intramural Research Programs of the NIA and NIAAA, NIH.

Title: Evidence that mitochondrial sirt3 protects the brain against Alzheimer’s disease by preventing aberrant neuronal hyperexcitability

Authors: *A. CHENG[1], N. GHENA[1], J. WANG[1], T. KING[2], R. VEECH[2], R. WAN[1], M. P. MATTSON[1]
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Abstract: Studies of human patients and experimental cell culture and animal models of Alzheimer’s disease (AD) suggest that an excitatory imbalance occurs in neuronal circuits of brain regions affected with amyloid β-peptide (Aβ) pathology, and that Aβ renders neurons vulnerable to mitochondrial dysfunction and excitotoxicity (*EMBO J.* 2015; 36:1474-1492). We

recently reported that the mitochondrial protein deacetylase SIRT3 protects neurons against excitotoxic and metabolic stress by mechanisms involving enhanced removal of mitochondrial superoxide and inhibition of apoptosis (*Cell Metab.* 2016; 23:128-142). AD patients exhibit a marked reduction in SIRT3 levels in vulnerable, but not in minimally vulnerable brain regions, suggesting a potential role for diminished SIRT3 in AD pathogenesis. We generated APP/PS1 double mutant transgenic AD mice with SIRT3 haploinsufficiency (APP/PS1 SIRT3+/-) and found that they suffer severe epileptic seizures and die within 5 months of age. EEG recordings revealed progressive aberrant subclinical spiking that was apparent several weeks before the onset of seizures and typical seizure bursting activity in the APP/PS1 SIRT3+/- mice. At this early age, APP/PS1 AD mice exhibited much less such neuronal hyperexcitability. Preliminary immunohistological analyses indicate that there is a reduction in numbers of parvalbumin- and calretinin-positive GABAergic interneurons in the frontal cortex of APP/PS1 SIRT3+/- mice. In addition, we found that the levels of SIRT3 are reduced in APP/PS1 SIRT3+/- mice compared to SIRT3+/- mice, and that exposure of cultured cerebral cortical neurons to aggregating Aβ reduces SIRT3 expression. Moreover, SIRT3 deficient neurons exhibit increased vulnerability to Aβ-induced death. Finally, feeding APP/PS1 SIRT3+/- mice a ketone ester diet increases SIRT3 expression and prevents their early death. Our findings show that, by constraining neuronal excitability, mitochondrial SIRT3 protects neuronal networks against dysfunction and degeneration in experimental models of AD. Interventions that increase SIRT3 expression and/or activity (exercise, fasting, ketone ester, NAD^+ precursors) would therefore be expected to protect the brain against AD.

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Poster

132. Alzheimer's Disease and Other Dementias: APP/Abeta: Animal and Cellular Models II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 132.05/R18

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: CYP51-sensitive cellular cholesterol pool increased by PS1 FAD mutation is associated with APP localization into lipid rafts

Authors: *Y. CHO, O.-H. KWON, S. CHUNG
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Abstract: Mutations in presenilin genes (PS1 and PS2) cause early onset familial Alzheimer's disease (FAD). Presenilin serves as a catalytic subunit of γ-secretase complex, and its mutation is responsible for the selective increase of Aβ42 over Aβ40. In addition to its proteolytic role, PS1

is involved in APP trafficking as well as caveolin and flotillin, which are components of lipid raft structures. Furthermore, either PS deficient and mutant cells elevate cholesterol level due to the increased expression of CYP51, which plays a critical role for the cholesterol synthesis. Since high cholesterol level is a well known risk factor for AD and lipid rafts has been considered as a platform for Aβ generation, there may exist addictive effects of FAD PS1-associated cholesterol elevations on cellular APP processing. In this study we investigate the effect of CYP51-sensitive cholesterol pool in PS1 mutant on APP distribution and Aβ generation. We found CHO PS1 ΔE9 mutant cell shows increased CYP51 expressions, which leads increased cellular cholesterol levels. We also showed that PS1 mutation is associated with a significant shift in localization of APP toward cholesterol-enriched lipid rafts. Reducing the cholesterol levels to the comparable level of WT cells by inhibitor of CYP51 significantly reduced lipid raft-associated APP as well as selectively decreased secreted Aβ42 from PS mutant cells. Our study suggests that CYP51-associated cholesterol elevation in PS1 mutant may contribute to the altered APP processing by increasing APP distribution in lipid rafts.

Disclosures: Y. Cho: None. **O. Kwon:** None. **S. Chung:** None.

Poster

132. Alzheimer's Disease and Other Dementias: APP/Abeta: Animal and Cellular Models II

Location: SDCC Halls B-H

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Program #/Poster #: 132.06/S1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Pennsylvania Dept. of Health CURE Award (420792 to J.W.E.).
NIH grant R01HL136954

Title: Genetic rescue of mitochondrial calcium efflux in Alzheimer’s disease preserves mitochondrial function and protects against cognitive decline and neuropathology

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[1]CTM, Temple Univ., philadelphia, PA; [2]Alzheimer’s Ctr. at Temple, Dept. of Pharmacol., Temple Univ., Philadelphia, PA

Abstract: Background. Alzheimer’s disease (AD) is characterized by the progressive loss of neuronal populations in the cortex and hippocampus. Numerous studies have suggested that impairments in neuronal intracellular calcium ($_{i}Ca^{2+}$) handling may be a primary contributor to AD development. In addition, metabolic dysregulation and progressive neuronal demise are cellular mechanisms shown to contribute to AD progression. Recently, our lab and others have shown that mitochondrial calcium ($_{m}Ca^{2+}$) signaling is a regulator of both of these cellular

processes. **Methods.** To discern if $_{m}Ca^{2+}$ exchange is causative in the development and/or progression of AD, we generated neuronal-specific genetic loss- and gain-of-function mice targeting the mitochondrial Na^{+}/Ca^{2+} exchanger (NCLX) in 3xTg-AD mice. These mice were examined for alterations in memory, amyloidosis, tau-pathology and oxidative stress. In addition, a neuroblastoma cell line stably expressing the human Swedish mutant amyloid precursor protein (N2a/APP*swe*) were examined for mitochondrial alterations in: Ca^{2+} handling, ROS generation, oxidative phosphorylation, amyloid beta production and cell death. **Results.** We found that impairment in $_{m}Ca^{2+}$ efflux precedes disease development in multiple experimental models of AD including human AD brain samples, 3xTg-AD mice and N2a/APPswe cell lines. Neuronal-specific deletion (Camk2a-Cre) of the mitochondrial Na^{+}/Ca^{2+} exchanger (NCLX) accelerated memory decline and increased amyloidosis, tau-pathology and oxidative stress in 3xTg-AD mice. Further, genetic rescue of neuronal NCLX (transgenic neuronal expression) in 3xTg-AD mice was sufficient to impede AD-associated pathology and memory loss. Mechanistically, we show that $_{m}Ca^{2+}$overload is a primary contributor to AD progression by promoting superoxide generation, metabolic dysfunction and neuronal cell death. Rescue of $_{m}Ca^{2+}$ extrusion in N2a/APP*swe* cells, via adenoviral expression of NCLX, enhanced the clearance of pathogenic $_{m}Ca^{2+}$, enhanced OxPHOS, decreased amyloid beta aggregation and beta secretase-1 (BACE1) activity and protected from ionomycin-, glutamate- and ROS-induced cell death. **Conclusions.** Our data suggest that impaired $_{m}Ca^{2+}$ efflux is a central contributor to neuronal cell death in AD and that NCLX represents a new therapeutic target to inhibit AD progression.

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Poster

132. Alzheimer's Disease and Other Dementias: APP/Abeta: Animal and Cellular Models II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 132.07/S2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Deutsche Forschungsgemeinschaft (SFB 870), to A.K.
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Title: A cellular mechanism of amyloid β-induced neuronal hyperactivity

Authors: *B. ZOTT[1,2], M. SIMON[1,2], J. HARTMANN[1,2], B. SAKMANN[1], A. KONNERTH[1,2]
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Abstract: Accumulating evidence, both from observations in mouse models and humans, indicates that an early dysfunction in Alzheimer's disease (AD) is an amyloid-β (Aβ)-dependent excessive level of activity, often referred to as 'hyperactivity', in a subset of neurons. In the *in vivo* hippocampus, hyperactive neurons are found in mouse models of AD prior to the formation of amyloid plaques, while, in wild-type mice, the application of soluble Aβ-dimers can directly trigger hyperactivity. Furthermore, hyperactivity can be efficiently prevented by the pharmacological inhibition of the production of Aβ. However, the cellular mechanism(s) underlying hyperactivity remained unknown.

During our initial experiments, we were puzzled by the fact that Aβ application can induce activity in hippocampal CA1 neurons *in vivo* but not in *in vitro* hippocampal slices. We then discovered that the ineffectiveness of Aβ in brain slices was due to the very low levels of spontaneous activity in such *ex vivo* preparations. Several lines of evidence support the notion of an activity-dependence of the Aβ action on neurons. Under *in vivo* conditions, Aβ application is ineffective when suppressing neuronal activity through blocking synaptic excitation by antagonists of glutamatergic transmission (CNQX, APV) or when blocking neuronal firing with TTX. Under *in vitro* conditions, Aβ can activate neurons only after raising the level of spontaneous neuronal activity to that observed *in vivo.* This can be achieved through manipulations such as raising the level of external K^+, applying glutamate or blocking GABAergic inhibition. What is the cellular mechanism underlying the activity-dependence of the Aβ-induced hyperactivity? In line with previous suggestions based on *in vitro* work, we found *in vivo* evidence that Aβ blocks glutamate reuptake. First, Aβ has a similar hyperactivity-inducing effect as the glutamate-reuptake blocker TBOA. Second, the hyperactivity-inducing action of Aβ can be occluded by the application of TBOA and vice versa. Third, the simultaneous application of glutamate receptor antagonists can abolish both the hyperactivity-inducing effect of TBOA or Aβ. Fourth, both the TBOA or Aβ effects are saturated in transgenic AD mice with high intrinsic levels of soluble Aβ.

In conclusion, we provide evidence that the mechanism of the pronounced activity-dependence of the Aβ-induced neuronal hyperactivity is a block of reuptake of synaptically released glutamate. Our results provide a mechanistic explanation for the observation that in early AD, especially brain areas with a high level of baseline activity and connectivity are highly susceptible to Aβ-induced functional impairment.

Disclosures: B. Zott: None. **M. Simon:** None. **J. Hartmann:** None. **B. Sakmann:** None. **A. Konnerth:** None.

Poster

132. Alzheimer's Disease and Other Dementias: APP/Abeta: Animal and Cellular Models II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 132.08/S3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: E07631.01

Title: Gender differences in olfactory dysfunction in a rat transgenic model of Alzheimer's disease

Authors: *E. CUEVAS, S. LANTZ, A. GUZMAN-LOPEZ, H. ROSAS-HERNANDEZ, J. RAYMICK, S. SARKAR
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Abstract: Olfactory dysfunction (OD) generally occurs at early stages in Alzheimer's disease (AD), with a prevalence of approximately 90%. Accumulation of amyloid beta (Aβ) and hyper-phosphorylated tau (pTau) proteins has been observed in the olfactory bulb (OB) of AD patients. However, little is known about potential gender differences in OD in AD pathology even though AD is more prevalent in women. In the current study, those AD-related proteins in the OB were quantified in a rodent AD model. The OB of Tg AD rats from both sexes, along with their wild type counterparts, were dissected and collected across different ages (6-24 months). The protein expression of APP (Aβ Precursor Protein), Aβ1-42 (mOC64; conformation specific), as well as pTau and the receptor for advanced glycation end products (RAGE), were evaluated using Western blot. Protein levels of APP, Aβ1-42, RAGE, and pTau were 1.5 - 2-fold higher in female rats compared to males from 9-12 months. Those levels were also significantly higher in female Tg rats compared to same-sex wild type animals from 9-12 months. In Tg male rats, APP levels were elevated from 13-17 months, without no changes in Aβ1-42 levels; RAGE levels, however, were elevated from 9-12 months, while pTau levels increased from 18-24 months, all relative to same-sex wild type rats. These data suggest that: 1) OD appears earlier in the AD progression of females than males in the Tg model of AD, and 2) that a direct correlation exists between key proteins and degeneration in female Tg AD rats. These data provide insight into gender-specific temporal expression of altered proteins in AD.

Disclosures: E. Cuevas: None. **S. Lantz:** None. **A. Guzman-Lopez:** None. **H. Rosas-Hernandez:** None. **J. Raymick:** None. **S. Sarkar:** None.

Poster

132. Alzheimer's Disease and Other Dementias: APP/Abeta: Animal and Cellular Models II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 132.09/S4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Research Funding for Longevity Sciences (28-27, 28-37) from the National Center for Geriatrics and Gerontology (NCGG), Japan.

Title: Elevated membrane cholesterol aggravates endocytic disturbance, resulting in enhanced Abeta accumulation: A potential mechanism underlying exacerbation of Abeta pathology by type 2 diabetes mellitus

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Abstract: Endocytic membrane trafficking system is altered in the brains of early-stage Alzheimer's disease (AD) patients, and endocytic disturbance affects the metabolism of beta-amyloid protein (Abeta), a key molecule in AD pathogenesis. It is widely accepted that type 2 diabetes mellitus (T2DM) is one of the strongest risk factors for development of AD. Evidently, experimentally induced T2DM enhances AD pathology in various animal models. Our previous study showed that T2DM enhances Abeta pathology, even in nonhuman primate brains and that age-related endocytic disturbance is also aggravated in T2DM-affected monkey brains. However, it remains unclear how T2DM exacerbates endocytic disturbance to enhance Abeta pathology. Here, we demonstrate that cholesterol synthesis-related genes are upregulated and that membrane cholesterol level is elevated in T2DM-affected monkeys. Moreover, our chemical treatment studies reveal that the manipulation of cellular cholesterol level disrupts lysosomal degradation and aggravates chloroquine-induced endocytic disturbance, resulting in increased accumulation of Abeta in neuronal cells. These findings suggest that alteration of cerebral cholesterol metabolism may be responsible for the exacerbation of age-related endocytic disturbance in T2DM-affected brains, which in turn may increase the risk for developing AD accompanied by enhanced Abeta pathology.

Disclosures: N. Kimura: None. **S. Takeuchi:** None. **N. Ueda:** None. **K. Suzuki:** None. **N. Shimozawa:** None. **Y. Yasutomi:** None.

Poster

132. Alzheimer's Disease and Other Dementias: APP/Abeta: Animal and Cellular Models II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 132.10/S5

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01AG030142
NCI CCSG P30 CA060553

NIH 1S10OD010398-01

Title: The role of CRAC channels in neuritic dystrophy in Alzheimer's disease

Authors: *K. R. SADLEIR[1], J. POPOVIC[1], A. SOMASUNDARAM[2], M. PRAKRIYA[2], R. VASSAR[1]
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Abstract: BACE1 is the β-secretase enzyme that initiates Aβ production and is a prime therapeutic target for Alzheimer's disease (AD). Drugs that inhibit BACE1 enzyme activity are in clinical trials for AD, but early termination of a recent trial raises concerns regarding safety and efficacy of these agents. Animal studies suggest that BACE1 inhibition may cause multiple neurological side effects. Thus, it is crucial to develop alternative therapeutic strategies that reduce BACE1 cleavage of APP without impairing essential BACE1 functions. We have shown that global BACE1 protein levels are markedly elevated in APP transgenic mouse and AD brains. Elevated BACE1 is concentrated within dystrophic axons and terminals surrounding amyloid plaques, and is associated with increased generation of BACE1-cleaved APP fragments and Aβ42. Our preliminary results show that Aβ elevates resting $[Ca^{2+}]i$ in primary neurons via Ca^{2+} release-activated Ca^{2+}(CRAC) channels. Moreover, we observe that axons of Aβ-treated primary neurons exhibit disrupted microtubules and impaired BACE1 axon transport. Peri-plaque dystrophic axons in 5XFAD mice also show elevated resting $[Ca^{2+}]i$ and disrupted microtubules. We hypothesize a feed-forward mechanism in which plaque-associated Aβ causes axonal dystrophy, BACE1 accumulation, and accelerated Aβ generation that drives amyloid progression.
To test the role of CRAC channels in dystrophic neurite formation, BACE1 accumulation and amyloid generation, we have generated a cohort 5XFAD mice with or without the conditional deletion of Orai1, the pore forming subunit of the CRAC channel, in the excitatory neurons of the forebrain using an iCre transgene driven by the CaMKII promoter. At six months of age, brains from 5XFAD Orai1 flox/flox CaMKII iCre and 5XFAD Orai1 flox/flox control mice are stained with Lamp1 and BACE1 to mark dystrophic neurites, and thiazine red to mark plaques. From these images, neuritic dystrophy and amyloid deposition are quantified. To measure $[Ca^{2+}]i$ in dystrophic neurites and neurons we use multiphoton imaging of live brain slices of 5XFAD Orai1 flox/flox CaMKII iCre and 5XFAD Orai1 flox/flox control mice who underwent postnatal day 0 intracerebral injections of AAV expressing the commonly used calcium sensor proteins GCaMP6f, or a ratiometric calcium sensor Twitch2B. We hypothesize that the 5XFAD Orai1 flox/flox CaMKII iCre mice will have reduced peri-plaque dystrophic neurites, and lowered resting calcium compared to 5XFAD Orai1 flox/flox control mice.

Disclosures: K.R. Sadleir: None. **J. Popovic:** None. **A. Somasundaram:** None. **M. Prakriya:** None. **R. Vassar:** None.

Poster

132. Alzheimer's Disease and Other Dementias: APP/Abeta: Animal and Cellular Models II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 132.11/S6

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: SynaNet - Twinning Action funded by H2020 (GA-692340)
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Title: Role of SEPT5 in the molecular pathogenesis of Alzheimer’s disease

Authors: *C. FERREIRA[1], K. PALDANIUS[2], P. MÄKINEN[2], A. SEBASTIÃO[3], A. DE MENDONÇA[3], M. DIÓGENES[3], M. HILTUNEN[4]
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Abstract: Septins (SEPT) have been proposed as potential candidates involved in the regulation of synaptic function in neurodegenerative diseases, such as Alzheimer’s disease (AD). These proteins are implicated in several cellular processes, including formation, growth and stability of axons and dendrites, synaptic plasticity, and vesicular trafficking. We have previously shown a transcript variant imbalance for SEPT8 in human temporal cortex in relation to AD-related neurofibrillary pathology. Furthermore, the SEPT8 transcript variant imbalance correlated with β-site amyloid precursor protein (APP) cleaving enzyme 1 (BACE1) enzymatic activity. Moreover, SEPT8 potentially serves as a sorting protein for BACE1 subsequently altering APP processing and Aβ production. Our phosphoproteomic analysis of human AD brain samples revealed that SEPT5, a direct interaction partner of SEPT8, has phosphorylation changes in functionally relevant amino acids at early stages of AD-related neurofibrillary pathology.To further, characterize the potential role of SEPT5 in AD, human SH-SY5Y neuroblastoma cells overexpressing the APP751 isoform were transfected with a control plasmid, human SEPT5 wild-type or SEPT5 phosphomutants. Immature and mature APP were quantified by Western blotting assay from the total protein lysates obtained from cells. Aβ40 and Aβ42 levels were quantified by ELISA from the cell culture medium.Our results show that overexpression SEPT5 did not significantly alter APP processing and Aβ generation.

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Poster

132. Alzheimer's Disease and Other Dementias: APP/Abeta: Animal and Cellular Models II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 132.12/S7

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant K99NR013593
NIH Grant R01NS096091

Title: Alzheimer’s disease pathology is a chronic sequela of ischemic stroke in two mouse models of mixed dementia

Authors: *T.-V. V. NGUYEN[1], M. HAYES[2], J. B. FRYE[2], J. C. ZBESKO[2], N. P. BELICHENKO[4], F. M. LONGO[4], K. P. DOYLE[1,3]
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Abstract: Post-mortem analyses indicate that chronic stroke infarcts exist alongside the pathological hallmarks of Alzheimer’s disease (AD) in nearly 50% of clinically diagnosed AD patients. Yet despite the significant co-existence of stroke and AD, the role of stroke infarcts in modulating AD progression remains insufficiently investigated. Prior studies examining the impact of stroke on AD pathogenesis have been mostly limited to an acute post-stroke timeframe, and focused solely on the overexpression of amyloid-beta (Abeta). In light of this gap in knowledge, we sought here to determine the chronic impact of stroke on the manifestation of AD pathology in two unique and translatable models of mixed dementia. First, we considered different ages in wildtype mice, to mimic the infarct and AD co-morbidities commonly seen in elderly mixed dementia patients with no known predisposition for AD. Second, we utilized an AD transgenic mouse model, APPL/S mice, to model infarct and AD co-morbidities in animals with a genetic predisposition for AD. We found that in wildtype mice, ischemia induces delayed motor recovery and an accelerated development of cognitive deficits in aged C57BL/6 mice compared to young adult C57BL/6 mice. This corresponds with increased brain atrophy, increased cholinergic degeneration, and increased Abeta42 and phosphorylated (p)-tau accumulation in areas of axonal and transneuronal degeneration in the ipsilateral hemisphere of the aged animals. In APPL/S mice, we found that ischemia induces aggravated behavioral deficits, and global increases in Abeta42, p-tau, and cholinergic pathology compared to APPL/S mice that undergo a sham stroke procedure. With regard to mechanism, in both models, we found that the stroke-induced AD pathology co-localized with an increased burden of Abeta

precursor protein (APP) cleavage enzyme, beta-secretase 1 (BACE1), and myelin-associated protein, neuregulin 1, both of which are proteins necessary for myelin repair. Based on these findings, we posit that the chronic sequelae of stroke ratchet-up myelin repair pathways, and that the consequent increase in BACE1 causes an inadvertent cleavage of APP, resulting in greater Abeta seeding and pathogenesis. **Efforts to ensure scientific rigor:** Studies were performed in male mice, with groups numbers determined by power analysis and experimenters blind to experimental condition wherever possible. All antibodies, kits, and chemicals were obtained from established vendors that authenticate their reagents prior to shipment as part of a standard quality control procedure, and were cross checked in the Resource Identification Portal.

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Poster

132. Alzheimer's Disease and Other Dementias: APP/Abeta: Animal and Cellular Models II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 132.13/S8

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: JSPS KAKENHI Grant Number JP26640023

Title: Behavioral and electrophysiological analyses of new Alzheimer’s disease model mouse that expresses amyloid beta oligomer intraneuronally

Authors: *T. OCHIISHI[1], M. KAKU[3], K. KIYOSUE[2], M. DOI[1], T. EBIHARA[1]
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Abstract: In Alzheimer’s disease (AD), recent studies suggest that the intracellular amyloid β protein (Aβ) oligomer has a strong cytotoxicity compared to extracellular Aβ accumulation in the nervous system, and possible roles in the synaptic transmission and cognitive function. We have developed a new animal model of AD, Aβ-GFP mouse, which expresses the GFP fused $A\beta_{1\text{-}42}$ peptide inside of the neuronal cells specifically. We confirmed that Aβ-GFP fusion molecules formed oligomer both *in vitro* and *in vivo*, using the techniques of electron microscopy, nuclear magnetic resonance, fluorescence correlation spectroscopy, and immunohistochemistry. Therefore, to see the effects of Aβ oligomer for the cognitive function in living animal, we performed the behavioral, electrophysiological, and biochemical analyses using the Aβ-GFP mouse. Firstly, we performed the open-field test and found that they did not show the impairments in this test, indicating that exploratory behavior/locomotor activity was intact in Aβ-

GFP mice. We have tested the cognitive function in object recognition task using 2-3 months and 18-20 months-old Aβ-GFP mice. Both young adult and aged Aβ-GFP mice exhibited impaired recognition memory compared to non-transgenic mice both in a short (1 hour) and long (24 hours) retention intervals. Furthermore, electrophysiological analysis showed that long-term potentiation in the hippocampus Schaffer-Collateral synapses was impaired in the young adult Aβ-GFP mice. Biochemical analysis showed that the reduced expression levels of NMDA receptors in the synaptosomal fraction of Aβ-GFP mouse. These results suggest that the intracellular Aβ oligomer induces the synaptic dysfunction from the early age of animals. These kinds of functional abnormality in young animals has not been reported in previously developed AD model mice, therefore, our newly developed Aβ-GFP mouse should be a useful tool for analyzing the function of Aβ oligomer *in vivo* and for finding a minute change in synapse at initial symptoms of AD.

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Poster

132. Alzheimer's Disease and Other Dementias: APP/Abeta: Animal and Cellular Models II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 132.14/S9

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01 NS086965
NIH R01 NS085171

Title: Restoration of sFRP3 expression improves spatial discrimination ability in a transgenic mouse model of Alzheimer's disease

Authors: *C.-H. FU, U. TOSI, J. PARK, Y. ZHENG, J. CHIN
Neurosci., Baylor Col. of Med., Houston, TX

Abstract: Alzheimer’s disease (AD) is associated with hippocampal dysfunction in patients and in transgenic mouse models of disease. Alterations in adult hippocampal neurogenesis may play a key role as it is critical for cognition as well as mood, both of which are dysregulated in AD patients and mice. We previously showed that early in disease progression, transgenic mice overexpressing a mutant form of human amyloid precursor protein (APP) exhibit seizure activity that increases the activation and proliferation of a finite pool of adult hippocampal neural stem cells (NSCs). Such increased activation of NSCs was associated with accelerated depletion of the NSC pool and impairments in spatial discrimination, a behavior that relies on adult-born neurons. All of these alterations were prevented by treatment with an antiepileptic drug.

However, the specific molecular mechanism that triggers seizure-induced proliferation and subsequent depletion of the NSC pool in APP mice is unknown. Our current findings suggest that a critical factor may be the expression of secreted frizzled-related protein 3 (sFRP3), a Wnt signaling inhibitor whose expression is decreased by neuronal activity. sFRP3 is an inhibitory niche factor constitutively expressed by mature dentate granule neurons that serves as a “brake” on adult hippocampal neurogenesis. We found that APP mice have reduced levels of sFRP3 relative to nontransgenic mice, and hypothesized that such reductions may initiate the seizure-induced proliferation and depletion of the NSC pool, and give rise to impairments in spatial discrimination. To test this hypothesis, we developed an adeno-associated virus (AAV) that expresses sFRP3 under control of a granule cell-specific promoter, and bilaterally injected it into the hippocampus of APP mice and nontransgenic littermates. We found that indeed, AAV-driven expression of sFRP3 in APP mice improved spatial discrimination ability. Our results suggest that sFRP3 may be a key protein whose expression is altered by seizure activity and may drive aberrant NSC proliferation and neurogenesis, with consequences for cognitive function, in APP mice and perhaps also in AD.

Disclosures: C. Fu: None. **U. Tosi:** None. **J. Park:** None. **Y. Zheng:** None. **J. Chin:** None.

Poster

132. Alzheimer's Disease and Other Dementias: APP/Abeta: Animal and Cellular Models II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 132.15/S10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: UK Dementia Research Institute, Imperial College, London, U.K.

Title: Faster responding of Tg2576 mice in the touch screen version of the progressive ratio task

Authors: *T. T. AHTONIEMI[1], J. P. JÄRVENPÄÄ[1,2], M. KOPANITSA[1,3], J. T. PUOLIVALI[1]
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Abstract: Mouse models of Alzheimer’s disease (AD) are the cornerstone of academic and industrial efforts to create effective treatments for this devastating disorder. Various transgenic lines of mice incorporating AD-relevant mutations have been found to exhibit altered performance in learning and memory tests that aimed to recapitulate cognitive deficits in humans. Remarkably, although apathy is a frequent neuropsychiatric symptom in individuals with AD, motivation has been rarely studied in mouse AD models. Here, we explored if 10-month-old Tg2576 mice, which overexpress a mutant form of amyloid precursor protein with the Swedish mutation (KM670/671NL), had altered motivational behaviour in the touch screen

version of progressive ratio (PR) reinforcement task. During operant pretraining in fixed ratio (FR) task, 21 Tg2576 and 15 age-matched WT mice were trained to make a fixed number (1, 2, 3 and 5) of touches to the illuminated window on a touch-sensitive screen in Bussey-Saksida chambers in exchange for small nutritional reward. Both genotypes required equal number of 30-trial daily sessions to reach the criteria for FR1, FR2, and FR3 stages. Notably, Tg2576 mice required slightly less time than WT animals (7.1 vs. 8.3 days, $P = 0.019$) to complete the FR5 stage. Immediately after attaining FR5 criterion, the animals were tested for PR responding for 3 consecutive days, during which in order to get the reward, they had to emit an increasing number of touches according to escalating PR4 schedule (1, 5, 9, $\cdots n + 4$). Tg2576 and WT mice kept interacting with the screen for similar periods until the first 5 min of inactivity. Both genotypes exhibited similar "breakpoints" (the number of touches in the last completed trial for which the reward was given), post-reinforcement pauses, and reward collection latencies. However, Tg256 mice demonstrated significantly faster total and target touch rates during the 3 days of PR4 testing. Following PR4 stage completion, the animals had a 3-day break from PR testing whereupon they were moved to PR8 schedule (1, 9, 17 $\cdots n + 8$). No significant differences in PR8 performance were noted except the rate of reward magazine visits was overall faster in mutants. Although Tg2576 mice were hyperactive during their first exposure to touch screen chambers, the rates of front and rear beam breaks were not statistically different by the time of PR testing. Therefore, we conclude that Tg2576 mice have generally unperturbed motivation to perform the touch screen version of the progressive ratio task and exhibit slightly enhanced responding and goal-tracking behaviour.

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Poster

132. Alzheimer's Disease and Other Dementias: APP/Abeta: Animal and Cellular Models II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 132.16/S11

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01AG047589

Title: Whole brain imaging reveals distinct spatial patterns of amyloid beta deposition and atrophy in mouse models of Alzheimer's disease

Authors: *J. D. WHITESELL[1], A. R. BUCKLEY[2], N. GRADDIS[1], L. KUAN[1], J. E. KNOX[1], M. NAEEMI[1], P. BOHN[1], A. MUKORA[1], K. E. HIROKAWA[1], J. A. HARRIS[1]
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Abstract: Amyloid beta (Aβ) plaques are not distributed uniformly across the brain, but instead form earlier in specific brain structures, and then spread in a predictable pattern through selectively vulnerable brain networks. A variety of Alzheimer's disease (AD) mouse models overexpress mutant forms of the human amyloid precursor protein (hAPP) to produce high levels of Aβ, but the degree to which they mimic the spatial and temporal patterns of Aβ deposition in patients remains an open question. We imaged and quantified brain-wide Aβ plaque density across age (4-18 months) in three mouse lines with mutant hAPP overexpression (APPswe/PS1dE9, hAPP-J20, Tg2576). Plaques were fluorescently labelled with methoxy-XO4 *in vivo*. Images were systematically acquired across the entire brain using serial 2-photon tomography. Labeled plaques were automatically detected using a custom-built segmentation algorithm, and each image series was registered to the fully annotated Allen Mouse Brain Common Coordinate Framework 3-D reference atlas. Patterns of brain-wide Aβ plaque deposition differed across the three mouse lines. APPswe/PS1dE9 mice accumulated plaques earliest, primarily in the isocortex in layers 2/3 and 5. hAPP-J20 mice had the most plaques in the hippocampus and retrosplenial cortex. Tg2576 mice had more vascular-associated plaques, with heavy accumulation in the subiculum. All three lines had dense plaques in the amygdala. Previous studies found that misfolded tau can increase the severity of Aβ pathology, so to determine the impact of pathological tau on brain-wide Aβ deposition patterns, we crossed APPswe/PS1dE9 mice with the rTg4510 tauopathy model. These mice had aggressive plaque deposition with a spatial pattern like the APPswe/PS1dE9 line. They also had more severe atrophy and cortical thinning than observed in either line alone, consistent with a synergistic role of Aβ and tau in the development and progression of AD pathology. Whole brain imaging of pathologies may thus provide critical information for whether a given model captures different aspects of AD pathologies. Future work will explore whether the Aβ patterns observed are related to pathological alterations in network activity.

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Poster

132. Alzheimer's Disease and Other Dementias: APP/Abeta: Animal and Cellular Models II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 132.17/S12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Brain Research New Zealand
Neurological Foundation of New Zealand
Health Research Council of New Zealand

Title: Circulating plasma microRNA are altered with amyloidosis in a mouse model of Alzheimer's disease

Authors: *M. M. RYAN[1], D. GUÉVREMONT[1], B. MOCKETT[2], W. C. ABRAHAM[2], J. M. WILLIAMS[1]
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Abstract: Alzheimer's disease (AD), the leading cause of dementia, develops decades before cognitive symptoms become evident but currently there are no non-invasive and easily obtainable preclinical biomarkers available. Recent studies have shown that stable circulating microRNA may be reflective of AD progression and thus may be ideal biomarkers for early-stage disease detection. Here we provide a novel, in-depth analysis of how plasma microRNA alter with aging, the most prominent risk factor for AD, and with development of amyloid-ß (Aβ) plaque deposition. We assessed the circulating microRNA in APPswe/PSEN1dE9 transgenic male mice and wild-type controls at 4, 8 and 15 m (n=8-10/group) using custom-designed Taqman arrays representing 185 neuropathology-related microRNA. The resulting Ct (threshold cycle) values from the arrays were analysed using the open source *HTqPCR* Bioconductor software package within the software environment R. Following filtering and normalisation, we performed a linear mixed model analysis on the data to investigate the effects of age and genotype on plasma microRNA expression. We identified significant changes in 8 age-related microRNA (Tukey's post-hoc tests; $p<0.01$), confirming the use of circulating microRNA to monitor aging-related processes. 12 microRNA were significantly altered in APPswe/PSEN1dE9 mice compared to wild-type controls (Tukey's post-hoc tests; $p<0.01$), either prior to Aβ plaque deposition (4 m) or during the development of AD-like pathogenesis (8 m or 15 m), with differing sets of microRNA identified at each time point. Plasma microRNA altered early, pre-amyloid deposition, may be easily detectable surrogates for events such as inflammation in the AD brain. Supporting this, functional analyses of the target genes of these microRNA (using Ingenuity Pathway Analysis and DAVID analysis) suggested early and sustained alterations of a number of common AD-related pathways including *Inflammatory Response.* Furthermore, pathways including *Immunological Disease and Apoptosis Signalling* were enriched from 8 and 15 m only, suggesting temporal effects on these functions that could be related to increased Aβ load. These studies highlight that plasma microRNA levels are likely reflective of changes in the brain and are dynamic, changing with the progression of AD.

Disclosures: M.M. Ryan: None. **D. Guévremont:** None. **B. Mockett:** None. **W.C. Abraham:** None. **J.M. Williams:** None.

Poster

133. Alzheimer's Disease and Other Dementias: Therapeutic Strategies: Preclinical Cellular Models

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 133.01/S13

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Lunbeck foundation R208-2015-3075

Title: New evidence on the role of tyrosine 682 residue on the amyloid precursor protein C-terminal domain in Alzheimer's disease

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Abstract: Alzheimer’s disease (AD) is the most common form of dementia in ageing societies. One of the cardinal signs of AD is the accumulation of the extracellular Aβ peptides. To be processed and generate Aβ, the Amyloid Precursor Protein (APP) needs to be trafficked to acidic neuronal compartments and this trafficking depends on the extent of phosphorylation of the Tyr682 residue, located on the C-terminal domain of APP.
We found that APP Tyr682 phosphorylation is increased in both neurons and fibroblasts from AD patients and that such increase is associated to Fyn tyrosine kinase activation.
Fyn binds APP and triggers APP Tyr682 phosphorylation, thus inducing APP mistrafficking and Aβ production in human neurons.
These results point on APP Tyr682 residue as potential crucial player in AD and suggest that keeping Fyn kinase activity under control might represent a promising strategy to slow or prevent neurodegenerative events in AD neurons.

Disclosures: F. Iannuzzi: None.

Poster

133. Alzheimer's Disease and Other Dementias: Therapeutic Strategies: Preclinical Cellular Models

Location: SDCC Halls B-H

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Program #/Poster #: 133.02/S14

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Dong-A ST
Korea Drug Development Fund KDDF-201606-03, Republic of Korea
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Title: Elucidating the mechanism of the protective effects of the botanical extract DA-9803 in Alzheimer's disease models

Authors: *A. LARIVIERE[1], G. PAGNIER[1], M. CALVO RODRIGUEZ[1], S.-Z. CHOI[2], S.-H. CHOI[2], H. SOH[2], B. J. BACSKAI[1], K. KASTANENKA[1]
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Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder characterized by deposition of amyloid plaques, progressive memory loss, and cognitive decline. Our recent results demonstrated that treatment of young APP/PS1 mice with DA-9803 (100 mg/kg), a multimodal natural extract, prevents amyloid plaque deposition and maintains neuronal calcium homeostasis in vivo, making DA-9803 a candidate as a preventative thearapeutic for AD. However, the mechanism(s) of action of DA-9803 remain unknown. Here, we studied the effect of DA-9803 on calcium homeostasis in primary cultures of cortical neurons and astrocytes after application of amyloid β (Aβ) oligomers. Aβ oligomers are known to be toxic and lead to intracellular calcium dyshomeostasis in neurons both in vitro and in vivo. 10-18 days in vitro (DIV) neuron-astrocyte co-cultures were loaded with the ratiometric calcium reporter Indo-1, the astrocytic marker SR-101, and were pretreated with either 300 μg/ml DA-9803 or vehicle for 45 minutes. Imaging was performed with multiphoton microscopy before and 1 hour after application of Aβ oligomers. We used transgenic conditioned media (TgCM) collected from Tg2576 neurons in culture as a source of naturally secreted Aβ oligomers that have a mix of oligomers containing human Aβ-40 and Aβ-42. Wildtype media (WtM) collected from wildtype littermate neurons in culture and lacking human Aβ-40 and Aβ-42 served as a control. As previously reported, exposure of cortical neurons to TgCM led to calcium overload. Pretreatment of the cell cultures with DA-9803 protected cells from TgCM-dependent calcium overload, while pretreatment with vehicle failed to do so. To test whether the neuroprotective effect of DA-9803 depended on DA-9803 interacting with the cells or with Aβ directly, DA-9803 was added to the Aβ oligomer containing TgCM and incubated for 3 hours at 37 degrees C. The mixture was centrifuged to separate the DA-9803 from the TgCM. TgCM pre-treated with DA-9803 failed to elicit calcium overload in cortical cells. Thus, DA-9803 interacts directly with Aβ oligomers to neutralize them independent of neurons and astrocytes. In summary, these results demonstrate that DA-9803 has protective effects on calcium homeostasis in vitro and that one mechanism of action involves direct interactions with soluble Aβ oligomers. Based on these results DA-9803 should be considered a promising therapeutic candidate for AD patients.

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Research Support (receipt of drugs, supplies, equipment or other in-kind support); Dong-A ST. **S. Choi:** A. Employment/Salary (full or part-time):; Dong-A ST. **S. Choi:** A. Employment/Salary (full or part-time):; Dong-A ST. **H. Soh:** A. Employment/Salary (full or part-time):; Dong-A ST. **B.J. Bacskai:** 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.; Dong-A ST. **K. Kastanenka:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Dong-A ST.

Poster

133. Alzheimer's Disease and Other Dementias: Therapeutic Strategies: Preclinical Cellular Models

Location: SDCC Halls B-H

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Chulabhorn Graduate Institute, Chulabhorn Royal Academy

Title: Melatonin regulates the amyloidogenic processing βAPP via Pin1/GSK3β/ NF-κB pathway in $A\beta_{42}$-induced cellular model of Alzheimer's disease

Authors: *V. CHINCHALONGPORN[1], M. SHUKLA[1], P. GOVITRAPONG[1,2]
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Abstract: The Amyloid β-peptide (Aβ) peptides are generated from sequential amyloid precursor protein (βAPP) proteolysis. These peptides are one of the most critical etiological factors that cause neuronal dysfunction in Alzheimer's disease (AD). Our previous studies demonstrated that under physiological conditions melatonin regulates the non-amyloidogenic and amyloidogenic processing of βAPP by stimulating the α-secretase (ADAM10) and down regulating both β-secretase (BACE1) and Presenilin1 (PS1). In the present study we evaluated the therapeutic potential of melatonin in $A\beta_{42}$ treated SH-SY5Y cell cultures. Results showed that pre-treatment with melatonin alleviated $A\beta_{42}$-induced alterations in the βAPP processing secretases. We also validated that the intrinsic mechanisms underlying the above effects occurred via regulation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and glycogen synthase kinase (GSK)-3β. Firstly, melatonin administration reversed $A\beta_{42}$-induced upregulation and nuclear translocation of NF-κBp65 as well as activation of GSK3β via its receptor activation and importantly specific blocking of the NF-κB and GSK3β pathways

partially abrogated the $A\beta_{42}$-induced reduction in the BACE1 and PS1 levels. In addition, GSK3β blockage affected α-secretase cleavage and modulated nuclear translocation of NF-κB. Importantly, our study proposed that peptidyl-prolyl cis-trans isomerase NIMA-interacting 1 (Pin1) is a crucial target of melatonin. The compromised levels of Pin1 are associated with age-dependent tau and Aβ pathologies and neuronal degeneration. We showed that $A\beta_{42}$ exposure decreased nuclear Pin1 levels which were abrogated by melatonin pre-treatments. Overall our present finding demonstrated that melatonin possibly prevented $A\beta_{42}$ induced alterations in βAPP processing secretases via Pin1/GSK3β/NF-κB signaling pathway.

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Poster

133. Alzheimer's Disease and Other Dementias: Therapeutic Strategies: Preclinical Cellular Models

Location: SDCC Halls B-H

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: VA I21BX002215
VA IO1 BX003527
Cure Alzheimer’s Fund

Title: Investigating medication combinations in Alzheimer’s disease patients by path analysis and plasma amyloid quantification

Authors: M. LI[1,2], B.-E. MORRIS-EPPOLITO[3], S. X QIAN[1,4], J. REISMAN[1], *J. M. WELLS[3], L. MOO[3], L. KAZIS[1], B. WOLOZIN[5], W. XIA[3,5]
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Abstract: Alzheimer’s disease (AD) is the number one cause of dementia in the elderly. To date, there exists no proven therapeutics that stop or prevent the disease. The growing elderly population in the U.S. makes drug discovery for AD a paramount concern. In this study, we developed an analytic method that can be utilized on a large population for generating and testing research hypotheses relevant to Alzheimer’s therapeutics. We applied a data mining method, exploratory path analysis, on prescribed drugs to predict potential combinations of medications as Alzheimer’s therapeutics. A subset of data was derived from veterans who had donated their blood samples for biomarker analysis. We collected these subjects’ medication history, clinical diagnoses, and quantified levels of plasma amyloid β protein (Aβ), the key

component of neuritic plaques found in brains of AD patients. The path analysis revealed a close link between statins and the other medications of interest for AD and cognitively normal subjects, indicating a feasible combination of two classes of drugs for re-purposing for AD. We found more AD patients are taking combination of drugs compared to cognitively normal subjects, and a higher percentage of AD patients who have taken or are currently taking individual or in combination of statins, metformin, beta blockers or angiotensin-converting enzyme (ACE) inhibitors, likely due to the prevalence of comorbidity in the AD patients. Furthermore, there was an alteration of plasma Aβ levels in subjects taking at least one of these medications relative to those taking no medication from the classes of interest. In conclusion, we illustrate a data mining approach that can be used in the future utilizing a larger data set for predicting combinations of medications that can be re-purposed as Alzheimer's therapeutics.

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Poster

133. Alzheimer's Disease and Other Dementias: Therapeutic Strategies: Preclinical Cellular Models

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Title: Knock-down of HDAC2 promotes expression of a unique neuronal endophilin-B1 isoform and contributes to neuronal maturity, neuroprotection and reduction of cellular AD phenotypes in hiPSC-derived neurons

Authors: *H. FRANKOWSKI[1], B. J. BERRY[2], C. KINOSHITA[3], R. S. MORRISON[3], J. E. YOUNG[2]
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Abstract: One of the most profound advances in the last decade of human disease research has been the development of human induced pluripotent stem cell (hiPSC) models for the identification of disease mechanisms and drug discovery. However, a major criticism of using hiPSC-derived neurons as a model of neurodegeneration is that these cells are functionally embryonic in nature. As most neurodegenerative diseases occur later in life, the use of functionally mature neurons is critical to study the pathobiology of aged cells. Recent studies

show that histone deacetylase 2 (HDAC2), an enzyme important in the epigenetic regulation of gene expression, is abnormally elevated in Alzheimer's disease (AD) and the aged brain leading to epigenetic repression of genes necessary for synaptic function. Our preliminary data demonstrates that decreasing HDAC2 levels in hiPSC-derived neurons increases levels of the neuron-specific isoform of Endophilin B1, EndoB1 b/c, previously shown to promote mitochondrial elongation and neuronal viability. We also demonstrate that knock-down of HDAC2 and EndoB1 b/c overexpression drives neuronal functional maturity as evidenced by decreased resting membrane potential, increased expression of NeuN and Tau, and increased expression of synaptic markers. We also observed significant neuroprotection against DNA damage-induced stress, decreased secreted Aβ peptides, and decreased tau phosphorylation in both HDAC2 knock-down and EndoB1 b/c overexpression conditions. We propose that this pathway may represent a novel therapeutic target for enhancing neuronal function in a human model of AD.

Disclosures: B.J. Berry: None. **C. Kinoshita:** None. **R.S. Morrison:** None. **J.E. Young:** None.

Poster

133. Alzheimer's Disease and Other Dementias: Therapeutic Strategies: Preclinical Cellular Models

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Program #/Poster #: 133.06/S18

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NRF-2017M3C7A1028945

Title: Zinc modulates autophagy flux and lysosomal function via transcription factor EB (TFEB) translocation

Authors: *K. KIM, Y.-H. KIM
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Abstract: We previously showed that increase of extracellular zinc enhances autophagy flux by upregulation of lysosomal protease expression and activity. Within 15 min after exposure to a sub-lethal dose of zinc, lysosomal pH was significantly reduced and after 1 hr of zinc treatment, the activity of lysosomal protease, cathepsin B and D, was markedly increased. From 2 hr later, zinc also elevates lysosomal protease expression in both mRNA and protein levels. However, it is not known that which molecules or pathway associates the increase of lysosomal function by zinc. Transcription factor EB (TFEB) is a master gene of lysosomal and autophagic protein biogenesis. TFEB activity is regulated by phosphorylation, which keeps TFEB inactive form in the cytosol. In contrast, dephosphorylated TFEB by phosphatase actives and moves to the nucleus to activate target transcription gene.

Therefore, in the present study, we examined whether the increase of intracellular zinc changes the localization of TFEB from the cytosol into the nucleus and which molecules are involved in zinc-mediated TFEB localization. First, we found that zinc rapidly increased TFEB expression level. From 1 hr after zinc treatment, protein levels of TFEB were noticeably enhanced. Furthermore, cytosolic TFEB was moved into the nucleus by zinc in cortical neuronal cultures, which was blocked by TPEN, zinc chelator. Next, we tested which kinase or phosphatase was involved in zinc-mediated TFEB translocation. Since it has been well known that the activation of AMPK inhibits mTOR, which prevents phosphorylation of TFEB, we observed zinc induces AMPK activation. From 1 hr, a sublethal dose of zinc increased AMPK phosphorylation. Compound C, the specific chemical inhibitor of AMPK, dramatically augmented p62 protein levels, showing the inhibition of autophagy flux. Thus, the modulation of intracellular free zinc has potential as therapeutics enhancing lysosome biogenesis for neurodegenerative disease in which abnormal protein aggregates are involved.

Keywords: Zinc, TFEB, translocation, AMPK, autophagy, lysosome
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Poster

133. Alzheimer's Disease and Other Dementias: Therapeutic Strategies: Preclinical Cellular Models

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Program #/Poster #: 133.07/T1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimerfonden AF-744871

Title: A high-throughput assay to assess the morphological and electrophysiological impact of neurodegenerative disease associated peptides on cortical neurons

Authors: M. KARLSSON[1], S. ILLES[1,2], J. PIHL[1], *J. SVENSSON DALÉN[1], E. ESBJÖRNER WINTERS[3], P. KARILA[1]
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Abstract: Neurodegenerative disease associated peptides (NDAPs) are causing neuropathological features in patients suffering from e.g. Alzheimer's or Parkinson’s disease, and thus, represent targets for therapeutic intervention approaches. The effective and fast

identification of promising candidate molecules that reduce, prevent or counteract NDAP-mediated morphological abnormalities and impaired electrophysiological function in neurons is urgently needed and require appropriate high-throughput platforms.
Here, we present an assay that allows a qualitative and quantitative assessment of the pathological impact of NDAPs on NDAP-uptake, NDAP spreading, neuronal survival, number of synapses, synaptic and neuronal network activity. We investigate these parameters simultaneously in a high capacity format based on a novel high throughput platform (see the abstract by Karila et al: *"In vitro modelling of prion-like mechanisms occuring in neurodegenerative diseases using a novel high throughput assay platform"*). In the assay, primary cortical neurons cultured in a multi-well format (96 and 384-well plates) were acutely or chronically exposed to NDAPs, e.g. the amyloid beta peptide Abeta42. During the first phase of assessment, spontaneous and electrical stimulation-evoked neuronal activity was visualised by multi-well calcium imaging and provided functional insights into NDAP-mediated altered synaptic and neuronal network activity in cortical neurons. During the second phase of the assessment, high-content imaging was used to quantify NDAP uptake and spreading as well as neuronal cell death and alterations of synaptic proteins.
Since key neuropathological features of neurodegenerative diseases are reflected in the presented high-throughput assay, the effective and fast evaluation of pre-clinical intervention approaches is feasible. To reduce the gap between pre-clinical animal-based model systems and clinical trials in patients, we are currently incorporating our established human cell-based neuronal model system in the presented high-throughput assay platform for neurodegenerative disease modelling (see also the abstract by Illes et al: "*Neurodegenerative disease associated peptides cause abnormal network function in human iPSC-cortical circuits*").

Disclosures: M. Karlsson: A. Employment/Salary (full or part-time):; Cellectricon AB. **S. Illes:** A. Employment/Salary (full or part-time):; Cellectricon AB. **J. Pihl:** A. Employment/Salary (full or part-time):; Cellectricon AB. **J. Svensson Dalén:** A. Employment/Salary (full or part-time):; Cellectricon AB. **E. Esbjörner Winters:** None. **P. Karila:** A. Employment/Salary (full or part-time):; Cellectricon AB.

Poster

133. Alzheimer's Disease and Other Dementias: Therapeutic Strategies: Preclinical Cellular Models

Location: SDCC Halls B-H

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Program #/Poster #: 133.08/T2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NUS grant

Title: Evaluation of humanin gene analogs for protection against amyloid-beta

Authors: *P. KUMAR, G. DAWE
Pharmacol., Yong Loo Lin Sch. of Medicine, NUS, Singapore, Singapore

Abstract: Different forms of amyloid-β have been implicated in the pathogenesis of AD, and the search for a therapeutic molecule has been a long-sought-after goal. A mitochondrially-derived peptide termed as Humanin has been shown to protect against the insults generated by Aβ and other familial AD (FAD) mutants. The modified forms of Humanin (HN) peptide have enhanced potency and are better alternatives to protect against Aβ. A major challenge in the use of HN derived peptides for the treatment of AD is their short half-life due to proteasomal degradation mediated by tripartite motif containing 11 (TRIM11). In the present pre-clinical study, we evaluated the biological functionality and therapeutic efficacy of HN gene analogs. To achieve this, we prepared coding-region DNA duplexes of HN gene analogs and cloned downstream of a constitutive mammalian promoter that would sustain the expression of these genes. We expressed these gene analogs in HEK293T cells, SH-SY5Y cells, and mouse primary hippocampal neurons to investigate the putative biological roles by using gene expression analysis and immunoblotting. We also evaluated the cytoprotective potential of the gene analogs against $A\beta_{25-35}$ using ATP-dependent viability assay and neuroprotective potential of Colivelin (CLN) gene against $A\beta_{1-43}$ using TUNEL assay. All the experiments were done independently in triplicates. We report that humanin gene analogs significantly upregulate the expression of SH3-binding protein 5 (SH3BP5) gene that mediates the therapeutic effect of HN by inhibiting c-Jun NH_2-terminal kinase (JNK). We also report that HN gene analogs activate MAPK p44/42 (ERK ½) and PI3K/AKT signalling pathways that are essential for neuronal survival. Most importantly, in our study, the cell viability assays show that the CLN gene has the highest efficacy as its expression completely prevents $A\beta_{25-35}$ and $A\beta_{1-43}$ mediated cell death. These results establish that the HN gene analogs preserve the biological activity after expression as their respective peptide counterparts. Our results suggest that the constitutive expression of the highly potent CLN gene (a hybrid variant of HN) can be used to provide long-term protection against Aβ and may alleviate the pathogenesis of AD.

Disclosures: P. Kumar: None. **G. Dawe:** None.

Poster

133. Alzheimer's Disease and Other Dementias: Therapeutic Strategies: Preclinical Cellular Models

Location: SDCC Halls B-H

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01NS075487
BrightFocus Foundation

Weston Brain Institute
Alabama Drug Discovery Alliance

Title: Tau-SH3 interactions are critical for amyloid-β toxicity in primary neurons

Authors: *J. ROTH, T. RUSH, S. THOMPSON, J. N. COCHRAN, E. ROBERSON
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Abstract: The microtubule-associated protein tau has been extensively studied because it aggregates into neurofibrillary tangles within neurons, which are one of the hallmarks of Alzheimer's disease (AD). Genetic knockout of tau is protective in several models of AD, making it an exciting therapeutic target for treatment of the disease. Interestingly, tau reduction also reduces network hyperexcitability, which may contribute to neurodegeneration, in these models. Similarly, in a primary neuron culture system, tau reduction protects from amyloid-β (Aβ) toxicity and glutamate or NMDA-induced excitotoxicity. Since tau reduction is protective, it is important to determine the mechanism by which it prevents Aβ toxicity and network hyperexcitability. While the microtubule-binding domain of tau has been studied heavily, less is known about tau's proline-rich region, which is hyperphosphorylated in AD. This region of tau has several PxxP motifs that mediate binding with SH3 domain-containing proteins, including the nonreceptor tyrosine kinase Fyn. Fyn is also an important mediator of network hyperexcitability, as it phosphorylates AMPA and NMDA receptors to strengthen their signaling and regulates dendritic spine dynamics. Exogenous Aβ activates Fyn in the postsynaptic density, leading to NMDAR phosphorylation and excitotoxicity in neurons. In mouse models, expressing a truncated form of tau excludes Fyn from dendrites and protects against cognitive deficits and seizure susceptibility, showing that these deficits may be influenced by tau-SH3 interactions like that with Fyn. We developed a peptide inhibitor of tau-SH3 interactions that mimics Fyn's primary binding site on tau, the 5th and 6th PxxP motifs, to competitively inhibit its interaction with SH3-containing proteins that bind to these motifs, including Fyn. We first confirmed that it blocks the tau-Fyn interaction in cells using proximity ligation assay. The peptide inhibitor of tau-SH3 interactions protected rat primary hippocampal neurons against Aβ toxicity by multiple outcome measures, including neurite loss and metabolic dysfunction. Our results show that tau-SH3 interactions contribute to Aβ-induced toxicity and inhibiting them could be a therapeutic target for AD.

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Poster

133. Alzheimer's Disease and Other Dementias: Therapeutic Strategies: Preclinical Cellular Models

Location: SDCC Halls B-H

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Program #/Poster #: 133.10/T4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01 AG032611
NIH R01 NS077239

Title: Antibody-mediated prevention of pathological tau spreading

Authors: Y. MA[1], J. CHUKWU[2], X. KONG[2], *E. E. CONGDON[1], E. M. SIGURDSSON[3]
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Abstract: Tau immunotherapy is a promising approach to treat Alzheimer's disease (AD) and other tauopathies, with eight clinical trials currently ongoing. Previously we showed that spreading of pathological tau can be reduced by antibodies (Congdon et al., 2016 Mol Neurodegener). Here, we examined the efficacy of 4E6, a phospho-selective antibody against Tau 396/404 region, and its partially humanized derivative (h4E6), in preventing the spread of pathological tau under several treatment conditions.
Neurons from JNPL3 tauopathy mice were grown in microfluidic chambers, with two cell populations plated in different compartments (Side A and B) connected by microgrooves. This allows axons to grow through but prevents fluid mixing. Fluorescently labeled PHF-enriched tau derived from AD brain (1 µg/mL) was added to Side A and images of Side B were collected 72 h later. Antibodies were applied using two dosing methods, PHF and antibody added together (PHF+Ab) or PHF first followed 24 h later by antibody (PHF→Ab), at 1 or 10 µg/ml. Respectively, the dosing methods represent extra- and intracellular antibody-tau binding.
After optimizing conditions, efficacy experiments were conducted. When added alone to Side A, PHF was visible intraneuronally in Side B 72 h later. Unmodified 4E6 added to Side A significantly reduced PHF spreading at either 1 or 10 µg/ml under both dosing conditions (PHF+Ab: 45% and 67% reduction, $p < 0.05$, 0.001; PHF→Ab: 53% and 80% reduction, $p < 0.01$, 0.0001). A partial humanization of 4E6 altered its binding properties, but h4E6 was also effective under both dosing conditions (PHF+Ab: 73% and 63% reduction at 1 and 10 µg/ml, $p < 0.0001$, 0.001; PHF→Ab (80% reduction at 10 µg/ml, $p < 0.0001$; not tested at 1 µg/ml).
These data show that prevention of PHF spreading is possible with an antibody working both extra- and intracellularly. Also, that a partially humanized antibody retains its efficacy in this assay. We have reported that, in contrast to 4E6, h4E6 did not prevent PHF-induced cell death in a different assay. Although this may in part be due to different experimental conditions, h4E6

may preferentially bind non-toxic tau aggregates, and thus prevent spreading but not toxicity. Our prior work indicates that tau seeding/spread is not necessarily linked to toxicity, which should be taken into consideration when studying tau pathogenesis and for evaluating antibody efficacy.

Disclosures: Y. Ma: None. **J. Chukwu:** None. **X. Kong:** None. **E.E. Congdon:** None. **E.M. Sigurdsson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); H. Lundbeck A/S, New York University.

Poster

133. Alzheimer's Disease and Other Dementias: Therapeutic Strategies: Preclinical Cellular Models

Location: SDCC Halls B-H

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Program #/Poster #: 133.11/T5

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant NS100459
NIH Grant AG039452

Title: Clearance of Aβ and tau by CNS pericytes

Authors: *A. P. SAGARE[1], D. LAZIC[2], C.-J. HSU[2], A. R. NELSON[3], Q. MA[2], Z. ZHAO[2], C. GRIFFIN[2], R. BAJPAI[2], B. V. ZLOKOVIC[2]
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Abstract: Recent studies suggest that brain vascular dysfunction plays a vital role in the development of neurodegenerative disorders including Alzheimer’s disease (AD). Pericytes are vascular mural cells embedded in the basement of brain microvessels and play an important role in the central nervous system (CNS) in regulating blood-brain barrier (BBB) integrity, angiogenesis, cerebral blood flow, and clearance of CNS neurotoxins. Previous studies by our group have shown that pericyte degeneration and loss occurs in AD patients. Moreover, we showed in mice overexpressing the Swedish mutation of human Aβ-precursor protein ($APP^{sw/0}$) that accelerated loss of pericytes leads to increased elevation of brain Aβ levels, the development of tau pathology, and early neuronal loss, which is not seen normally in $APP^{sw/0}$ transgenic mice. Here, we studied the role of primary cultured murine brain pericytes, adult human brain pericytes and human iPSC-derived cranial pericytes (iPSC-PC) in clearance of Aβ and tau. By silencing different LDL receptors, LDLR, VLDLR, and LRP8, we show that the iPSC-PC clear extracellular Cy3-labeled Aβ from multi-spot glass slides and Cy3-labeled human recombinant tau from the medium mainly via low-density lipoprotein receptor-related protein 1 (LRP1). Thus,

development of novel therapies to prevent loss of CNS pericytes and preserve their functions may significantly alter disease progression in the AD.

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Poster

133. Alzheimer's Disease and Other Dementias: Therapeutic Strategies: Preclinical Cellular Models

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Weston Brain Institute
Krembil Foundation
Alzheimer's Society of Canada
Canadian Institutes of Health Research

Title: Design and optimization of indoleamine-2,3-dioxygenase inhibitors as putative therapeutics for Alzheimer's dementia

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Abstract: Clinically, Alzheimer's disease (AD) manifests as progressive deterioration in multiple cognitive domains; pathologically, these symptoms initially arise from cytotoxic oligomerization of β-amyloid, concomitant with microglial activation and neurotoxic immuno-inflammation, inducing the release of TNF-α and IL-1β pro-inflammatory cytokines and contributing to concurrent proteopathic and immunopathic neural toxicities. Targeting the immunopathy has emerged as a viable target for AD therapeutics.
To modify brain innate immunity, we targeted the indoleamine-2,3-dioxygenase (IDO) enzyme – the rate limiting step in producing immuno-regulatory kynurenines via tryptophan catabolism and leading to trp depletion. By inhibiting IDO we anticipate downregulating the release of neurotoxic cytokines. To design IDO inhibitors, we used known crystal structures (4PK5, 6F0A, 5EK2, 6AZU) to identify a 3-pocket receptor site: Pockets A, B and a heme moiety. Structural insights of this receptor coupled with 3D QSAR studies, multiparameter optimization scores (MPO, TEMPO, LLE) and extensive molecular modelling were used to design drug-like brain-penetrant IDO inhibitors. Starting with imidazole derivatives of because of their ability to coordinate the heme iron, we achieved the structure-based design of two related series of

molecules: *N*1-substituted 5-indoleimidazoles (Series 1) and *N*1-substituted 5-phenylimidazoles (Series 2); the latter (more potent) series was accessed through a rearrangement of an imine intermediate during a Van Leusen imidazole synthesis reaction. 75 Series 2 analog molecules were synthesized. The most potent compound from Series 2 was DWG1267 (IDO IC_{50} 33.8 nM, IDO EC_{50} 260 nM, in enzyme and cell-based assays, respectively). DWG1267 is twice as potent as the clinical candidate epacadostat, and has 5000-fold selectivity for IDO over TDO, another trp catabolic enzyme. We optimized a murine pharmacokinetic-pharmacodynamic model in which brain IDO is upregulated by systemic lipopolysaccharide administration and demonstrated that brain kynurenine levels can be lowered by our brain penetrant IDO inhibitors. Markers of neuroinflammation were also assessed to examine downstream effects of IDO inhibition with a focus on microglia and astrocytes. Multiple compounds in this series have good oral bioavailability and favorable pharmacokinetic profiles in mice. We are now refining our lead compound to improve pharmacokinetic properties and to collect pre-clinical data.

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Poster

133. Alzheimer's Disease and Other Dementias: Therapeutic Strategies: Preclinical Cellular Models

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 133.13/T7

Topic: C.02. Alzheimer's Disease and Other Dementias

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Title: The neuroprotective role of Amidated-Kyotorphin on Alzheimer's disease pathophysiology

Authors: *R. BELO[1,2], J. FONSECA-GOMES[1,2], H. VICENTE MIRANDA[3], T. FLEMING OUTEIRO[3,4,5], A. M. SEBASTIÃO[1,2], V. NEVES[1], M. A. R. B. CASTANHO[1], M. J. DIÓGENES[1,2]
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Lisboa, Lisboa, Portugal; [4]Dept. of Exptl. Neurodegeneration, CNMPB, Ctr. for Biostructural Imaging of Neurodegeneration, Univ. Med. Ctr. Göttingen, Göttingen, Germany; [5]Max Planck Inst. for Exptl. Med., Göttingen, Germany

Abstract: Kyotorphin (KTP) is an endogenous dipeptide, L-tyrosyl-L-arginine, initially described as an analgesic. Interestingly, in cerebrospinal fluid of AD patients the levels of KTP are decreased, and chronic administration of an amidated form (KTP-NH_2) that crosses the blood brain barrier, to a rat AD model prevents learning and memory impairments by an unknown mechanism.
In this work, we studied the effects of both KTP and KTP-NH_2 upon hippocampal synaptic plasticity and loss of endogenous BDNF neuroprotection.
Field-excitatory post-synaptic potentials were recorded from the CA1 area of mice hippocampal slices. The effects of both forms of KTP (50nM) upon long-term potentiation (LTP) and post-tetanic stimulation (PTP) were evaluated. In addition, we analysed the effect of KTP-NH_2 (co-)incubation on slices exposed to amyloid-β (Aβ, 200nM) for 3h. Since the loss of endogenous BDNF neuroprotection is associated by TrkB-FL receptors cleavage leading to the formation of TrkB-ICD fragment, we evaluated TrkB-ICD/TrkB-FL ratio by western-blot, using primary neuronal cultures incubated with KTP-NH_2 (50nM) and/or Aβ (25μM) for 24h.
Our results show that the incubation with KTP-NH_2 restores LTP impairment in slices exposed to Aβ ($p<0.05$, N=4). KTP-NH_2 also prevents TrkB-FL cleavage induced by Aβ ($p<0.05$, N=6), whereas KTP has no effect. Interestingly, the superfusion of both KTP and KTP-NH_2 does not affect LTP magnitude (N=6-8), however KTP-NH_2 increases PTP ($p<0.05$, N=6).
In summary, these findings reveal that KTP-NH_2 can rescue synaptic plasticity deficits, caused by Aβ peptide-mediated TrkB-FL decreased levels. This suggests that the memory deficits induced by Aβ in AD patients may be ameliorated by kyotorphin derivatives, highlighting a putative therapeutic role for this class of peptides.

Disclosures: R. Belo: None. **J. Fonseca-Gomes:** None. **H. Vicente Miranda:** None. **T. Fleming Outeiro:** None. **A.M. Sebastião:** None. **V. Neves:** None. **M.A.R.B. Castanho:** None. **M.J. Diógenes:** None.

Poster

133. Alzheimer's Disease and Other Dementias: Therapeutic Strategies: Preclinical Cellular Models

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 133.14/T8

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimerfonden (AF-744871 to S.I.)

Title: Neurodegenerative disease associated peptides cause abnormal network function in human iPSC-cortical circuits

Authors: *S. ILLES[1,2], P. KARILA[1], J. PIHL[1], E. ESBJÖRNER WINTER[3], M. KARLSSON[1]
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Abstract: Neurodegenerative disease associated peptides (NDAPs) are causing neuropathological features within the cortex and are associated with impaired cognitive function as described in patients suffering from e.g. Alzheimer's or Parkinson's disease. The causal role of NDAPs in impaired human cortical circuit function is still enigmatic. Synchronous neuronal activity represents a neuronal network activity stage essentially involved in cognitive, memory and motoric functions. By using synchronously active human cortical circuits, obtained from human iPSCs, we analysed the functional impact of NDAPs, e.g. amyloid beta peptides, on human neuronal synchronous activity. For this purpose, human iPSC-derived neurons were cultured on microelectrode arrays and after the formation of synchronously active neuronal networks (two to three weeks) different concentrations of NDAPs were applied. Here, we describe the acute and chronical impact of NDAPs on synchronous human neuronal activity. Complimentary immunocytochemistry and high-resolution imaging were used to describe NDAP uptake and spreading, as well as neuronal survival and alterations of synaptic proteins.Here, we provide novel insights in how NDAPs cause morphological and functional alterations in human cortical circuits. Since neuropathological features of neurodegenerative diseases are reflected, the presented human cell-based model system may hold promise to bridge the gap between pre-clinical animal-based model systems and clinical trials in patients. For the evaluation of therapeutical intervention approaches, we are currently incorporating our established human cell-based neuronal model system in our high-throughput platform for neurodegenerative disease modelling. (see the abstract by Karila *et al*: "*In vitro modelling of prion-like mechanisms occuring in neurodegenerative diseases using a novel high throughput assay platform*").

Disclosures: S. Illes: A. Employment/Salary (full or part-time):; Cellectricon AB, Mölndal, Sweden. **P. Karila:** A. Employment/Salary (full or part-time):; Cellectricon AB, Mölndal, Sweden. **J. Pihl:** A. Employment/Salary (full or part-time):; Cellectricon AB, Mölndal, Sweden. **E. Esbjörner Winter:** None. **M. Karlsson:** A. Employment/Salary (full or part-time):; Cellectricon AB, Mölndal, Sweden.

Poster

133. Alzheimer's Disease and Other Dementias: Therapeutic Strategies: Preclinical Cellular Models

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 133.15/T9

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: *In vitro* modelling of prion-like mechanisms occurring in neurodegenerative diseases using a novel high throughput assay platform

Authors: *P. KARILA[1], S. ILLES[2,1], C. NODIN[1], J. PIHL[1], E. ESBJÖRNER WINTERS[3], M. KARLSSON[1]
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Abstract: Spreading of neurodegenerative disease associated peptides (NDAPs) within the brain is considered as the major pathological mechanism in progressive neurodegenerative diseases, such as Alzheimer's and Parkinson's disease. In this concept, pathological soluble forms of NDAPs, such as amyloid beta, alpha synuclein and tau proteins, are incorporated by neurons where they cause protein misfolding, synapse elimination and neuronal cell loss. Moreover, a plethora of literature reports a prion-like mechanism of intracellular NDAPs, i.e. the intracellular transport of NDAPs and spreading from one neuron to another. Since neurodegenerative diseases are still non-treatable, a high throughput assay platform that reflect all these complex neuropathological features in vitro and that allows screening and profiling of larger compound sets to prevent this neurodegenerative cascade, represents an urgent unmet clinical need.
We combined our previously established Cellaxess® Elektra optical electrophysiology system with a newly developed microfluidics approach and created a unique high throughput in vitro platform that reflects all hallmarks of the neurodegenerative disease cascade, including prion-like spreading of NDAPs within CNS neuronal circuits. Mouse cerebral cortical neuronal cultures were used since neurons in these cultures develop extensive processes and form functional synaptic connections in vitro.
In detail, cortical neurons plated in wells of custom-developed microplates were exposed to different NDAPs and high-content imaging was used to describe NDAP uptake, intraneuronal spreading and NDAP-mediated alteration of synapses and neuronal survival. Optical electrophysiological assessment by using a calcium-sensitive probe was used to describe NDAP-mediated alterations of synaptic and neuronal network function.
To our knowledge, this is the first report of an approach that shows sufficient capacity and robustness to allow screening and profiling of larger compound sets in the search for molecules preventing the prion-like spreading of NDAPs across synaptically coupled neurons.

Disclosures: P. Karila: A. Employment/Salary (full or part-time):; Cellectricon AB. **S. Illes:** A. Employment/Salary (full or part-time):; Cellectricon AB. **C. Nodin:** A. Employment/Salary (full or part-time):; Cellectricon AB. **J. Pihl:** A. Employment/Salary (full or part-time):; Cellectricon AB. **E. Esbjörner Winters:** A. Employment/Salary (full or part-time):; Chalmers University of Technology. **M. Karlsson:** A. Employment/Salary (full or part-time):; Cellectricon AB.

Poster

134. Parkinson's Disease: Molecular and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 134.01/T10

Topic: C.03. Parkinson's Disease

Support: NS045962

Title: Reduction of SPN firing highly impacts motor behavior in animal models of Parkinson's disease

Authors: *S. M. PAPA[1], A. SINGH[2], G. BECK[3]
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Abstract: Dopamine replacement is an effective treatment for motor symptoms of Parkinson's disease (PD); however, long-term dopaminergic therapy can induce further motor complications, such as L-DOPA-induced dyskinesia (LID). Previous studies have shown that striatal projection neurons (SPNs) are hyperactive at the baseline parkinsonian state ("OFF" state) across animal models and human patients with PD. Hyperactive neurons respond to dopaminergic stimulation with unstable firing changes at the peak of the response, which are associated with the expression of LID. The objective of the current study was to investigate whether decreasing the baseline SPN firing rates reduces LID. We used two methods to lower acutely the SPN activity: (I) striatal injection of selective NMDAR antagonist (LY235959) in advanced parkinsonian NHPs (n=3); and (II) treatment with clozapine-N-oxide (CNO) after expression of inhibitory DREADDs (designer receptor exclusively activated by designer drugs) using AAV-hSyn-hM4D(Gi)hM4Di in hemiparkinsonian rats (n=9). In addition, we suppressed the gene expression of NMDAR2B (GluN2B) using AAV-NR2B shRNA in the putamen of one advanced parkinsonian NHPs in order to reduce chronically the SPN activity. The whole motor response and LID or AIMs (abnormal involuntary movements) were assessed using standardized rating scales for NHPs and rodents. Virus transduction in the rat striatum was confirmed with IHC, and protein reduction was analyzed with WB. Results in all three experiments showed significant changes in responses to L-DOPA; i.e. reduction of LID/Aims scores without affecting the antiparkinsonian action of L-DOPA. Furthermore, after striatal gene silencing of the NMDAR subunit, a noticeable improvement in the baseline motor disability was observed. These results support the development of therapeutic strategies that specifically reduce SPN hyperactivity for PD therapy.

Disclosures: S.M. Papa: None. **A. Singh:** None. **G. Beck:** None.

Poster

134. Parkinson's Disease: Molecular and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 134.02/T11

Topic: C.03. Parkinson's Disease

Support: NIH Grant P50-NS098685; The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health

Title: Intrinsic excitability and synaptic transmission of neurons in basal ganglia input receiving motor thalamus in 6-OHDA-lesioned mice

Authors: *E. K. BICHLER, D. JAEGER
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Abstract: The motor symptoms of Parkinson's disease results from pathological information processing in the motor loop of the basal ganglia following the loss of dopamine innervation. We hypothesize that intrinsic excitability and synaptic transmission (specifically through NMDA channels) in neurons from the motor thalamus that receive afferents from the basal ganglia (BGMT neurons) could be affected in 6-hydroxydopamine- (6-OHDA-) lesioned mice.
Whole-cell patch-clamp recordings were performed in BGMT neurons from control and 6-OHDA-lesioned mice. GABAergic terminals were visualized (through AAV-EYFP vector injection into substantia nigra pars reticulata) and cortical glutamatergic afferents projecting to BGMT neurons were transfected with ChR2 (AAV-Syn-CamkIIa-hchR2-mcherry into the anterior lateral motor cortex). AMPA- and NMDA- mediated EPSCs were evoked by 2ms exposure to blue light at various holding potentials. Our results show that BGMT neurons show a mixed AMPA/NMDA conductance and that the EPSC peak amplitude and voltage-dependence of both AMPA and NMDA components remains statistically unchanged in 6-OHDA lesioned mice.
In contrast, intrinsic excitability of BGMT neurons in mice 8-16 weeks post lesion was increased. The average rheobase current was reduced up to 40% of control assessed as the minimal constant current injection necessary to evoke tonic spiking (Control: 57.1 pA vs. Treated: 108.0 pA; $p = 0.023$). Additionally, the F-I relationship (action potentials frequency as a function of injected current) was left shifted.
We further tested the effect of dopamine depletion on T-type calcium channel-mediated rebound spike bursts by evaluating the voltage threshold in response to rapid change of voltage from a hyperpolarizing current step (rebound burst threshold). The average rebound burst threshold was significantly less depolarized compare to controls (Control: -40.92 mV vs. Treated: -45.98 mV; $p=0.041$).
Taken together, these results suggesting that BGMT neuronal responses to the anterior lateral

motor cortex afferents remain normal but voltage dependent conductances are altered. In particular, increases in excitability may provide a compensatory mechanism for increased inhibitory input rates from SNr likely to occur in Parkinsonism.

Disclosures: E.K. Bichler: None. **D. Jaeger:** None.

Poster

134. Parkinson's Disease: Molecular and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 134.03/T12

Topic: C.03. Parkinson’s Disease

Support: NIH grant P50NS091856

Title: Enhancing striatal cholinergic interneuronal function rescues performance of rats modeling falls in Parkinson’s disease

Authors: *A. J. KUCINSKI, M. SARTER
Psychology, Univ. of Michigan, Ann Arbor, MI

Abstract: In addition to the disease-defining motor symptoms of Parkinson's Disease (PD), resulting from striatal dopamine loss, about half of the patients also suffer from falls and related balance and complex movement deficits. Falls in PD are associated with the presence of executive impairments and with decline in the basal forebrain cholinergic projection system. We previously established a rat model of PF falls. Rats with partial losses of striatal dopamine and cortical acetylcholine (ACh; “dual lesions”, DL) exhibit relatively high fall rates when traversing dynamic surfaces such as a rotating rod. Loss of cortical ACh is hypothesized to impair the cortical processing of exteroceptive and interoceptive balance and movement cues, including errors, and thus the transfer of this information to the striatum. In the striatum, cholinergic interneurons (ChIs) are positioned to integrate cortico-striatal cue information with the dopaminergic neuromodulation of action selection. Therefore, we first hypothesized that silencing of ChIs in the dorsomedial striatum, the prefrontal projection field, would increase falls in otherwise intact rats. Rats received bilateral infusions of inhibitory DREADD into the dorsomedial striatum and performed attention-demanding traversals on the Michigan Complex Motor Control Task (MCMCT), including navigation of rotating and zig-zag rods. Administration of CNO (5 mg/kg; i.p.) fully reproduced the types and frequency of falls seen in DL rats. Next, we expressed an excitatory DREADD in dorsomedial striatal ChIs of fall-prone DL rats. CNO-induced activation of these DREADDs attenuated falls by these rats when traversing the straight rotating rod by 78%, and the zig zag rotating rod by 79%. CNO did not affect falls in DL control rats not expressing a DREADD. These findings indicate that ChIs in the striatum play a critical role in the integration of cognitive-motor information that is normally

required to maintain balance and forward movement across dynamic surfaces. Furthermore, enhancing striatal cholinergic interneuronal function may serve as a therapeutic strategy to reduce fall rates in PD patients.
Supported by NIH grant P50NS091856 (Morris K. Udall Center for Excellence in Parkinson's Disease Research)

Disclosures: A.J. Kucinski: None. **M. Sarter:** None.

Poster

134. Parkinson's Disease: Molecular and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 134.04/T13

Topic: C.03. Parkinson's Disease

Title: The uncompetitive, low affinity NMDA-receptor channel blocker, amantadine, reduces LTP in multiple brain regions

Authors: *A. MITRA[1], J. HOLT[1], K. VAN[1], R. TEYSSIÉ[2], B. BUISSON[2], J. NGUYEN[1]
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Abstract: ADS-5102 (amantadine) extended release capsules (GOCOVRI™, Adamas Pharmaceuticals, Inc.) is the only approved treatment for dyskinesia in patients with Parkinson's disease (PD). Previous studies have shown that amantadine is an NMDA receptor antagonist that binds to the open NMDA receptor channel and inhibits cation flux, including calcium, through the channel pore. However, more physiologically relevant functional outcomes of synaptic NMDA receptor antagonism have not been investigated with amantadine. Long-term potentiation (LTP), a form of synaptic plasticity that is dependent on NMDA receptor activation, is thought to be aberrant at corticostriatal synapses in dyskinesia due to excessive glutamatergic signaling. In this study, we evaluated the ability of amantadine to block LTP in corticostriatal rat brain slices, *ex vivo*. We also measured the effect of amantadine on NMDA receptor mediated LTP in rat hippocampal CA3-CA1 synapses, a brain structure classically used to study LTP. Our results showed that amantadine exhibited a concentration-dependent inhibition of LTP in both corticostriatal and hippocampal brain slices in the 50-500 μM concentration range. Ongoing studies are focused on demonstrating amantadine as a low affinity, use-dependent NMDA receptor blocker, by measuring changes in amantadine potency with increasing agonist (NMDA) concentration in rat primary dissociated cortical cultures, *in vitro.* We hypothesize that in disease conditions such as dyskinesia, with excessive glutamatergic signaling and overactivity of NMDA receptors, a use-dependent NMDA receptor blocker such as amantadine could normalize aberrant NMDAR-mediated synaptic plasticity by targeting high, pathological levels of NMDA receptor

activation more effectively than lower NMDA receptor activation associated with normal physiology.

Disclosures: A. Mitra: A. Employment/Salary (full or part-time):; Adamas Pharmaceuticals, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Adamas Pharmaceuticals, Inc. **J. Holt:** A. Employment/Salary (full or part-time):; Adamas Pharmaceuticals, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Adamas Pharmaceuticals, Inc. **K. Van:** A. Employment/Salary (full or part-time):; Adamas Pharmaceuticals, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Adamas Pharmaceuticals, Inc. **R. Teyssié:** A. Employment/Salary (full or part-time):; Neuroservice. **B. Buisson:** A. Employment/Salary (full or part-time):; Neuroservice. **J. Nguyen:** A. Employment/Salary (full or part-time):; Adamas Pharmaceuticals, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Adamas Pharmaceuticals, Inc..

Poster

134. Parkinson's Disease: Molecular and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 134.05/T14

Topic: C.03. Parkinson’s Disease

Support: S. Cameron was supported by a University of Otago Doctoral Scholarship

Title: Pathophysiological and anatomical changes of the deep cerebellar nuclei in a chronic rat model of Parkinson’s disease

Authors: *S. CAMERON, L. C. PARR-BROWNLIE
Univ. of Otago, Dunedin, New Zealand

Abstract: Parkinson’s disease (PD) is hallmarked by progressive degeneration of dopamine neurons in the substantia nigra pars compacta that reduces dopamine in the brain and leads to severe movement deficits. The degree of PD motor symptoms strongly correlates to altered activity in the basal ganglia (BG). However, BG dysfunction does not fully explain PD pathogenesis. Despite the cerebellum being crucial for motor control, its role in PD has been largely ignored. Imaging studies indicate that the cerebellum is hyperactive in PD patients. In addition, grey matter volume in the cerebellar cortex is reduced, which is associated with increased connectivity between the cerebellum and motor cortex that predicts the level of motor impairment in PD patients. We hypothesised that hyperactive and irregular activity in the subthalamic nucleus (STN) in the dopamine depleted brain drives cerebellar changes by a

disynaptic connection from the STN to the cerebellar cortex via pontine nuclei. Supporting this, Purkinje neurons, the inhibitory output neurons of the cerebellar cortex to the deep cerebellar nuclei (DCN), in a primate model of PD show increased cFos expression, which is an indirect marker of neuronal activity. To date, PD-associated changes in the DCN remain unknown. To address this, DCN single-unit and local field potential (LFP) activities were recorded in the lesioned hemisphere of unilateral 6-hydroxydopamine (6-OHDA, $n = 12$) and sham-lesioned rats ($n = 10$) under urethane anaesthesia. DCN neuron firing rate was significantly decreased ($p < 0.0001$) and the incidence of bursty firing was increased ($p < 0.0001$). Analysis of the timing of neuronal spikes, with respect to LFPs, showed spiking occurred earlier in 6-OHDA rats ($p < 0.001$). This is important as the timing and pattern of DCN firing correlates with the onset of muscle contractions. In addition, we immunohistochemically labeled GABAergic and putative glutamatergic neurons in the DCN using antibodies against glutamic acid decarboxylase 67 (GAD67) and calmodulin-dependent protein kinase type II alpha chain (CaMKIIα), respectively, in 6-OHDA ($n = 4$) and sham-lesioned rats ($n = 5$). Stereological quantification of neuron numbers using the Cavalieri's and optical disector methods, revealed a significant reduction in GAD67-positive neurons in the DCN of 6-OHDA rats ($p < 0.005$). For the first time, we show that anatomical and physiological changes occur in the DCN of parkinsonian rats. Data support cerebellar involvement in the pathogenesis of PD and validates further research into the cerebellum as a therapeutic target for treating PD movement deficits.

Disclosures: S. Cameron: None. **L.C. Parr-Brownlie:** None.

Poster

134. Parkinson's Disease: Molecular and Cellular Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 134.06/T15

Topic: C.03. Parkinson's Disease

Support: NIH Grant R01NS082565

Title: Analysis of cortico-striatal glutamatergic transmission in PINK1 KO rats

Authors: *R. B. CREED[1], L. L. MCMAHON[2], M. S. GOLDBERG[3]
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Abstract: PTEN induced kinase 1 (PINK1) targets dysfunctional mitochondria for degradation via autophagy, and PINK1 mutations cause autosomal recessive Parkinson's disease (PD). The main pathological hallmarks of PD are loss of dopaminergic neurons in the substantia nigra pars compacta, which are required for normal movement, and the formation of α-synuclein-rich

aggregates termed Lewy body inclusions. Accordingly, PINK1 knockout (KO) rats have mitochondrial dysfunction, locomotor deficits, and α-synuclein aggregates in multiple brain regions including the substantia nigra, striatum, and cortex. The appearance of α-synuclein aggregates in idiopathic PD and the genetic linkage of α-synuclein mutations to inherited PD both implicate α-synuclein abnormalities in PD pathogenesis. How and why α-synuclein-immunoreactive aggregates appear in PINK1 KO rats remains uncertain. α-Synuclein is one of the most abundant synaptic proteins and is important for synaptic vesicle movement and synaptic transmission. Thus, decreased spontaneous excitatory postsynaptic currents (EPSCs) in medium spiny neurons appear in α-synuclein transgenic mice even at early ages when α-synuclein aggregates are first appearing. The α-synuclein abnormalities in PINK1 KO rats leads us to predict that defects in excitatory transmission will occur in PINK1 KO rats. To test this hypothesis, we conducted whole-cell, voltage-clamp recordings of medium spiny neurons in acute slices of dorsal striatum from PINK1 KO and wild-type (WT) littermate controls at various ages. We measured spontaneous and mini excitatory postsynaptic currents (sEPSC and mEPSCs, respectively) and paired-pulse ratios of evoked glutamatergic transmission to assess the efficacy of corticostriatal synaptic transmission. This work advances the characterization of PINK1 KO rats as a model of PD and can provide important insight into the mechanisms by which PD-linked loss-of-function mutations in PINK1 cause dysfunction and neurodegeneration.

Disclosures: R.B. Creed: None. **L.L. McMahon:** None. **M.S. Goldberg:** None.

Poster

134. Parkinson's Disease: Molecular and Cellular Mechanisms

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Program #/Poster #: 134.07/T16

Topic: C.03. Parkinson’s Disease

Support: APDA
NINDS R21NS095253
RISE, CUNY

Title: The coordination of Sonic Hedgehog signaling across the cholinergic interneuron centered connectome within the striatum and its implication for Parkinson’s disease

Authors: *L. B. MALAVE[1], D. ZUELKE[2], V. ANOSIKE[1], A. H. KOTTMANN[3]
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Abstract: Mesencephalic dopamine (DA) neurons are “multilingual” signaling centers that communicate with their targets with their namesake neurotransmitter dopamine and Sonic Hedgehog (Shh). In the striatum, Cholinergic (CIN) and Fast Spiking (FS) interneurons are the

only neuronal subtypes that express the Shh receptor patched (Ptch1). In CINs, Shh from DA neurons (Shh_{DA}) is critical for trophic support, regulation of activity, and gene expression. Recently, we found that Shh_{DA} facilitates reinforcement learning, inhibits habit formation, and is critical for the maintenance of small, plastic glutamatergic synapses on CINs. Importantly, we found that the absence of Shh in L-Dopa therapy contributes to the formation of debilitating, and uncontrollable L-Dopa induced dyskinesias (LIDs) and that pharmacological activation of the Shh signaling pathway activates CIN and attenuates LID in murine and monkey models of PD. These results implicate Shh_{DA} as an important regulator of neuroplasticity in the basal ganglia during learning and disease, but Shh_{DA} is only one of several potential sources of Shh for the striatum. A population of cortical pyramidal tract (PT) neurons also express Shh (Shh_{PT}) and project to the striatum. We hypothesize that Shh_{PT} represent an additional Shh source and that Shh_{PT} contributes to trophic support and neuromodulation of CIN. We further hypothesize that loss of multiple Shh sources contributes to the severity of LID expression. The conditional ablation of Shh_{DA} results in a reduction of extracellular ACh tone in the striatum and a late adult onset of partial, progressive degeneration of CINs in aged animals. We find in preliminary results that aged Shh_{PT}-/- animals have fewer CIN, reduced cholinergic neurite density, and swollen CIN soma in the dorsal lateral striatum. In the genetic aphakia model of PD, in which DA neurons fail to project into the striatum and thus Shh_{DA} cannot reach the striatum, we find an increased expression of Shh_{PT}. Together these observations reveal the existence of a rheostat feedback mechanism that senses Shh signaling and allows compensatory upregulation of Shh expression in response to reduced Shh signaling in the adult striatum. Our results also point to the possibility of additive effects of Shh signaling when delivered to the striatum from several sources. In future experiments we will investigate whether coincident signaling by Shh from PT and DA neurons leads to changes in CIN physiology that is distinct from changes in CIN physiology induced by Shh signaling from one particular source.

Disclosures: L.B. Malave: None. **D. Zuelke:** None. **V. Anosike:** None. **A.H. Kottmann:** None.

Poster

134. Parkinson's Disease: Molecular and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 134.08/T17

Topic: C.03. Parkinson’s Disease

Support: NINDS R21NS095253
APDA

Title: Chronic optogenetic stimulation of dopamine neurons induces optical induced dyskinesia (OID) due to an imbalance of sonic hedgehog and dopamine signaling

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Abstract: Mesencephalic dopamine (DA) neurons communicate with their targets by secreting dopamine and several other factors including Sonic Hedgehog (Shh). Progressive DA neuron degeneration causes the cardinal features of Parkinson's Disease (PD) and eventually leads to a diminishment of all DA neuron signaling factors. Exploiting unilateral optogenetic stimulation of DA neuron cell bodies in the midbrain with Shh specific pharmacology, we show that Shh signaling to cholinergic interneurons neurons (CIN) inhibits contralateral rotation. An hour of intermittent DA neuron stimulation leads to the exhaustion of Shh and an increase in rotational behavior. This optogenetic induced locomotion asymmetry is mitigated by VU0467154, an M4 positive allosteric modulator (PAM), previously found to attenuate L-Dopa induced dyskinesia (LID) in animal PD models. LID occur in over 90 % of patients on semi-chronic L-Dopa substitution therapy for more than 8 years. LIDs are thought to form from out of context L-Dopa induced dopamine highs that reinforce random, non-purposeful movements in the dopamine hypersensitive brains of PD patients. We hypothesized that if acute optogenetic stimulation leads to exhaustion of Shh and out of context striatal dopamine release, then chronic stimulation should model PD patients that receive L-dopa but not Shh agonists. We find that repeated, daily one hour forced optogenetic stimulation leads to the display and gradual increase of LID-like behaviors selectively during stimulation. We scored these LID-like behaviors, which we termed Optical Induced Dyskinesia (OID), using the Abnormal Involuntary Movement (AIM) scale. OIDs are attenuated by treatment with M4 PAM or the Shh agonist SAG. These findings suggest Shh signaling from DA neurons attenuates LID by counteracting D2 receptor mediated inhibition of CIN. Biochemically, pERK is unchanged between stimulated and non-stimulated striata indicating that OID is in absence of striatal dopamine hypersensitivity. However, like classic models of LID, OID is associated with increased expression of c-Fos selectively in the ipsilateral striatum and this activation is attenuated in SAG treated animals. Consistent with the pharmacological experiments we find that phosphorylation of ribosomal protein S6 (P-RpS6), a marker for burst firing activity, is decreased in CIN of the stimulated striata in animals with high OID. P-RpS6 increases in CIN with SAG treatment when compared to untreated controls. These findings reveal that in an unlesioned brain that lacks dopamine hypersensitivity LID-like OIDs can be induced by optogenetic stimulation that reduces Shh signaling relative to dopamine signaling.

Disclosures: D.R. Zuelke: None. **L.B. Malave:** None. **A.H. Kottmann:** None.

Poster

134. Parkinson's Disease: Molecular and Cellular Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 134.09/T18

Topic: C.03. Parkinson's Disease

Support: NIH-NINDS R21NS087496
NIH-NIDA R01036612
FWF J3656-B24

Title: Role for VGLUT2 in the selective vulnerability of midbrain dopamine neurons

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Abstract: Degeneration and dysfunction of midbrain dopamine (DA) neurons play a causal and contributing role in Parkinson's disease (PD). Although other neuron types are also affected, the cardinal symptoms of PD are a consequence of progressive DA neurodegeneration in the substantia nigra *pars compacta* (SNc). The precise mechanisms underlying DA neuron vulnerability remain unclear, but include mitochondrial dysfunction, oxidative stress, and alpha-synuclein aggregation. More recently, a glutamate-driven process has been implicated in disease progression, and there is now molecular and physiological proof that DA neurons themselves express the vesicular glutamate transporter VGLUT2 and co-release glutamate. There is evidence for a presynaptic role of VGLUT2, whereby VGLUT2 can increase the vesicular driving force for loading DA into synaptic vesicles, especially at times of high metabolic demand. This may enable tuning of DA release in response to activity changes. More recently, we discovered that >90% of SNc DA neurons express VGLUT2 in development, but most shut down VGLUT2 transcription in the adult. Interestingly though, VGLUT2 can re-emerge in response to neuronal insult. Re-emergent VGLUT2 expression may provide a beneficial compensatory adaptation for example through sequestration of endogenous or exogenous neurotoxins, and may contribute to the native resistance of ventral tegmental area (VTA) DA neurons that express more VGLUT2. Consistent with this, we find that midbrain DA neurons are more sensitive to neurotoxin-induced cell death in conditional knockout mice that lack VGLUT2 in DA neurons. On the other hand, we find that ectopic expression of VGLUT2 causes profound and selective toxicity to SNc DA neurons *in vivo*. Overall, our findings suggest that VGLUT2 expression in DA neurons is

dynamically regulated, and that the balance of VGLUT2 expression has important consequences on DA neuron survival *in vivo*. We thus speculate that VGLUT2 expression is actively repressed in adult SNc DA neurons, de-repressed with injury and that dysregulated VGLUT2 expression contributes to DA neuron vulnerability in PD.

Disclosures: T. Steinkellner: None. **V. Zell:** None. **Z. Farino:** None. **M. Sonders:** None. **M. Villeneuve:** None. **R. Freyberg:** None. **S.E. Przedborski:** None. **W. Lu:** None. **Z. Freyberg:** None. **T.S. Hnasko:** None.

Poster

134. Parkinson's Disease: Molecular and Cellular Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 134.10/U1

Topic: F.03. Neuroendocrine Processes

Support: NIH Grant NS091359

Title: NOX 1 and IP3, targets for membrane androgen receptor-induced neurodegeneration

Authors: M. TENKORANG[1], *R. L. CUNNINGHAM[2]
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Abstract: Parkinson’s disease (PD) is the second most common neurological disease. Oxidative stress (OS) plays a key role in the pathogenesis of PD. Several studies have established that Parkinson’s disease (PD) is sex biased, affecting more men than women. Testosterone, a primary male sex hormone and a known oxidative stressor, has been implicated in PD. Previous studies in our lab have shown that testosterone via a non-genomic mechanism exacerbates OS damage in dopaminergic neurons. However, the mechanism by which testosterone increases OS is unknown. We found testosterone acts through a membrane associated androgen receptor (mAR) variant - AR45, leading to increased OS. NADPH Oxidase 1 (NOX 1) is a major OS generator in cells, hence a potential target for testosterone-induced OS. We hypothesize that in dopaminergic cells, testosterone increases oxidative stress by activating NOX 1. We used a dopaminergic cell line (N27 cells). For an oxidative stressor, we used hydrogen peroxide (H2O2) prior to testosterone (100nm) administration. All inhibitors (androgen receptor -AR, NOX1, and IP3) were administered before H2O2 exposure. To examine mAR, we used cell impermeable DHT-BSA (500 nM) to confirm that NOX 1’s effect is through a non-genomic mechanism. Cell viability and OS were quantified using the MTT and Reduced Thiols assays respectively. NOX 1 and AR protein expression were also quantified. To determine if NOX 1 interacts with a mAR, we immunoprecipitated the mAR and probed for NOX 1. Using classical AR antagonists did not block testosterone’s negative effects in an OS environment. However, testosterone’s negative effects were blocked by degrading AR, indicating the involvement of the non-genomic mAR.

We found the mAR complexes with NOX 1 protein. To investigate the involvement of NOX 1, we used the NOX 1 inhibitor, Apocynin. Apocynin did not alter H2O2-induced cell loss, indicating that H2O2 increases OS via a non-NOX 1 mechanism. However, Apocynin blocked testosterone induced cell loss and OS generation, suggesting that NOX 1 mediates testosterone's damaging effects in an OS environment. Inhibition of NOX 1 also blocked DHT-BSA's damaging effects on cell viability in an OS environment. Since mAR also complexes with the Gαq/IP3 intracellular calcium pathway, we inhibited IP3 receptors and blocked testosterone's negative effects on cell viability. We conclude that testosterone-induced cell loss is mediated by a NOX 1/mAR complex that activates an IP3-mediated calcium pathway.

Disclosures: M. Tenkorang: None. **R.L. Cunningham:** None.

Poster

134. Parkinson's Disease: Molecular and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

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Topic: C.03. Parkinson's Disease

Support: Department of Defense Grant W81XWH-17-1-0386
NIH 1R01NS082650
NeuroDiscovery Pilot Grant from the Pittsburgh Brain Institute
NIH NINDS T32 NS086749

Title: Dopamine-induced spine plasticity may mediate normalized motor function in a 6-OHDA model of Parkinson's disease

Authors: *J. BRAGUE, R. P. SEAL
Neurobio., The Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Dopaminergic signaling is critically important to the regulation of motor functions in the mammalian nervous system. For example, the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpC) leads to severe motor deficits, as seen in patients with Parkinson's Disease (PD). These dopamine neurons densely innervate the dorsal striatum and profoundly influence motor function through actions on medium spiny neurons (MSN), the major projection neurons of the striatum. These projection neurons form the direct (go) and indirect (no go) output pathways of the basal ganglia, hypothesized to coordinate motor behavior. Loss of striatal dopamine in PD is thought to affect motor behavior by altering the output of these two pathways. Our lab has previously reported that mice lacking vesicular glutamate transporter 3 (Vglut3KO) show a circadian-dependent hyperlocomotion, hyperdopaminergia, and upregulation of immature dendritic spines on dopamine 1 receptor (D1R; direct) containing MSNs in the dorsal striatum. Unexpectedly, Vglut3KO animals do not show motor impairments in a 6-hydroxydopamine (6-

OHDA) model of PD throughout the circadian cycle, suggesting a more permanent form of plasticity is normalizing motor output. However, the exact mechanisms underlying the improved motor function in the 6-OHDA model are unknown. We hypothesize that dopamine depletion, in Vglut3KO animals, triggers the maturation of the elevated immature spines to normalize motor function. To test this hypothesis, we injected wild type and Vglut3KO males and females (3-4 months) with the retrograde tracer, cholera toxin B-488, into the SNp reticula, to differentiate D1R from D2R MSNs, and 6-OHDA in the dorsal striatum for depletion. The data indicate that depletion upregulated D1R mature spines ($n=12$; $p<0.01$) while decreasing D1R immature spines ($n=12$; $p<0.01$). Unlike control mice ($n=3$; $p<0.01$), Vglut3KO animals exhibit normalized motor function throughout the circadian cycle ($n=6$; day $p=0.98$; night $p=0.42$). Preliminary data also indicate is an increase in immature spines on D1R MSNs with selective stimulation of the dopamine neurons, mimicking what is observed in the VGLUT3KO. These data suggest that dopamine upregulation, prior to depletion, provides a potential therapeutic effect in PD by altering dendritic spines on D1R MSNs. Understanding how striatal dopamine levels affect spine plasticity within basal ganglia motor circuits will help the development of more effective treatment strategies for patients suffering from PD.

Disclosures: J. Brague: None. **R.P. Seal:** None.

Poster

134. Parkinson's Disease: Molecular and Cellular Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 134.12/U3

Topic: C.03. Parkinson’s Disease

Support: W911NF-10-1006
F30MH106293
T32HL007909

Title: A CDK1-NUCKS1-regulated striatal dopamine-responsive gene network links motor, sleep, and affective phenotypes in Parkinson’s disease

Authors: *P. JIANG[1,2], J. R. SCARPA[3], V. D. GAO[1,2], M. H. VITATERNA[1,2], A. KASARSKIS[3], F. W. TUREK[2,4,1]
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Abstract: In addition to the characteristic motor symptoms, Parkinson’s disease (PD) often involves a constellation of sleep and mood symptoms, although the mechanisms underlying the linkage among symptoms are largely unknown. Using a systems approach, we integrated data

from multiple public sources to characterize gene networks that are perturbed in PD and are associated with sleep and affective phenotypes, since these networks may contribute to symptomatic changes in sleep and mood when disrupted by PD pathology. Our analysis focused on the striatum, which is one of the primary brain regions affected by the dopamine deficiency in PD, leading to PD symptoms. We first established a robust differential gene expression signature by performing a meta-analysis combining five transcriptomic datasets collected from the striatum of PD patients. To understand how altered gene expression in PD may perturb sleep and affective functions leading to non-motor symptoms, we integrated the PD differential expression signature with functional gene networks in the striatum reconstructed in our previous study of sleep and affective phenotypes in a population of (C57BL/6J x A/J) F2 mice. Four gene networks were found enriched with upregulated genes in PD. Elevated gene expression in one of those networks was also widely supported by published data in a range of mouse models of PD. This network was enriched with genes involved in the circadian clock and mitotic regulations, and in our F2 mouse population, it was associated with sleep fragmentation and despair-related phenotypes, which are relevant to PD non-motor symptoms. In addition, network driver genes in this network are known to affect motor behaviors when mutated. Therefore, this striatal gene network might be an important nexus linking PD pathology to symptoms. We further demonstrate that this network was responsive to dopamine signaling, and may function as a postsynaptic effector that is perturbed by the nigrostriatal dopamine deficiency in PD. We identified a potential upstream regulator of this striatal gene network, CDK1, which may modulate the gene expression in this gene network via a network of transcription factors, including NUCKS1, which was associated with genetic susceptibility of PD. Our preliminary data indicated that inhibition of CDK1 led to improved locomotor activities in a mouse MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) model of PD. Taken together, our findings provide novel insights into the mechanisms by which dopamine deficiency in PD leads to a complex constellation of motor, sleep, and mood symptoms.

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Poster

135. Neurotoxicity, Inflammation, and Neuroprotection: Animal Models

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 135.01/U4

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH/NNRS R01 NS097313-01
Intramural support from NIDA and NIAAA to K.C.R.

Title: Low-dose rat and mouse experimental autoimmune encephalomyelitis (EAE) models for unconfounded testing of complex behaviors

Authors: *E. H. MITTEN[1], A. J. KWILASZ[1], A. E. W. SCHRAMA[1], L. S. TODD[1], J. C. DURAN-MALLE[1], S. M. GREEN FULGHAM[1], A. VAN DAM[2], H. P. PATEL[1], S. F. MAIER[1], K. C. RICE[3], L. R. WATKINS[1]
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Abstract: Multiple sclerosis (MS) is a debilitating and lifelong disease of the central nervous system. MS leads to demyelination of neurons and neuroinflammation, ultimately leading to an inability for neurons to communicate effectively. The primary symptom associated with MS involves paralysis to varying degrees. However, patients with MS also experience many additional secondary neurological symptoms, such as neuropathic pain, deficits in cognition/social interaction, and depression including anhedonia. Importantly, the most common preclinical rodent model of MS, experimental autoimmune encephalomyelitis (EAE), causes paralysis similar to MS which can confound testing in many standard assays of rodent behavior, particularly in assays that are optimized to study many of the aforementioned secondary symptoms of MS. In this study, we utilize a low-dose EAE model in which rats and mice develop minimal motor impairment throughout disease progression, allowing for extensive and repeated testing of complex behaviors. Male and female Dark Agouti (DA) rats, female C57Bl/6J mice, and female SJL mice were administered reduced doses of standard EAE-inducing agents (myelin oligodendrocyte glycoprotein [MOG] for DA rats and C57BL/6J mice and proteolipid protein (PLP] for SJL mice). Paralysis and mechanical allodynia (central neuropathic pain) were then assessed for up to 2 months post-EAE induction. Results indicate that by reducing the MOG or PLP doses used, a low-level EAE disease progression can be induced that minimizes confounding motor impairment yet allows robust expression of pain behavior in both rat and mouse EAE models. Future studies will utilize our newly-developed low-dose EAE models to study additional complex behaviors that model secondary symptoms associated with MS.

Disclosures: E.H. Mitten: None. **A.J. Kwilasz:** None. **A.E.W. Schrama:** None. **L.S. Todd:** None. **J.C. Duran-Malle:** None. **S.M. Green Fulgham:** None. **A. Van Dam:** None. **H.P. Patel:** None. **S.F. Maier:** None. **K.C. Rice:** None. **L.R. Watkins:** None.

Poster

135. Neurotoxicity, Inflammation, and Neuroprotection: Animal Models

Location: SDCC Halls B-H

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Program #/Poster #: 135.02/U5

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Intramural Support from NIDA and NIAAA to K.C.R
NIH/NNRS R01 NS097313-01

Title: The toll-like 2 and 4 receptor antagonist (+)-naltrexone reverses neuropathic pain and associated spinal inflammation in male and female Dark Agouti rats in a model of multiple sclerosis

Authors: *A. E. SCHRAMA[1], A. J. KWILASZ[1], J. C. DURAN-MALLE[1], E. H. MITTEN[1], L. S. TODD[1], S. M. GREEN FULGHAM[1], H. P. PATEL[1], X. WANG[2], A. VAN DAM[3], S. F. MAIER[1], K. C. RICE[4], L. R. WATKINS[1]
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Abstract: Up to 92% of patients with multiple sclerosis (MS) report frequent and disabling neuropathic pain. In MS, neuropathic pain develops after demyelination, neuroinflammation, and damage to axons in the central nervous system. Although several treatments for MS-related neuropathic pain exist, many patients' symptoms are refractory to current treatments. Recent research has provided evidence that toll like receptors 2 and 4 (TLR2/4) are implicated in propagating the inflammatory response of MS. Moreover, previous research in our lab has shown that TLR2/4 antagonists are effective at reversing neuropathic pain in various rodent models. In this study we thus investigated the effects of the non-opioid TLR2/4 antagonist, (+)-naltrexone, on mechanical allodynia and transcription levels of spinal TLR2/4-related inflammatory markers (i.e. TLR2/4, NLRP3, IL-1β, TNF, IκBα) as well as the Th17 cell signalling molecule, IL-17, using the experimental autoimmune encephalomyelitis (EAE) model of MS. Male and female Dark Agouti rats were induced with EAE and 14 days later began 14 days consecutive treatment with subcutaneous (+)-NTX or saline. (+)-Naltrexone treatment successfully reversed neuropathic pain in both male and female rats compared to the rats receiving saline treatment. Moreover, (+)-naltrexone treatment resulted in significantly lower inflammatory mRNA markers (i.e. TLR2/4, NLRP3, IL-1β, TNF, IκBα, and IL-17) in the spinal cord relative to the saline-treated animals. Lastly, administration of intrathecal interleukin-1 receptor antagonist (IL-1ra) on day 15 and 29 post EAE induction demonstrated that ongoing spinal IL-1β signaling is necessary for EAE-induced mechanical allodynia both early and late in disease development. Collectively, our findings provide the first evidence supporting both TLR2/4 and intrathecal IL-1β antagonism as effective interventions against EAE related chronic neuropathic pain in both males and/or females and suggests decreased spinal IL-1β and other related inflammatory signals as important mechanisms by which (+)-naltrexone exerts its therapeutic effects.

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Poster

135. Neurotoxicity, Inflammation, and Neuroprotection: Animal Models

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 135.03/U6

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: UBACYT 20020130100673BA
UBACYT 20020120100216
CONICET PIP567
CONICET PUE060

Title: Immunomodulatory action of bone marrow cell transplant in sciatic nerve injury

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Abstract: Bone marrow mononuclear cells (BMMC) include different cell types, containing a minority multipotent fraction. For this reason, BMMC have recently become a therapeutic alternative to mesenchymal stem cells, as culture is not required and phenotypic transformations can be hence avoided. Wallerian degeneration induced by nerve sectioning or compression is a simple and extremely useful experimental approach to study the pathophysiology of peripheral nervous system degenerative disorders. In this model, our group has shown systemically transplanted BMMC to spontaneously migrate to and remain in the injured nerve for as long as 60 days post injury. BMMC were also shown to enhance axonal regeneration and remyelination, and to prevent lesion-induced hyperalgesia. In this context, the aim of the present work is to evaluate whether BMMC exert their well-established beneficial effect on sciatic nerve regeneration through immunomodulatory actions. Adult C57BL/6 mice received intravenous transplantation of either BMMC or vehicle after 8-second sciatic nerve crush and were then sacrificed 1, 3 or 7 days after transplant to perform immunohistochemistry, qPCR and flow cytometry analyses. So far, immunohistochemical analyses carried out after recovery showed a small number of BMMC upregulating markers unexpressed before transplant, which led to cell phenotypic changes and transdifferentiation to Schwann cells to participate in axon ensheathment and remyelination. However, a significantly larger proportion may be speculated to have left the tissue after the inflammatory phase had finished. In addition, qPCR results have shown animals transplanted with BMMC to undergo a downregulation of pro-inflammatory signals and an upregulation of anti-inflammatory cytokines. Nevertheless, further studies are required to fully corroborate immunomodulation effects.

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Poster

135. Neurotoxicity, Inflammation, and Neuroprotection: Animal Models

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 135.04/U7

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH/NNRS R01 NS097313-01
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Title: The non-opioid TLR2/4 antagonist (+)-naltrexone blocks contextual long-term memory deficits in experimental autoimmune encephalitis and associated neuroinflammation in hippocampus

Authors: *A. J. KWILASZ[1], L. S. TODD[1], J. C. DURAN-MALLE[1], A. E. W. SCHRAMA[1], E. H. MITTEN[1], A. VAN DAM[2], S. F. MAIER[1], K. C. RICE[3], L. R. WATKINS[1], R. M. BARRIENTOS[1]
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Abstract: Approximately half of individuals suffering from multiple sclerosis (MS) express cognitive deficits in the form of memory impairments. Previously we have shown that administration of toll-like 2/4 (TLR2/4) antagonists such as (+)-naltrexone [(+)-NTX] block neuropathic pain and associated spinal inflammation in rats with a model of MS, experimental autoimmune encephalomyelitis (EAE). In this study, we examined the effects of (+)-NTX on memory impairments and associated brain inflammation in EAE. Male Dark Agouti rats were induced with EAE and 14 days later received subcutaneous (+)-NTX or saline for 14 days. Contextual- and auditory-fear conditioning were then conducted to assess memory impairments one week after beginning (+)-NTX administration. We found that EAE induced impairments in long-term contextual fear memory but not short-term contextual- or auditory-related fear memory. This effect was associated with increased interleukin-1β (IL-1β) mRNA expression in the hippocampus but not the amygdala. Importantly, (+)-NTX blocked EAE-induced impairments in long-term contextual fear memory and the associated increased hippocampal IL-1β expression. Moreover, (+)-NTX blocked hippocampal expression of TLR2, TLR4, NLRP3, and IL-17 mRNA, suggesting blockade of the TLR2/4, NLRP3 inflammasome and Th17 cell signaling as additional mechanisms by which (+)-NTX exerts its therapeutic effects. These findings support the role of TLR2/4 antagonists as potential treatments for MS-related cognitive

deficits and encourage future research on TLR2/4 antagonists as treatments for various symptoms associated with inflammatory-related diseases, including MS.

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Poster

135. Neurotoxicity, Inflammation, and Neuroprotection: Animal Models

Location: SDCC Halls B-H

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Program #/Poster #: 135.05/U8

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: *In vivo* brain imaging following organophosphate exposure utilizing 2-photon microscopy in mice

Authors: *K. LAITIPAYA, J. K. CHANDLER, C. E. KAROLENKO, D. D. PALMER, D. L. SPRIGGS, E. A. JOHNSON, J. W. SKOVIRA
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Abstract: Organophosphate (OP) cholinesterase inhibitors cause excessive excitatory signaling in the central nervous system that can result in persistent seizures and progressive brain damage. A robust neuroinflammatory response is associated with seizure activity. The cellular neuroinflammatory response following OP exposure is characterized by the activation of astrocytes and microglia and the infiltration of circulating monocytes. These changes occur before the first signs of overt cellular pathology, suggesting that they are in response to signals from neurons injured during seizure activity and indicate a role for inflammatory cells in OP-induced neural damage. The objective of this project was to monitor the cortex and hippocampus of mice *in vivo*, before and after OP exposure, to track and characterize the neuroinflammatory response to OP exposure. This was achieved by utilizing a 2-photon microscope and harness system to view a small region of the brain *in vivo* via a window surgically implanted on the skull. Three different genetically modified strains of mice were used in these experiments, enabling observation of astrocytes (FVB/N-Tg[GFAPGFP]14Mes/J) and microglia (B6.129P-Cx3cr1tm1Litt/J) via green fluorescent protein (GFP) and neurons (B6.Cg-Tg[Thy1-YFP]HJrs/J) via yellow fluorescent protein (YFP). Mice were imaged 1 week prior to OP exposure (sarin, 256 µg/kg or 400 µg/kg; IP) and then for 30 days following exposure. The oxime HI-6 (50 mg/kg; IP) was given prior to OP exposure, and atropine (1 mg/kg; SC) was given immediately following exposure to increase survival. Images taken over 30 days following exposure within individual animals indicate distinct changes in microglia and astrocytes (increase in size and number) and neurons (increased expression of Thy-1 in axonal and dendritic processes) as observed via increase or decrease in GFP or YFP signaling.

Disclosures: K. Laitipaya: None. **J.K. Chandler:** None. **C.E. Karolenko:** None. **D.D. Palmer:** None. **D.L. Spriggs:** None. **E.A. Johnson:** None. **J.W. Skovira:** None.

Poster

135. Neurotoxicity, Inflammation, and Neuroprotection: Animal Models

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 135.06/U9

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: 2R15NS060117-02

Title: Brassicaceae and asteraceae plants (kale, arugula, dandelion) mediates gut microbiota in maintaining phylogenetic bacterial diversity and reduces cognitive decline in diet-induced obese pre-diabetic C57BL/6 mice

Authors: *B. TENG, D. FOSTER, A. A. OYETUNDE, V. PEÑA-GARCIA, S. SHATELA, T. SIMON, D. HICKS, G. FLORES, L. BANNER
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Abstract: World obesity rates have increased in the last few decades. An increase in high fat consumption along with a decrease in physical activity is the primary driver leading the obesity epidemic. Diet-induced obesity predisposes individuals to type-2 diabetes and contributes to complications including those of the nervous system and the immune system. Diabetes is associated with an elevated risk for neurodegeneration and dementia and changes in hippocampal plasticity. Studies have shown that neurodegeneration caused by diabetes is related in part, to elevated levels of inflammatory cytokines involved in brains of animals fed a high fat diet (HFD). In addition, it is well documented that a high fat diet causes changes in gut microbiota. The dysbiosis of gut microbiota triggers a pro- inflammatory response and may also disrupt neuronal signaling. While diabetes caused by diet-induced obesity is largely preventable by reducing high fat intake, individuals often find it difficult to radically change unhealthy aspects of their diet. Instead, we are proposing to alter the imbalance of gut microbiota through supplementation of kale, arugula (f. Brassicaceae) and dandelion (f. Asteraceae) plants to mediate the inflammatory response, potentially taper neurodegeneration, and thus stymie cognitive decline. To address this issue, C57BL/6 mice were fed either a control or high-fat diet (HFD)(60% fat) for 18 weeks until the high-fat group reached a pre-diabetic stage. After 18 weeks, mice on a HFD weighed significantly more than the control mice, displayed elevated blood glucose levels, and showed deficits in spatial learning. During weeks 18 to 40, the diets of all the mice were supplemented daily with 1.0 gram of kale, arugula, or dandelion. During the 22- weeks when the mice were fed their supplemental kale, arugula, or dandelion diets, the mice were subjected to multiple repetitions of the Morris Water Maze, Barnes Maze, and Nonconditioned Social Discrimination Procedure, to probe for changes in their memory. Fecal

samples before and after supplementation of Brassicaceae and Asteraceae plants were collected and changes in gut microflora were characterized by 16S rRNA gene sequencing for bacterial identification. Preliminary data shows that mice fed a HFD and supplemental greens maintained their phylogenetic bacterial alpha diversity, while mice solely fed a HFD saw a decrease. This suggests that the supplemental greens may protect or slow the progression of the dysbiosis of the gut microbiome that is typical of a HFD. Analyses are ongoing; the brains of the subject animals will also be analyzed for inflammatory, neuronal markers and changes in dendritic spine morphology.

Disclosures: D. Foster: None. **A.A. Oyetunde:** None. **V. Peña-Garcia:** None. **S. Shatela:** None. **T. Simon:** None. **D. Hicks:** None. **G. Flores:** None. **L. Banner:** None.

Poster

135. Neurotoxicity, Inflammation, and Neuroprotection: Animal Models

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 135.07/DP05/U10

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Inhibition of RIP1 reduces EAE-induced disease symptoms via a necroptosis-independent process

Authors: *S. ZHANG, Y. SU, Z. YING, J. GUO, C. PAN, D. GUO, Z. ZHANG, Z. ZHANG, X. WANG
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Abstract: Background & Abstract
Experimental autoimmune encephalomyelitis(EAE) has long served as an animal model for human Multiple sclerosis (MS), a demyelination disease in which the insulating covers, myelin structure, of nerve cells in the brain and spinal cord are affected. The main process of EAE could be classified into three steps, the immune response is the first step of disease, followed by the blood brain barrier (BBB) break down, eventually demyelination.
Previous study, from another group has already told that RIP1 inhibitor could decrease the disease symptoms through blocking the necroptosis pathway, however, in my recent work, I found the RIP1 inhibitor 1165, a small molecule created by our cooperator, could significantly reduce the disease symptoms in mice challenged by EAE, through necroptosis independent pathway.
I divided the whole project into four parts to discover the target of RIP1 inhibitor in EAE mouse model: I firstly found that the function of 1165 is truly repeatable, but necroptosis has little contribution in the disease process. Moreover, in the second part, I found that the structure of BBB is still intact if treating the mice with 1165 in the EAE disease model, so the function of 1165 should be at the initial steps of disease progression. In the third part, I started to test the

immune response, comparing the EAE or EAE+1165 groups: the ability of antibody generation is no difference, however, the whole blood testing showing that the number of monocyte decreased a lot if treated with 1165. Monocyte is the most important part of EAE, previous study has already shown that if block the function of monocyte the disease symptoms could decrease significantly. To figure out, if 1165 also has a function in the demyelination process. In the last part, two alternative demyelination models have been used, however, 1165 has no significant protective function in all three models.
In conclusion, I found the RIP1 inhibitor 1165 directly or indirectly influencing the monocyte function to block the disease process which has no relation with necroptosis or demyelination in EAE mouse model.

Disclosures: S. Zhang: None. **Y. Su:** None. **Z. Ying:** None. **J. Guo:** None. **C. Pan:** None. **D. Guo:** None. **Z. Zhang:** None. **Z. Zhang:** None. **X. Wang:** None.

Poster

135. Neurotoxicity, Inflammation, and Neuroprotection: Animal Models

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 135.08/U11

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: FDA OSEL CDRH DBP

Title: Evaluation of neuroinflammation and behavioral outcomes following high intensity therapeutic ultrasound to the brain

Authors: *H. RAFI[1], K. SOLARANA[2], M. R. MYERS[4], C. G. WELLE[5], M. YE[3]
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Abstract: High intensity therapeutic ultrasound (HITU) has been used for many years with FDA-approved applications for diverse indications. Within the brain, HITU is used to treat patients with essential tremor that do not respond to drug treatment. Newer areas of application are under investigation, including the use of HITU for blood brain barrier disruption to facilitate drug delivery into the brain, the ablation of brain tumors; focused ultrasound surgery or FUS, and as a neuromodulation technique to stimulate or suppress neural activity in the treatment of epilepsy or chronic pain. With the expansion of HITU modalities under development, it is important to understand the effect of HITU on the brain at both a structural and functional level. To elucidate the spatial and temporal tissue effects of HITU, we delivered high intensity focused ultrasonic pulses with an output pressure approximately 12 MPa to the left motor cortex of the brain in mice. Behavioral performance was evaluated using a rotarod to assess locomotor

deficits, and an open field test (OFT) to assess changes in exploratory behavior. Behavioral tests were repeated at 2-hours, 24-hours, 1-week, and 1-month post-HITU or a sham procedure. The neuroinflammatory response was then investigated postmortem using immunohistochemical (IHC) staining at 24-hours and 1-month post-HITU or sham, with a focus on astrocytes (GFAP) and microglia (Iba-1). We comprehensively examined multiple anatomic locations in brain slices from 1 mm anterior to bregma to 2 mm posterior to bregma to further evaluate the spatial extent of HITU effects.
Behavioral analysis revealed significantly poorer performance in HITU mice, with an impaired ability to remain on the rotarod and a decrease in distance traveled in the OFT arena. We saw a significant increase in astrocyte and microglial reactivity in the cortex of HITU-treated mice, indicating a neuroimmune response to the HITU procedure. Interestingly, although focused ultrasound pulses were delivered, immune responses indicated a more diffuse effect of HITU beyond the cortex, with increases in astrocytes evident in the thalamus and anterior commissure, and microglial reactivity in the hypothalamus and caudate putamen. The data also suggest a possible correlation between rotarod performance and the neuroimmune response. By analyzing the effects of HITU on the behavioral and histological changes seen in mice, we can gain a more comprehensive understanding of the safety profile of HITU on the brain and identify parameters that minimize unintended tissue effects.

Disclosures: H. Rafi: None. **K. Solarana:** None. **M.R. Myers:** None. **C.G. Welle:** None. **M. Ye:** None.

Poster

135. Neurotoxicity, Inflammation, and Neuroprotection: Animal Models

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 135.09/U12

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: INNOVATE PERU
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.)
NIH training grant FIC/NIH D43 TW001140

Title: Neurocysticercosis:Neurological disability in a novel animal model

Authors: *L. E. BAQUEDANO[1], A. D. DELGADO[1], D. G. DÁVILA[1], R. GILMAN[2], M. R. VERASTEGUI[1]
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Abstract: Neurocysticercosis (NCC) is a parasitic disease caused by the Taenia solium larval stage (cyst) in the central nervous system. NCC is the leading cause of acquired epilepsy in endemic countries. The clinical manifestations of NCC are pleomorphic, and seizures are the most common clinical manifestation; cognitive decline is also observed, involving multiple memory deficits or dementias in some cases. Motor and sensorial disabilities have been reported in 15% of people worldwide caused by this disease. Previously studies were realized in rat model for neurocysticercosis and was confirmed the relationship between NCC and impairment cognitive represented as memory deficit. The aim of this study was to use the animal model to evaluate the motor and sensorial ability in a novel animal model to NCC using rats that was developed in our laboratory. The Holtzman rats between 12 to 15 days after birth were intracranial infected with activated T. solium oncospheres or saline solution for control group. Then, we use the Neurobehavioral severity scale (NSS) to evaluate balance, motor coordination (including movement and postures), as well as motor and sensory reflexes in rats at 12 months after infection. The brains were stained with Hematoxylin and eosin, Masson's trichrome stain and GFAP to histological and immunohistochemistry examination. The results indicate that rats with NCC had sensorimotor deficits that are statically significant compare to control group ($p<0.04$), using NSS task. We found statistical differences in tail raise ($p=0.041$) and drag test ($p=0.000$). The histological damage showed that the localization of cyst in cerebellum was associated with the motor disability in infected rats. Also, 5% of rats showed cerebellar injures, gait abnormality, ataxia, visual changes and pyramidal signs. a layer of collagen tissue, infiltrate cells, perivascular infiltrate and gliosis in the surrounding the cyst. This study is the first systemic review of clinical manifestations associated with NCC in rat as animal model, which can have a wide spectrum of neurologic manifestations including seizures, cerebrovascular disorders, motor deficits. In the future we hope to look for new treatment schemes to avoid or decrease this disease.

Disclosures: L.E. Baquedano: None. **A.D. Delgado:** None. **D.G. Dávila:** None. **R. Gilman:** None. **M.R. Verastegui:** None.

Poster

135. Neurotoxicity, Inflammation, and Neuroprotection: Animal Models

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 135.10/V1

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Sodium phenyl butyrate neuroprotective effect over status epilepticus induced damage in immature rats

Authors: *C. E. GALLARDO FLORES[1], A. S. VEGA-GARCÍA, Jr.[2], A. TALEVI[3], S. OROZCO-SUAREZ[4]

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Abstract: Neuroinflammation and seizures are two related processes. In previous works, it's been found that proinflammatory cyclooxygenase 2 (Cox-2) is overexpressed during seizures and in patients with epilepsy. The objective of this work is to determine whether Sodium Phenyl Butyrate (SPB) inhibits Cox-2 expression and evaluate the neuroprotective effect on the hippocampus.
Kainic acid was used to induce *status epilepticus* in 12 post-natal days (PN) rats, and one hour later, 6 different doses of SPB were administered to find the effective dose 50. A day later, the hippocampus was extracted and Cox-2 expression was analyzed by Western Blot method. Subsequently, *status epilepticus* was induced with KA in another group of rats and the effective dose of SPB (50ug/m) was administered for 5 days every 24 hours. On the 6th day, rats were perfused and the brains were extracted and sectioned in 12 micron sections by cryostat. Fluorojade and Immunofluorescence were used to determine neuroprotective effect and Cox-2 expression in dorsal and ventral hippocampus respectively.
Results showed that FBS (50 mg/Kg) dose treatment reduced the expression of Cox-2 in hippocampus. Furthermore, higher neuroprotective effect was observed in dorsal hippocampus than in ventral hippocampus as determined by Fluorojade and Cox-2 immunofluorescence. FBS reduced the cells death process in CA1 mainly and the Cox-2 expression in ventral hippocampus where it was observed in blood vessels and dentate gyrus neurons, while Cox-2 expression in dorsal tissue was limited to blood vessels only. This also supports that FBS had better neuroprotective effect in dorsal hippocampus than in ventral hippocampus.
In conclusion, results suggest that intervention during post-*status epilepticus* inflammation may have neuroprotective effects that would reduce epilepsy risk.

Disclosures: C.E. Gallardo Flores: None. **A.S. Vega-García:** None. **A. Talevi:** None. **S. Orozco-Suarez:** None.

Poster

135. Neurotoxicity, Inflammation, and Neuroprotection: Animal Models

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 135.11/V2

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Rotary Club of Narellan

Title: Bilateral orchiectomy preserves peripheral immune organs in cuprizone treated mice

Authors: *P. J. SHORTLAND[1], M. S. ALMUSLEHI[2], M. K. SEN[3], D. A. MAHNS[3], J. R. COORSEN[4]
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Abstract: Multiple sclerosis (MS) is a common disabling neurological disorder affecting about 2.5 million people worldwide. We tested whether a variant of the established cuprizone (CPZ) mouse model yielded a pattern of disease development and progression more closely resembling that seen clinically in human MS. Recently, we found that CPZ had a prominent suppressive effect on peripheral immune organs (thymus and spleen); T lymphocytes could not be detected in the CNS of CPZ-treated mice even when the integrity of the blood brain barrier had been disrupted by pertussis toxin treatment. Here, we hypothesised that castration (Cx) could protect the thymus and spleen from atrophy during CPZ treatment. Four week old C57BL/6 mice were surgically orchidectomized followed by CPZ feeding for two weeks. Six groups of mice were treated as follows: group (1) control, (2) 0.1% CPZ, (3) 0.2% CPZ, (4) Cx, (5) Cx+0.1% CPZ, and (6) Cx+0.2% CPZ. There were significant changes ($p \leq 0.05$) in thymus and spleen wet weight between CPZ treated mice and Cx mice. Western blot analysis confirmed a clear suppression of CD4 and CD8 signal intensity in thymus and spleen of CPZ fed mice whereas CD4 and CD8 signals were preserved in the Cx groups. CNS histology indicated that Cx increased the severity of demyelination in Cx+CPZ groups relative to CPZ treatment alone. Immunohistochemically, there was no synergistic/additive effect of Cx+CPZ on microglial or astrocyte activation. By confirming peripheral immune suppression, the data here provide new insight to the long-standing question of why CPZ can induce demyelination but fails to recruit peripheral immune cells into the CNS. Moreover, the findings identify a novel and important variant of the CPZ model of Multiple Sclerosis.

Disclosures: P.J. Shortland: None. **M.S. Almuslehi:** None. **M.K. Sen:** None. **D.A. Mahns:** None. **J.R. Coorsen:** None.

Poster

135. Neurotoxicity, Inflammation, and Neuroprotection: Animal Models

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 135.12/V3

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Field Neurosciences Institute
Department of Chemistry and Biochemistry
John G. Kulhavi Professorship in Neuroscience
Neuroscience Program and CMU

CMU College of Medicine

Title: Therapeutic effect of curcumin entrapped dendrimer nanoparticles on a mouse model of glioblastoma

Authors: *N. MUNRO[1], B. SRINAGESHWAR, 48858[2], M. FANA[3], C. MALKOWSKI[2], S. CLIMIE[2], B. KATHIRVELU[2], D. SWANSON[2], A. SHARMA[2], G. DUNBAR[2], J. ROSSIGNOL[4]
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Abstract: Glioblastoma (GBM) is an aggressive form of brain tumor. Currently there are no viable treatment options outside of radiation therapy and chemotherapy for this disease. Dendrimer nanoparticles are multi-branched, star-shaped macromolecules with nanometer-scale dimensions. Dendrimers can be defined by three components. The first being a central core, the second being an interior dendritic structure, and finally an exterior surface with functional surface groups. What makes dendrimers potentially very useful for the treatment of many diseases is that fact that the shielded interior cores can carry cargo within them. For this study we used cystamine dendrimers (D-Cys) which have anti-inflammatory properties due to reduced forms and thiol groups formation resulting of their splitting when entering the cytosol. This dendrimer can also contain and release their cargo once reduced. In this study, D-Cys were loaded with curcumin (D-Cys-Cur). Curcumin is an extract of the turmeric plant and has anti-inflammatory properties itself. Curcumin has been shown to cause cell death of the GBM cells while sparing stem cells and neurons at certain concentrations. The anti-inflammatory effects of the dendrimers combined with the possibility of curcumin, causing GBM cell death, will hopefully emphasize tumor shrinkage and help to facilitate a longer lifespan. Our lab has observed significant results of D-Cys-Cur on GBM cells *in vitro*. This study focuses on the possible *in vivo* benefits of D-Cys-Cur on GL261 glioblastoma cells in C57BL/6J mice. Two groups were designed, the survivability group of animals serves the purpose of observing if the treatment helps to lengthen the lifespan of the mouse, the histology group serves the purpose of looking at the tumor size and the inflammation that is present around the tumor.
The mice treated with D-Cys-Cur are living significantly longer when compared to non-treated mice. A trend towards significance, when comparing the inflammatory markers between treated and untreated mice, was observed and will be confirmed with addition of more animals.

Disclosures: N. Munro: None. **B. Srinageshwar:** None. **M. Fana:** None. **C. Malkowski:** None. **S. Climie:** None. **B. Kathirvelu:** None. **D. Swanson:** None. **A. Sharma:** None. **G. Dunbar:** None. **J. Rossignol:** None.

Poster

136. Ischemia: Neuroprotection

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 136.01/V4

Topic: C.08. Ischemia

Title: Exploring neuroprotective potential of chlorogenic acid in ischemic stroke rat model: An *in vivo* and *in silico* approach

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Abstract: Stroke is life-threatening neurological disorder and a leading cause of adult disability. Several clinical trials have been performed using potential neuroprotective agents, none of them has proven to be useful for providing functional recovery from ischemic stroke. Therefore, present in vivo and in silico study was designed to investigate the neuroprotective potential of chlorogenic acid in middle cerebral artery occlusion (MCAO) rat model, and further molecular docking simulation was performed to evaluate its inhibition potential for NMDA, nNOS, and iNOS. Chlorogenic acid (10mg/Kg dose) was administered intranasally before 30 min of MCAO. Brain infarction, % brain water content, blood-brain barrier (BBB) disruption along with biochemical and molecular biomarkers of neuronal dysfunction were observed in the different regions of rat brain after 4 hrs. of reperfusion. Further, chlorogenic acid was docked with mediators of neuronal dysfunction, i.e., NMDA, nNOS and iNOS active sites along with their inhibitors. We found that % brain water content, brain infarction, and BBB disruption were significantly reduced in the treated group ($p<0.005$). As well as the level of nitrate, glutamate, and calcium were substantially restored in the treated group ($p<0.005$). Chlorogenic acid has formed more number of H-bonds and established many hydrophobic contacts with the active site of NMDA, nNOS and iNOS as compared to their inhibitors. Hence, we can say that chlorogenic acid confers neuroprotection in ischemic stroke and can be developed as a potential neurotherapeutic candidate.

Disclosures: S. Mukherjee: None. **R. Patnaik:** None.

Poster

136. Ischemia: Neuroprotection

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 136.02/V5

Topic: C.08. Ischemia

Title: Prophylactic zinc and therapeutic selenium administration increases the antioxidant enzyme activity in the rat temporoparietal cortex and improves memory after a transient hypoxia-ischemia

Authors: *C. T. SANCHEZ[1], V. BLANCO-ALVAREZ[2], D. MARTINEZ-FONG[3], J. A. GONZALEZ-BARRIOS[4], A. GONZALEZ-VAZQUEZ[2], A. K. AGUILAR-PERALTA[2], M. TORRES-SOTO[2], G. SOTO-RODRIGUEZ[2], I. D. LIMON-PEREZ DE LEON[2], E. BRAMBILA[2], L. MILLAN-PEREZ PEÑA[2], B. A. LEON-CHAVEZ[2]
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Abstract: In the cerebral hypoxia-ischemia rat model, the prophylactic administration of zinc can cause either cytotoxicity or preconditioning effect, whereas the therapeutic administration of selenium decreases the ischemic damage. Herein, we aimed to explore whether supplementation of low doses of prophylactic zinc and therapeutic selenium could protect from a transient hypoxic-ischemic event. We administrated Zinc (0.2 mg/kg of body weight; i.p.) daily for 14 days before a 10-min common carotid artery occlusion (CCAO). After CCAO, we administrated sodium selenite (6 μg/kg of body weight; i.p.) daily for 7 days. In the temporoparietal cerebral cortex, we determined nitrites by Griess method and lipid peroxidation by the Gerard-Monnier assay. qPCR was used to measure mRNA of nitric oxide synthases, antioxidant enzymes, chemokines and their receptors. We measured the enzymatic activity of SOD and GPx, and protein levels of chemokines and their receptors by ELISA. We evaluated long-term memory using Morris-Water maze test. Our results showed that prophylactic administration of zinc caused a preconditioning effect, decreasing nitrosative/oxidative stress and increasing GPx and SOD expression and activity, as well as eNOS expression. The therapeutic administration of selenium maintained this preconditioning effect up to the late phase of hypoxia-ischemia. Ccl2, Ccr2, Cxcl12, and Cxcr4 were upregulated, and long-term memory was improved. Pyknotic cells were decreased suggesting prevention of neuronal cell death. Our results show that the prophylactic zinc and therapeutic selenium administration induces effective neuroprotection in the early and late phase after CCAO.

Disclosures: C.T. Sanchez: None. **V. Blanco-Alvarez:** None. **D. Martinez-Fong:** None. **J.A. Gonzalez-Barrios:** None. **A. Gonzalez-Vazquez:** None. **A.K. Aguilar-Peralta:** None. **M. Torres-Soto:** None. **G. Soto-Rodriguez:** None. **I.D. Limon-Perez de Leon:** None. **E. Brambila:** None. **L. Millan-Perez Peña:** None. **B.A. Leon-Chavez:** None.

Poster

136. Ischemia: Neuroprotection

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 136.03/V6

Topic: C.08. Ischemia

Support: NIH Grant NS078791
NIH Grant NS101564
CIHR Doctoral Research Award 394353

Title: Influence of subventricular zone-derived precursors on neurovascular remodeling after ischemic cortical lesions

Authors: *M. R. WILLIAMSON[1], M. R. DREW[2], T. A. JONES[3]
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Abstract: Focal brain injuries cause proliferation and ectopic migration of newborn cells from the subventricular zone towards the site of injury. Increased cytogenesis is associated with improved outcome following many types of nervous system injury. However, precise mechanisms of cytogenesis-mediated recovery remain to be defined. We show that pharmacogenetic ablation of neural stem and progenitor cells (NSCs) worsens recovery of skilled forelimb use in mice after photothrombotic ischemic lesions in sensorimotor cortex, a model of stroke-induced upper limb impairments. Surprisingly, we found that despite ablation of $GFAP^+$ stem cells in both canonical rodent cytogenic niches, subventricular and subgranular zones, there was a slow, but complete, repopulation of progenitors specific to the subventricular zone. Delayed repopulation of this progenitor pool in animals with previously ablated NSCs was temporally associated with behavioral gains. Post-stroke migration of progenitor cells is thought to depend on migratory cues from remodeling vasculature around the injury. We found rapid increases in peri-infarct vascular density after cortical infarcts that were largely confined to a narrow region within ~400 µm from the infarct border. Vascular patterning was altered by the ablation of NSCs, suggesting bidirectional interactions between newborn subventricular zone-derived progenitors and remodeling vasculature. Ongoing studies are investigating the altered vascular patterning associated with NSC ablation and characterizing the influence of cytogenesis on structural plasticity of surviving peri-infarct neurons that have a critical role in behavioral recovery.

Disclosures: M.R. Williamson: None. **M.R. Drew:** None. **T.A. Jones:** None.

Poster

136. Ischemia: Neuroprotection

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 136.04/V7

Topic: C.08. Ischemia

Support: Veterans Affairs Merit Program

Title: Triggering receptor expressed on myeloid cells-2 expression derived from microglia contributes to neurological recovery in experimental ischemic stroke

Authors: Z. ZHENG[1], K. KURISU[1], J. KIM[1], J. SHI[1], A. KANOKE[2], J. LIU[2], C. HSEIH[3], *M. A. YENARI[1]
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Abstract: Background and Purpose: Triggering receptor expressed on myeloid cells-2 (TREM2) is a novel receptor that promotes phagocytosis of microglia and macrophage. We previously reported that TREM2 expression promotes post-ischemic phagocytosis and leads to neurological recovery. However, the precise underlying mechanism is not fully understood yet. In present study, we aimed to clarify which pool of myeloid cells (brain resident microglia versus macrophage derived from circulation) are contributing to its beneficial role in post-ischemic recovery, with the usage of bone marrow (BM) chimeric mice. **Materials and Methods:** BM chimeric mice were created by BM transplanting BM from TREM2 knockout (KO) and wild type (Wt) mice into KO and Wt recipient mice. Thus, 4 groups (Wt (donor) to Wt (recipient), KO to Wt, Wt to KO, KO to KO; n=6/each) of BM chimeric mice were produced, then were subjected to ischemic stroke surgery (distal middle cerebral artery occlusion (dMCAO)). Neurological function was observed until 14 days after surgery. Infarct volume and immunohistological analysis (myeloid cell activation and infiltration: isolectin B4 (IB4) and CD11b; phagocytosis: oil red o and CD68; TREM2 expression) was also undergone. **Results:** Compared to mice lacking brain TREM2 (WT to KO and KO to KO), mice with intact TREM2 in brain microglia (KO to WT and WT to WT) showed better neurological recovery ($p<0.05$), and smaller infarct volume ($p<0.01$). Myeloid cell activation and infiltration (IB4) was significantly decreased in order of WT to WT, KO to Wt, Wt to KO, KO to KO ($p<0.05$). Likewise, number of TREM2 positive myeloid cells (CD11b) showed similar decreases. Oil red o staining demonstrated that mice with intact TREM2 in microglia (KO to WT and WT to WT) showed more accumulation of foamy macrophages, compared to mice lacking TREM2 in brain (Wt to KO and KO to KO). Furthermore, both the number and proportion of TREM2 positive phagocytes (CD68) was significantly higher in mice with intact TREM2 in the brain, compared to mice lacking brain TREM2. **Conclusions:** Although our results suggest TREM2 expression in

both microglia and circulating macrophage were crucial for post-ischemic recovery, its beneficial effect was greater when TREM2 was present in brain resident microglia. These findings might be useful to develop new therapeutic strategy for various cerebrovascular diseases.

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Poster

136. Ischemia: Neuroprotection

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 136.05/V8

Topic: C.08. Ischemia

Title: Resveratrol suppresses SUR1 expression in human brain endothelial cells and prevents cell swelling

Authors: *I. M. ALQUISIRAS BURGOS[1], P. AGUILERA HERNANDEZ[2]
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Abstract: Cerebral edema is an important clinical problem that frequently accompany ischemic infarcts. Cerebral edema is resolutely prognostic of patient's functional outcome and account for up to 50% of patient death. In order to find therapies that attenuate the molecular mechanism of cerebral vascular endothelial cells (EnVC) swelling and prevent edema progress, we studied the effect of resveratrol on SUR1 expression, a protein which regulate cellular osmolyte influx. In the initial phase of edema formation, SUR1-NCCa ion channel increases its *de novo* expression favoring the massive internalization of Na^2+ and water into the EnVC. Expression of the *Abcc8* gene encoding SUR1 depends on transcriptional factors which are sensitive to oxidative stress. Since reactive oxygen species are generated during ischemia, we hypothesized that transcriptional activity might be blocked by antioxidants and therefore, be able to reduce the *Abcc8* gene expression. Previously, we demonstrated that the potent antioxidant resveratrol, significantly reduced the cerebral edema on the *in vivo* model of Middle Cerebral Artery Occlusion. **Objective**. Evaluate the effect of resveratrol on EnCV swelling and on regulation of SUR1 *de novo* expression induced by oxygen and glucose deprivation (OGD). **Material and methods**. Human brain endothelial cell line were submitted to OGD for 2 h followed by different times of recovery. Then, the cellular response to different concentrations of resveratrol administrated at the onset of recovery was assess. **Results.** OGD increased SUR1 protein expression (13.0-fold ±5.2) in EnCV. SUR1 over-expression correlated with cell swelling and cell death. Both cellular responses were prevented by the administration of resveratrol, with the maximum effect observed with 5 μM. In addition, we found that transcriptional factors Sp and NF-κB were involved on regulation of SUR1 expression. **Conclusions**. Our findings represent an

advance in the description of the molecular mechanism of action of resveratrol on cerebral edema. Its effect on cell swelling prevention seems to be at the level of gene expression regulation of proteins that participate on the ionic edema formation.

Disclosures: I.M. Alquisiras Burgos: None. **P. Aguilera Hernandez:** None.

Poster

136. Ischemia: Neuroprotection

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Program #/Poster #: 136.06/V9

Topic: C.08. Ischemia

Support: Mid-career Research Grant 2015R1A2A2A01003395
Medical Research Center Grant 2014R1A5A2009392

Title: Proangiogenic capacity of the RGD-SLAY-containing osteopontin icosamer in the endothelial cells and in the postischemic brain

Authors: *H. LEE[1], S. KIM[1,2], S. PARK[1], J.-K. LEE[1]
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Abstract: Osteopontin (OPN) is a phosphorylated glycoprotein secreted into the various body fluids by many different types of cells. OPN contains arginine, glycine, aspartate (RGD) and serine, leucine, alanine, tyrosine (SLAY) motifs which bind to several integrins and mediate a wide range of cellular processes such as adhesion, migration, and proliferation. In the present study, the proangiogenic effects of the 20 amino acid OPN peptide (OPNpt20) containing RGD and SLAY motifs were examined in human umbilical vein endothelial cells (HUVECs) and in a rat model of focal cerebral ischemia/reperfusion injury. OPNpt20 exerted robust proangiogenic effects in HUVECs by promoting proliferation, migration and tube formation. These effects were significantly reduced in OPNpt20-RAA (RGD→RAA)-treated cells, but only barely reduced in OPNpt20-SLAA (SLAY→SLAA)-treated cells. Interestingly, a mutant peptide without both motifs failed to induce all of these proangiogenic processes, indicating that the RGD motif is crucial and that SLAY also has a role. In OPNpt20-treated HUVECs, AKT and ERK signaling pathways were activated, but activation of these pathways and tube formation were suppressed by anti-$\alpha_v\beta_3$ antibody, indicating that OPNpt20 stimulates angiogenesis via the $\alpha_v\beta_3$-integrin/AKT and ERK pathways. The proangiogenic function of OPNpt20 was further confirmed in a rat middle cerebral artery occlusion model. Total vessel length and vessel densities were markedly greater in the cortical penumbra of OPNpt20-treated ischemic brains, accompanied by induction of proangiogenic markers such as vascular endothelial growth factor (VEGF) and alpha smooth muscle actin (α-SMA). Together, these results demonstrate that the 20

amino acid OPN peptide containing RGD and SLAY motifs exerts proangiogenic effects in the endothelial cells, wherein both motifs have important roles, and these effects appear to contribute to the neuroprotective effects of this peptide in the postischemic brain.

Disclosures: H. Lee: None. **S. Kim:** None. **S. Park:** None. **J. Lee:** None.

Poster

136. Ischemia: Neuroprotection

Location: SDCC Halls B-H

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Program #/Poster #: 136.07/V10

Topic: C.08. Ischemia

Support: NIH grant: AR-067667

Title: Neuroprotective effect of vitamin -d on ischemia-reperfusion brain injury

Authors: N. K. MONDAL, J. BEHERA, A. GEORGE, K. E. KELLY, *N. TYAGI
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Abstract: Ischemia reperfusion (I/R) injury is a major cause of stroke and is found to be associated with 1, 25-dihydroxyvitamin D3 (vitamin D_3) deficiency. Several studies indicated that treatment with vitamin D_3 could have neuroprotective effects in ischemic stroke. High levels of homocysteine (Hcy) known as hyperhomocysteinemia (HHcy), a co-morbid condition due to abnormalities in its metabolizing enzyme systems. However, the HHcy induced alternation of epigenetic mechanism and how Vitamin D_3 protect ischemic stroke is not clear yet. Therefore, the objectives of this study are to understand the molecular mechanisms underlying ischemic stroke-induced alternation of chromatin landscape in the brain leading to inflammation-mediated neurovascular dysfunction (NVD). In our study, 8-10 weeks old C57BL/6 wild-type mice were subjected to middle cerebral artery occlusion (MCAO) for 40 min, followed by reperfusion for 72 hours. Vitamin D_3 (50mg/kg-BW/day) was administrated by oral gavage once daily for 3 days after 6 hour of ischemia. Interestingly, our result suggest that status of oxidative stress, cerebral blood flow and blood-brain barrier (BBB) integrity are compromised during ischemic stroke (MCAO model), causing neuronal damage by increasing the total Hcy (tHcy) mediated TLR4 (Toll-Like Receptor 4) activation promoting pro-inflammatory responses and neurovascular dysfunction via alternation of chromatin landscapes and modulation of NF-κB-OPG (Nuclear Factor κB-Osteoprotegerin) signaling. The results also demonstrate that Vitamin D_3 treatment activates Vitamin D receptor (VDR)-RANKL (Receptor Activator of Nuclear Factor Kappa-B Ligand) signaling in brain calvarial osteoblasts and attenuates inflammation mediated NVD via paracrine signaling of osteoblast-derived RANKL. In conclusion, increases in tHcy levels during ischemic stroke may cause an imbalance in redox homeostasis leading to cerebrovascular impairment by inducing inflammation via NF-κB histone acetylation and transcriptional

regulation of OPG expression. Vitamin D_3 treatment attenuates hyperhomocysteinemia, modulates TLR4 mediated inflammation and ultimately ameliorate neurovascular dysfunction by restoring vascular and neuronal functions.

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Poster

136. Ischemia: Neuroprotection

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 136.08/V11

Topic: C.08. Ischemia

Title: Brain stroke neuroprotection by the mitochondrial mitoneet agonist nl-1

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Abstract: Stroke is a devastating brain injury and leading cause of adult disability. Current management of stroke patients is aimed at preventing the progression of at-risk cerebral tissue towards infarction by restoring blood supply to ischemic penumbra in timely manner. Thrombolytic therapy (t-PA) and surgical clot removal (thrombectomy) are the most common and only one approaches to this effect, however they do not address local and systemic neuroprotection in brain stroke pathology. When the ischemic tissue is re-perfused with new restoring blood, secondary tissue damage is observed, which is generally referred to as ischemia-reperfusion (IR) injury. One of the key role-players in the IR injury is mitochondria, which contributes to the oxidative stress seen during the re-perfusion. Therefore, an urgent need exists to develop compounds which can mitigate the damage from IR injury. mitoNEET is a novel mitochondrial iron-sulfur containing protein located on the outer membrane of mitochondria which regulates mitochondrial bioenergetics. Recently, the anti-diabetic drug pioglitazone (thiazolidinedione) was found to be a ligand for mitoNEET and has been also shown in vivo to be neuroprotective against IR injury. In this study, our goal was to evaluate a mitoNEET ligand NL-1, a derivative of pioglitazone, with its neuroprotective effects in MCAO (middle cerebral artery occlusion) model of brain stroke. In our prior studies we have shown that NL-1 has neuroprotective effects in MCAO murine stroke model when NL-1 was administered 30min before stroke (pre-treatment), also we shown neurogenic effects of NL-1 on neural stem cells proliferation in hippocampal dentate gyrus. To mimic clinical relevance objective of this study was to investigate a mitoNEET ligand NL-1, as a therapeutic agent against ischemia-reperfusion IR injury. Stroke was induced in male CD-1 mice (25-30g) by occluding MCA and removing the occlusion after 1h to allow blood reperfusion (transient MCAO). NL-1 (10mg/kg) was

administered 10-15min after reperfusion (post-treatment). Control-stroke group received vehicle. The mice were euthanized 24h after stroke to collect the brains. The brains was sectioned for TTC-staining to determine histological brain stroke pathology parameters - brain ischemia and edema. We found that in the NL-1 treated group compared to the untreated stroke group, the infarct volume, as assessed by TTC-staining, was reduced by 41%, and the stroke-associated edema also was significantly reduced by 63%. Taken together, these data suggests that NL-1 targeted toward mitoNEET could be used in the development of therapies to prevent IR injury after a brain stroke.

Disclosures: A. Mdzinarishvili: None. **W.J. Geldenhuys:** None.

Poster

136. Ischemia: Neuroprotection

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 136.09/V12

Topic: C.09.Stroke

Title: Ischemic stroke protective MAPK10 rs17008675 variants affect the blood proinflammatory profile via FOS involved gene-gene interaction mechanism

Authors: *K. TADEVOSYAN[1,2], A. SOGHOYAN[3], K. MAYILYAN, 0005[2], A. BOYAJYAN, 0005[2]
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Abstract: A number of studies indicate a significant role of the JNK/AP1 signaling in the development of ischemic stroke (IS). Recently, we described putative associations of *FOS* (encoding c-Fos) rs7101, and *MAPK10* (encoding JNK3) rs17008675 and rs6815306 SNPs to the IS-risk, which diverged by their biological significance. Particularly, the *MAPK10* rs6815306*A/A genotype protective effect was linked to the > 12 years delay in IS onset, while rs17008675*A carriage contributed to the defense against atrial fibrillation and was significantly associated with the excessively reduced average infarct volume in subgroup of patients with this comorbidity. On the other hand, the carriage of *FOS* rs7101*T allele conferred the risk for IS. Thus, in the present study, we searched for molecular correlates of those variations to the inflammatory processes, aiming to identify a possible gene-gene interaction molecular mechanism, underlining those genetic associations with the IS. For this aim, the levels of proinflammatory cytokines IL-1β, IL-6 and TNFα were measured in the blood plasma samples of 83 controls from Armenian population using ELISA. *MAPK10* rs17008675 and rs6815306 and *FOS* rs7101 SNPs were genotyped using PCR-SSP and genomic DNA samples. According to the data obtained, *MAPK10* rs6815306 genotype did not affect the blood cytokines level significantly either in univariate term (P>0.33) or in interaction with *FOS* rs7101 genotype

(P>0.085). However, we found that *MAPK10* rs17008675*A carriage had significant multi-factorial association with subjects' proinflammatory profile (Wilks' $\lambda = 0.83$, P<0.026). Particularly, T/A and A/A genotypes of rs17008675 SNP were associated with significantly decreased average levels of IL-1β (P<0.004) and IL-6 cytokines (P<0.025), while the level of TNFα showed a decline on the trend level (P<0.19). In the model, *FOS* rs7101 genotype interaction did not affect the significant *MAPK10* rs17008675 SNP effect on proinflammatory profile, but rather helped to explain more than 25% of cytokines level variation (Wilks' $\lambda = 0.75$, P<0.026). Remarkably, we also found gender and *MAPK10* SNPs interaction effect associated with IL-1β average level (sex*rs17008675: P<0.027; sex*rs6815306: P<0.040), where females had lower levels than males with IS-risk associated rs6815306 A/A genotype largely linked to rs17008675*T/T genotype. Considering that the stress kinase JNK3 is the major mediator of neuronal apoptosis and neuroinflammation, *MAPK10* rs17008675 IS-protective genotypes down-regulating effect on the blood proinflammatory profile may underlie the mechanism by which it can affect the IS-risk and IS-related blood-reperfusion injury.

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Poster

136. Ischemia: Neuroprotection

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 136.10/V13

Topic: C.09.Stroke

Title: Endothelial Nox4 deteriorates infarct volume and neurologic functions, albeit with enhanced angiogenetic responses in peri-infarct areas, in acute brain ischemia

Authors: *Y. YOSHIKAWA, T. AGO, J. KURODA, Y. WAKISAKA, M. TACHIBANA, T. SHIBAHARA, M. KOMORI, K. YAMANAKA, T. KITAZONO
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Abstract: Purpose: Reactive oxygen species (ROS) are important modulators of cellular functions. NADPH oxidase 4 (Nox4), one of ROS-producing Nox family protein, is expressed in endothelial cells, and is highly upregulated upon ischemic or hypoxic insults. In the present study, we investigated the roles of Nox4 on acute brain ischemia in mice, as well as on cultured endothelial cells. **Method:** Permanent MCAO (pMCAO) stroke was induced in mice with endothelium-specific overexpression of Nox4 using a tie2 promoter (Tg-EC-Nox4) and their littermate mice. We examined infarct volume by TTC staining, breakdown of the blood-brain barrier (BBB) by the leakage of Evans blue dye, and post-stroke infiltration of inflammatory cells into infarct areas by flow cytometry (FC). In FC, monocytes/macrophages were identified as lineage (CD90.2/B220/CD49b/NK1.1/Ly-6G)^{low}, CD11b^{high}, while neutrophils were identified

as lineage^{high}, CD11b^{high}. Neurologic functions were assessed by modified neurologic severity scores (mNSS). In vitro, we overexpressed Nox4 in cultured human umbilical vein endothelial cells (HUVEC) by using adenovirus, and examined the expression of pro-inflammatory cytokines and angiogenic molecules by real-time PCR and the proliferation of HUVEC by MTT assay. **Results:** Nox4 was expressed on endothelial cells at baseline in the brain, and the expression on endothelial cells was upregulated in ischemic core and peri-infarct areas after pMCAO. Infarct volume and breakdown of the BBB were significantly greater with worse neurologic functions on day 1 after pMCAO in Tg-EC-Nox4 than in wild-type littermates. The numbers of lineage^{high}/CD11b^{high} neutrophils and lineage^{low}/CD11b^{high} monocytes/macrophages infiltrating into infarct areas were significantly greater in Tg-EC-Nox4 mice than in wild-type littermates. In vitro, Nox4 overexpression increased the expression of chemokines related to cell survival (IL-8), angiogenesis (angiopoietin 2), and pro-inflammation (CXCL2/IL-8). Furthermore, Nox4 overexpression increased the proliferation of endothelial cells, while the enhancement was canceled in the presence of a CXCR2 or a Tie2 inhibitor. On day 7 after pMCAO, the number of CD34-positive tube-forming endothelial cells in peri-infarct areas were greater in Tg-EC-Nox4 mice than in wild-type littermate. **Conclusion:** In acute brain ischemia, endothelial Nox4 deteriorates infarct volume and neurologic functions, probably through enhanced breakdown of the BBB and recruitment of pro-inflammatory cells. While there remains a possibility that Nox4 may also enhance post-stroke angiogenetic responses in peri-infarct areas around day 7 after stroke.

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Poster

136. Ischemia: Neuroprotection

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 136.11/V14

Topic: C.09.Stroke

Support: California Table Grape Commission grant

Title: Grape enriched diet protects axon function against ischemia by preserving mitochondrial dynamics

Authors: *C. BASTIAN[1], D. SCERBO[2], J. DAY[2], S. BRUNET[1], S. BALTAN[3]
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Abstract: White matter (WM) of the brain is highly vulnerable to stroke injury. Our studies showed that preservation of white matter components by histone deacetylase (HDAC) inhibition reduces stroke-induced brain damage. Previous studies have demonstrated that a grape powder diet produces activity consistent with HDAC inhibition and protects gray matter structure and function against ischemic injury. However, the effects of a grape-enriched diet on WM integrity remain unexplored. Therefore, we hypothesize that a grape powder diet will promote axon function recovery following oxygen and glucose deprivation (OGD) by promoting oligodendrocyte survival and preserving axonal mitochondrial structure and motility. Groups of male C57BL6/J or Thy-1 mito CFP mice (3-5 months of age) were placed on 5% or 10% grape powder diet or on control diets supplemented with fructose and glucose (Teklad 2018 rodent diet) for up to 10 weeks. Mouse optic nerves (MONs), which are pure white matter tracts, were used to evaluate axon function recovery by quantifying the area under evoked compound action potentials (CAPs) following OGD (60 min). MONs were also fixed for immunohistochemical experiments. Thy1-CFP (+) mito mice on a similar diet were used to quantify CFP (+) mitochondrial pixel intensity or live imaging of mitochondrial motility. Grape powder diet caused baseline changes in glial cells such that astrocytes became longer, thicker, and oriented their processes transversally along axons compared to mice fed with the control diet. Moreover, there were greater numbers of microglia and oligodendrocytes in MONs obtained from mice on the grape powder diet. Subsequently, when exposed to OGD, MONs obtained from mice on the grape powder diet showed better recovery of axon function and better preservation of oligodendrocytes starting at 8 weeks and continuing up to 10 weeks. Grape powder diet at 5% or 10% equally protected and promoted axon function. Likewise, CFP (+) fluorescent mitochondria were preserved in the grape diet groups, which was associated with a reduction in mitochondrial fragmentation. Mitochondria remained motile during and after OGD in MONs from mice on grape powder diets, while mitochondria became stationary and fragmented in MONs obtained from mice on control diets. Therefore, we propose that a grape-enhanced diet confers protection to axon function and WM integrity that could improve functional recovery following a stroke. However, whether epigenetic changes mediate the protective mechanisms conferred by a grape-rich diet requires further investigation.

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Poster

137. Stroke, Damage, or Disease: Assessment and Treatment II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 137.01/V15

Topic: C.09.Stroke

Support: ORF RE-04-047

CIHR PDF

Title: A robotic unloading perturbation task to assess fast corrective responses in the upper limb following stroke

Authors: *C. R. LOWREY[1], T. C. BOURKE[2], S. D. BAGG[5,3], S. P. DUKELOW[6], S. H. SCOTT[4]

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Abstract: *Background:* Robotic technologies to measure human behavior are emerging as a new approach to assess brain function and dysfunction. Our recent Load Task assessed corrective responses to mechanical disturbances applied to the arm and found impairments in the performance of participants with stroke compared to a large cohort of healthy controls (Bourke et al JNER 2015). However, a striking feature of our findings was the large range and skewed distribution of performance across healthy controls. This likely reflects the use of different strategies across the control population, making it more difficult to identify impairments in subjects with stroke. Here, we developed a more intuitive "Unload Task" with more straightforward instructions. We hypothesized this would reduce healthy control performance variability, and as a result, improve our ability to detect impairments in participants with stroke. *Methods:* Performance on the Load Task and Unload Task in the KINARM exoskeleton robot was directly compared for control participants (n=107) and participants with sub-acute stroke (n=31; mean 24 days post-stroke, range 2-61 days). The task goal was to keep a cursor representing the hand inside a virtual target and return "quickly and accurately" if the robot applied an unexpected load for the Load Task (or removed a load in the Unload Task) that moved the hand away from the target. A number of kinematic parameters quantified subject performance, and impairment was defined as performance outside the 95th percentile for control subjects, corrected for age, sex and handedness. A Task Score was also calculated using standardized parameter scores that reflects overall performance on the task. *Results:* The distribution of healthy control performance was smaller and less skewed on each parameter for the Unload Task compared to the Load Task. As well, fewer outliers were removed from the Unload Task (3.7%) compared to the Load Task (7.4%) when developing normative models of performance. More participants with stroke failed the Unload Task based on Task Score with their clinically-defined most affected arm (68%) compared to the Load Task (23%). In the Unload Task, ~one-third of participants who were impaired with their affected arm, were also impaired with their "unaffected" arm. The Task Scores for affected arm in the Unload Task significantly correlated with standard clinical measures such as the Chedoke-McMaster Stroke Assessment (r = -0.41). *Conclusions:* The Unload Task provides an improved behavioural task to assess fast corrective responses in the arm. We found that corrective responses are impaired in persons living with stroke, and are often equally impaired in both arms.

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collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; BKIN Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BKIN Technologies.

Poster

137. Stroke, Damage, or Disease: Assessment and Treatment II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 137.02/V16

Topic: C.09.Stroke

Support: ORF RE-04-047

Title: Quantifying rapid online corrective responses in the stroke population using a novel upper limb task

Authors: *K. PARK[1], S. H. SCOTT[2]
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Abstract: Sensorimotor impairments after stroke can have debilitating effects on everyday activities. Clinical scales used to assess upper limb impairment examine relatively slow voluntary movements, but are susceptible to floor and ceiling effects. This can lead to some people with stroke having few clinical impairments but still exhibit visible impairments. Furthermore, studies have shown that people who have suffered from a stroke can be more prone to injury from falls that may at least partially be due to slowed sensory responses used to maintain balance and control. These impairments of rapid processing are difficult to be objectively and accurately quantified using current clinical tools.
We created a new task to compare and contrast impairments after stroke for rapid motor responses to different sensory feedback modalities. Using robotic technology (KINARM), we quantified upper limb deficits after stroke using an interception task. This task required subjects to hold their cursor at a specific point while a ball moved towards them. On random trials, we applied proprioceptive or visual perturbations to the limb or the ball that required a rapid corrective response to successfully intercept, or, if instructed by an abrupt change in the color of the ball, to avoid the ball. These perturbations were applied through sudden changes in the spatial position of the ball or hand, sudden mechanical perturbations of the hand, or sudden changes in the chromatic properties of the ball.
We recruited 10 patients with stroke and a control population of 21 healthy participants. We found that this task was able to show impairments in proprioceptive and visual sensory processing. Furthermore, we found some patients with stroke had specific impairment in one or two types of sensory feedback, whereas others could have impairments in all types of feedback. This interception task takes approximately 5 minutes to complete X trials making it a simple and

fast approach to quantify the ability of subjects to perform different types of fast feedback processing.

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Poster

137. Stroke, Damage, or Disease: Assessment and Treatment II

Location: SDCC Halls B-H

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Program #/Poster #: 137.03/W1

Topic: C.09.Stroke

Support: JES Edwards Foundation

Title: Influence of ovarian hormone deprivation length on the neuroprotective effects of genistein in stroke

Authors: *A. OPPONG-GYEBI, D. METZGER, J. HAN, T. DOAN, C. SMITH, N. SUMIEN, D. A. SCHREIHOFER
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Abstract: Background: Estrogen (E) deprivation increases the risk of stroke, cardiovascular disease, and cognitive decline in women. Studies in rodents demonstrate that the beneficial effects of E in the brain are lost 10 weeks after ovariectomy (Ovx). Similar effects are seen in women after several years without E, and many observed benefits require early intervention with hormones after menopause. Unfortunately, E also has undesirable effects (such as breast cancer) that lead women to alternative treatments for menopause, including plant estrogens such as genistein. Natural products are perceived to be safe even though their benefits are not well established. Previous studies demonstrate neuroprotective effects of genistein following experimental stroke. This project sought to investigate genistein's ability to protect the brain at varying lengths of ovarian hormone deprivation. **Hypothesis:** We hypothesized that dietary genistein will maintain the ability to provide neuroprotection in the brain and improve functional recovery after long-term hormone deprivation associated with Ovx. **Method:** Adult female Sprague-Dawley rats (n=5-10) were placed on a genistein-free diet, Ovx, and randomly assigned to 2- (Short, ST) or 12-weeks (Long, LT) E deprivation. After deprivation, rats were placed on genistein-free or genistein diet (500 ppm) 2 weeks before 60 minutes transient middle cerebral

artery occlusion (MCAO) or sham surgery. Neurological (neuroscore), motor (rotarod) and cognitive function (Morris water maze, MWM) were used to assess post-MCAO function over 21 days. Data was assessed with 2-way ANOVA and Fisher's LSD. Significance was set at $p<0.05$. **Results:** Neuroscore and Cylinder test score showed a significant effect of stroke, but not diet in both ST and LT groups. Rotarod showed a significant effect of stroke, but not diet, on both the learning phase and plateau phase for the ST group and effect of stroke on the learning phase in the LT group. Comparison between ST and LT stroke subgroups showed a significant effect of hormone deprivation length in the learning phase of rotarod. MWM showed a significant effect of stroke in the genistein-free subjects of the ST group, an effect which was not observed in the genistein-treated group. Comparison between ST and LT stroke subgroups further showed a significant effect of hormone deprivation length in the retention phase of MWM. **Conclusion:** Our results suggest that long-term estrogen deprivation worsens MCAO outcomes including higher mortality and greater behavioral deficits. Although genistein did not preserve motor function, it may preserve cognitive abilities, even after long-term estrogen deprivation.

Disclosures: A. Oppong-Gyebi: None. **D. Metzger:** None. **J. Han:** None. **T. Doan:** None. **C. Smith:** None. **N. Sumien:** None. **D.A. Schreihofer:** None.

Poster

137. Stroke, Damage, or Disease: Assessment and Treatment II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 137.04/W2

Topic: C.09.Stroke

Support: ORF RE-04-047

Title: Robotic assessment to identify impairments in individuals with transient ischemic attack or migraine

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Abstract: Background: Transient ischemic attack (TIA) and migraine both cause a brief alteration to the brain's normal function. However, deficits in both cases have not historically been thought to persist far beyond symptom resolution. Migraine and TIA often present with similar symptoms, and thus differentiating them in a clinical setting can be difficult. Therefore, we performed a quantitative robotic assessment of people who had migraine or a TIA, with the goal of determining 1) if people who have had TIA or migraines demonstrate persisting deficits on robotic tasks, and 2) if people that have had a TIA or a migraine display different patterns of impairment. **Methods:** We assessed 42 individuals who had a first-ever TIA/minor stroke, and

24 individuals who had a migraine within 2 weeks of symptom resolution. Robotic assessment was performed using a KINARM exoskeleton robot (BKIN Technologies, Kingston, ON, Canada). Eight tasks were used to test cognitive performance, as well as upper limb motor and sensory performance. Impairment was defined as performance below the 5^{th} percentile of a large population of healthy controls. We also collected traditional clinical assessments: Montreal Cognitive Assessment (MoCA), Chedoke-McMaster Stroke Assessment (CMSA), Behavioural Inattention Test (BIT), and the National Institutes of Health Stroke Scale (NIHSS). In people who had TIAs, we also measured diffusion-weighted imaging (DWI), age-related white matter change (ARWMC), and cella-media index (CMI). **Results:** All participants scored 0 on the NIHSS. However, many individuals in both the migraine and TIA groups demonstrated impairments on robotic tasks. Performance on the reverse visually guided reaching task identified 72.7% of people who had a TIA as impaired, and 57.9% of those who had migraine. However people who had migraines only displayed impairments related to motor function. In contrast, people that had TIAs were impaired on a broader range of behavioural tasks across sensory, motor and cognitive domains. **Conclusions:** Quantitative robotic assessment highlights measurable impairments in the majority of subjects with TIA or migraine and different patterns of impairments.

Disclosures: L.E. Simmatis: None. **S.H. Scott:** 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.; BKIN Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BKIN Technologies. **A.Y. Jin:** None.

Poster

137. Stroke, Damage, or Disease: Assessment and Treatment II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 137.05/W3

Topic: C.09.Stroke

Support: R00 HD073240

Title: StartReact increases reaching distance in severe stroke survivors by activating paralyzed muscle inaccessible during volitional reaching

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Abstract: StartReact - the involuntary release of planned movement - has garnered recent attention for its capacity to enhance movement in stroke survivors. During single-joint reaching

movements, startReact normalizes muscle activity patterns. Specifically, the reaction time and agonist/antagonist firing are not statistically different from unimpaired older adults (Honeycutt et. al. 2012). Still, this initial study was completed during single-joint tasks begging the question if startReact could enhance movements in an unrestricted range of motion. We evaluated the startReact response of 17 stroke survivors of varying impairment (UEFM 6-59) and spasticity (MA 0-4) while they performed two dimensional reaching to three distinct targets.
We evaluated 6 severe stroke survivors (UEFM: All < 25/66) ranging in spasticity (MA: 0-4/4) during unrestricted, multi-joint reaching tasks. Subjects were instructed to reach from a HOME position near their body to three TARGETs. During voluntary movement, subjects cannot leave the HOME. During StartReact, subjects generate reaching movement away from the body. StartReact increases muscle activation in terms of the probability of muscle activity, measured as a quantifiable onset latency, and amplitude of muscle activity. Increased muscle activation is functional leading to increased reaching distance and decreased minimum distance to target. In conclusion, StartReact represents a viable method for enhancing movement in stroke survivors - particularly severe stroke survivors.

Disclosures: C.F. Honeycutt: None. **M. Rahimi:** None.

Poster

137. Stroke, Damage, or Disease: Assessment and Treatment II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 137.06/W4

Topic: C.09.Stroke

Support: Heart and Stroke Grant

Title: Sex-related changes of cerebrovascular function in two mouse models of ischemic stroke

Authors: *X. TOUSSAY[1], C. H. COMIN[3], M. YIN[4], R. DANIEL[5], C. A. SIMADA[1], J. OUELLETTE[1], L. DA F. COSTA[3], B. LACOSTE[2]
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Abstract: Cerebral function relies on a steady supply of oxygen and nutrients from the blood stream, thus relying on healthy blood vessels and cerebral blood flow (CBF). As such, the brain is particularly vulnerable to vascular failure. Aging, poor diet and other risk factors affect vascular health and promote the onset and/or progression of neurological disorders including ischemic stroke (IS), the most prevalent form of human stroke. IS represents a major cause of infirmity and death worldwide, with more than 400,000 people living with disabling consequences in Canada. While neuronal plasticity represents a central focus in stroke research,

vascular responses to IS remain poorly understood. This study aims at better understanding the spatio-temporal profile of hemodynamic responses to IS in female and male mice. Indeed, as IS differentially affects females and males, it is crucial to investigate sex differences by building in experimental groups that better represent the reality of a human population. Our multidisciplinary approach combines sophisticated imaging modalities (laser-Doppler flowmetry and photoacoustic imaging), as well as anatomical and molecular methods to investigate cerebrovascular responses following photothrombotic (PT) or endothelin-1 (ET-1)-mediated IS in the somatosensory cortex of adult outbred (Swiss Webster) mice. Immediately (15 min) after PT stroke induction, we measured a significant decrease in baseline CBF in females (-32% ± 6.5; n=5), while no change was observed in males (+1.6% ± 12.1; n=5) within the direct vicinity of the injury. However, 48 hours post-stroke, a similar decrease in baseline CBF was found in both females and males (-55% and -45%) but not 3 weeks post-stroke. Following ET-1-induced IS, we found comparable reductions in baseline CBF in both females and males, as well as similar reperfusions 48 hours post-stroke. Intriguingly, 3 weeks after ET-1 stroke, the diminution in baseline CBF was maintained only in females. Our data thus suggest sex-related changes in the CBF responses to IS in two mouse models, suggesting a sex-dependent CBF regulation and resistance to IS. This work is complemented by anatomical and molecular readouts to assess the underlying mechanisms of these changes observed after IS. For instance, in the cerebral cortex of both females and males, microvascular branching and density, as well as changes in the expression of gene candidates related to angiogenesis and CBF, are being quantified. This original study will provide a novel understanding in cerebrovascular remodeling following IS and its associated sex-difference in mice.

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Poster

137. Stroke, Damage, or Disease: Assessment and Treatment II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 137.07/W5

Topic: C.09.Stroke

Support: NS056839

Title: Effects of remote limb ischemic conditioning in conjunction with rehabilitation training after motor cortical infarcts in rats

Authors: *B. R. BARKSDALE[1,3], A. K. LEE[2], D. F. MIRANDA-SOHRABJI[2], T. A. JONES[1]
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Abstract: The most common lasting disability suffered by stroke survivors is hemiparesis of the upper extremity. Complementary therapies that can enhance the neuroplasticity of effective rehabilitation is a current focus in the field. Remote limb ischemic conditioning (RLIC) is a non-invasive procedure that in two recent studies by the C.E. Lang group was shown to enhance motor learning in healthy adults. The aim of this study was to determine the effect of remote ischemic limb conditioning on motor recovery of the upper extremity when added to rehabilitative training (RT) during the subacute phase of stroke recovery. Male Long-Evans rats were trained on the single pellet reaching (SPR) task until proficiency was reached and then a surgery was performed to model a cortical stroke by applying endothelin-1 to the caudal forelimb area of the motor cortex. Rehabilitative training was started 7 days post-op and consisted of SPR for 60 trials every day for 14 days. One group was given RLIC every 72 hours of the RT period. RLIC consisted of a blood pressure cuff being applied to the hindlimb and inflated above 200 mmHg for 3 cycles of 5 minutes on and 5 minutes off under isoflurane anesthesia. A control group did not have the blood pressure cuff inflated but was kept under isoflurane for the same amount of time. Ischemia was confirmed by skin temperature drop and/or color change of the hindlimb paw. Performance on the SPR, Schallert Cylinder, and Foot Fault tasks was assessed pre-op, post-op, and post-RT. Preliminary results suggest that RLIC when given at this dosing regimen, does not improve motor recovery in the subacute phase of stroke.

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Poster

137. Stroke, Damage, or Disease: Assessment and Treatment II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 137.08/W6

Topic: C.09.Stroke

Title: Stroke: Perception of risk factors and warning signs amongst survivors in an herbal centre

Authors: *P. U. NWOHA, I. AYOOLA
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Abstract: Stoke is a major health concern worldwide because of its high mortality and morbidity. It is increasingly becoming more devastating in developing countries, where the economy is very depressing. Unfortunately, this should not be so, because stroke is a non-communicable disease that can be prevented with appropriate life-style. Good knowledge of its risk factors and warning signs will drastically reduce the occurrence. The current spate of stroke in the developing countries shows some emerging risk factors, different from the established. In depressed economies like Nigeria, there is now increasing drift to the herbal/traditional centres for stroke management. The stroke survivors attending these centre need to be investigated with

a view to understanding their perception of risk factors and warning signs for stroke. Information obtained from this study will be very helpful for the sufferers and others at risk. A herbal centre in Umunomo Ihitteafoukwu, Ahiazu Mbasie, Nigeria, is an attraction for most stroke survivors. Cross-section of those attending this centre was interviewed by two trained staff after Ethical approval and informed consent were obtained. The patients were asked to recall what they thought could be responsible for their stroke and what things that can lead to stroke; they were also asked to recall unusual sudden experience before the stroke happened. Their results were analysed using IBM SPSS Statistics version 23. Results showed that of the 149 participants, stroke was highest (36, 24.2%) amongst those in 70-74 years age bracket, highest (37, 24.8%) amongst those with 5 to 8 children, highest (83, 54.6%) amongst those with primary education only, highest amongst traders (45, 38.5%), occurred most during resting (85, 57.4%). Amongst the established risk factors, hypertension was the highest (80, 53.7%) suffered by survivors but only 29 (19.5%) of the survivors could point to it as a risk factor; perceived causes included attack or witchcraft (20, 13.4%) and large number (59, 39.9%) could not identify anything as leading to their stroke or as risk factor. There was significant association of sex with level of education and occupation, smoking, alcohol ($p<0.05$) but not with hypertension and diabetes. Age significantly associated with hypertension and diabetes but not with other parameters. Nearly all (99.4%) of the participants claimed they got better within three months of treatment. That 99.4% of them expressed satisfaction with treatment at the centre calls for interest of policy makers in the centre. The results call for urgent need of vigorous education of the populace on stroke prevention.

Disclosures: P.U. Nwoha: None. **I. Ayoola:** None.

Poster

137. Stroke, Damage, or Disease: Assessment and Treatment II

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Topic: C.09.Stroke

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Title: Protective effects of pharmacological hypothermia against reperfusion injury after severe ischemic stroke in adult mice

Authors: Y. Y. ZHAO[1,2], Z. Z. WEI[1], L. WEI[1], Y. B. ZHANG[2], *S. YU[1]
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Abstract: Recent clinical trials demonstrate that delayed thrombolytic or thrombectomy treatment is beneficial for some ischemic stroke patients. The restoration of the local blood flow in the ischemic brain region ensure sustainable cell survival and repair process. Meanwhile, it is well-known that reperfusion injury is a deteriorating event following an ischemic insult. In order to improve the therapeutic effect of delayed reperfusion treatments and benefit more stroke patients, it is essential to develop effective strategies to prevent reperfusion injuries. Mild to moderate therapeutic hypothermia is protective against tissue damage and functional impairments after strokes. We showed that pharmacological hypothermia using neurotensin receptor-1 agonists reduced post-stroke inflammation and protected multiple brain cells after ischemic and hemorrhagic stroke. Specifically, the pharmacological hypothermia is protective on the blood-brain barrier and attenuates hemorrhagic transformation. The present investigation tested the hypothesis that the pharmacological hypothermia can be applied to prevent the reperfusion injury after ischemic stroke. In adult C57BL/6 mice, severe ischemic stroke was induced by inserting a filament into the right middle cerebral artery for 60 min, followed by reperfusion of withdrawing the inserted filament. The hypothermic compound HPI-201 (2mg/kg, i.p.) was administered 30 min after reperfusion and more injections at 1 mg/kg were given to maintain the body temperature at 33±1.5°C for 6 hrs. At 3 days after stroke, the HPI-201 post-reperfusion treatment significantly reduced the infarct volume and neuronal cell death. The apoptotic genes Bax and Caspase-3 decreased in the HPI-201 group compared to stroke vehicle controls. The expression and phosphorylation of MMP-9, a pro-hemorrhagic protein, decreased in the HPI-201 group. HPI-201 decreased the expressions of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-12, inducible nitric oxide synthase (iNOS), and increased the expression of arginase-1, a marker of M2 phenotype microglia/macrophages. One and two weeks after stroke and reperfusion, the functional outcomes in different functional assessments were significantly improved compared to stroke vehicle controls. Taken together, the data support that the pharmacological hypothermia using HPI-201 shows multifaceted benefits and may be used as a protective treatment after brain ischemia and reperfusion.

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Poster

137. Stroke, Damage, or Disease: Assessment and Treatment II

Location: SDCC Halls B-H

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Program #/Poster #: 137.10/W8

Topic: C.09.Stroke

Support: NIH Grant R01NS053606-05A1

Title: Distribution analysis variables that best capture individual differences in motor deficits due to stroke

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Abstract: The goal of this study was to investigate which kinematic variables better reveal individual differences of upper limb impairment in stroke survivors. Our work has shown that distribution analysis of motor exploration data, or self-directed movement practice, provides a reliable tool for identifying an individual's unique patterns of endpoint motion (Huang et al. 2016). However, motor impairments manifest in the inability to coordinate limb movement intrinsically. Thus, we hypothesized that distributions of joint variables (angular position, velocity and acceleration) provide a better prediction. Stroke survivors (n = 22) completed a motor exploration task in which they were instructed to move their affected limb at various speeds and directions, and to not repeat the same movements. For each participant, we computed two-dimensional probability distributions of endpoint variables (in Cartesian coordinates) variables and joint (shoulder and elbow angles) variables which were estimated from inverse kinematic calculations. We performed classification of participant's distributions using a linear discriminant analysis wherein the selected features directly employed the raw probabilities of kinematic variables. Our results show that, overall, joint variables better predicted individuals than endpoint variables. Also, similar to our previous results, we found more accurate classification for high derivatives. The LDA-classifier identified participants for each trial correctly 73%, 46% and 37% accuracy for joint angular acceleration, velocity and position, respectively; compared to 50%, 44% and 36% accuracy for endpoint position, velocity and acceleration, respectively. The overall sensitivity for correctly identifying a participant's distributions was 100%, 95% and 92% joint angular acceleration, velocity and position, respectively; and 98%, 96% and 92% for endpoint position, velocity and acceleration, respectively.

Disclosures: Z.A. Wright: None. **J. Patton:** None. **F. Huang:** None.

Poster

137. Stroke, Damage, or Disease: Assessment and Treatment II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 137.11/W9

Topic: C.09.Stroke

Support: FNS

Title: Caveolin-1 involvement in early tissue remodeling after stroke: Effects on angiogenesis and astrogliosis

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Abstract: A series of events occurs within the neurovascular unit (NVU) early on after stroke such as blood-brain barrier dysfunction, inflammation and oxidative stress, all contributing to neuronal cell death, neurological deterioration and mortality. Caveolin-1 (Cav-1) is present in endothelial cells in the brain and has been reported in astrocytes in culture. Cav-1 has distinct physiological roles such as caveolae formation associated with endocytosis, transcytosis and exocytosis. Cav-1 can also modulate signaling pathways in endothelial cells. Therefore, Cav-1 is likely to be an important player in the context of NVU dysfunction. To date, its role after stroke is still controversial and poorly understood.
The main goal of the study was to investigate the role of Cav-1 during the first week after stroke with reperfusion. We compared wild type (WT) and genetically modified Cav-1 knock-out (Cav-1 KO) mice in a transient Middle Cerebral Artery Occlusion (tMCAO) model. Outcome measures including lesion volume, behavioral (neuroscore, rotarod, and adhesive removal) tests, and immunofluorescence staining were collected at various time-points and up to 7 days after brain injury.
After tMCAO, Cav-1 expression was increased in new blood vessels within the lesion and we showed for the first time its presence in reactive astrocytes in tissue of the peri-lesion areas. Cav-1 KO mice exhibited more severe post-stroke outcomes with larger lesion volumes (97% increase) and worse behavioural scores in all the tests than WT mice at all time points. Cav-1 KO mice exhibited reduced angiogenesis (36% decrease) and modified astrogliosis (changes in astrocytes morphology: decrease of 16% of processes length) compared to WT mice 3 days post injury associated with the aggravated functional deficits.
All together, these results point towards a potential protective role of endogenous Cav-1 in the first few days after ischemia by promoting both angiogenesis and astrogliosis.

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Poster

137. Stroke, Damage, or Disease: Assessment and Treatment II

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Program #/Poster #: 137.12/W10

Topic: C.10. Brain Injury and Trauma

Support: Research Excellence Proposal, University of Connecticut

Title: Effects of caffeine and sex on behavioral outcomes following neonatal hypoxia-ischemia in P6 rats

Authors: *R. M. MCLEOD[1], T. ROSENKRANTZ[2], R. H. FITCH[1]
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Abstract: Approximately 10% of newborns are born preterm (≤ 36 weeks gestation) with pulmonary and cardiovascular dysfunction. As a result these infants are at high risk for hypoxic (reduced oxygen) and ischemic (reduced blood flow) brain injury with subsequent cell death and tissue loss, as well as long-term cognitive and behavioral deficits. Although whole-body or head cooling (hypothermia) is now standard therapy for full-term infants with moderate/severe brain injury, no such therapy has proven safe or effective following cerebral hypoxic-ischemic (HI) events in preterm infants. However, approved therapy for other conditions (e.g., anemia and apnea of prematurity) may have a neuroprotective effect. Caffeine, an adenosine antagonist, is used as a respiratory stimulant to aid in removing infants from mechanical ventilation and for the treatment of apnea. Caffeine treated human preterm infants have been shown to have superior outcomes when compared to untreated preterm infants. Animal research has also shown that caffeine acts as a neuroprotectant in postnatal day (P) 6-7 induced-HI male rats, as measured by both behavioral and anatomic outcomes. However, pre-clinical investigation has not assessed caffeine therapy in female rats following neonatal HI, nor have clinical trials prospectively addressed possible benefits of caffeine in male as compared to female infants. This is unfortunate since it is well known that male infants experience more complications of pregnancy and preterm birth, and worse outcomes when compared to matched females with or without HI-related brain injuries. To assess this issue, we tested male and female rats with HI induced on P6 (simulating a moderate preterm brain injury (GA = 32-35 wks)), using the Rice- Vannucci method. HI and sham rats were then treated with caffeine or saline. We predicted that caffeine would ameliorate HI injury as measured by later motor, sensory, cognitive and neuropathologic assessments. We also predicted that female P6 HI rats would show a greater benefit from treatment than male P6 HI rats. Results from behavioral assessments and histological results will be presented.

Disclosures: R.M. McLeod: None. **T. Rosenkrantz:** None. **R.H. Fitch:** None.

Poster

137. Stroke, Damage, or Disease: Assessment and Treatment II

Location: SDCC Halls B-H

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Program #/Poster #: 137.13/W11

Topic: C.10. Brain Injury and Trauma

Support: Internal funding, Research Excellence Proposal, University of Connecticut.

Title: Early biomarkers and outcomes following neonatal hypoxic-ischemic injury

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Abstract: The study of biomarker levels following neurologic injury as predictors of outcome is an emerging area of interest, particularly for neonatal brain injuries associated with birth trauma or prematurity. The ability to screen infants for therapeutic intervention would greatly enhance individualized care for at-risk newborns, and could reduce long-term consequences of brain injury as well as the incidence of cognitive/behavioral impairments. CCL5 (chemokine c-motif ligand 5, aka RANTES) is a chemo-attractant and activating cytokine for T cells, monocytes, eosinophils and basophils that can signal through CCR1, CCR3 or CCR5. In both animal and clinical studies of traumatic brain injury (TBI), CCL5 mRNA has been found to be up-regulated in the cortex, blood, and CSF following injury. In one study, elevated serum CCL5 concentrations in TBI patients at admission were found to correlate with heightened mortality. However, to date there are no functional or mechanistic studies of which we are aware regarding this long-term association. The current study sought to assess an animal model of neonatal hypoxic-ischemic (HI) injury simulating neurologic events that characterize moderately preterm infants, and more specifically to establish associations between post-injury biomarkers collected from serum obtained 48 hours after injury, and long-term outcomes including behavioral performance and neuropathology. To accomplish this, postnatal day 6 (P6) male pups received either induced HI or sham injuries in matched like-treated littermate pairs. At 48 hours post-injury, blood samples were obtained by heart-puncture under anesthesia from one out of each littermate pair. The remaining littermate served to provide matched repeated measure for long-term behavioral and neuropathologic outcomes. This design was based on robust physiologic and behavioral correlations between same-sex like-treated rat littermates, and the fact that the blood sample needed from a 6g pup would cause fluid loss compromising health and any subsequent assessment. Results showed that late or “chronic” inflammatory factors (Rantes, MCP-1, MIP) were significantly elevated at 48 hrs in HI pups, and relative concentrations were highly predictive of subsequent behavioral and neuropathologic outcomes. Specifically, when HI-injured subjects were divided according to high and low chronic inflammatory levels, we saw robust differences in sub-group outcomes, with far greater neuropathologic damage and impaired behavioral scores in the High Rantes expression group. Possible implications for use of serum biomarkers from at-risk preterm infants with suspected HI injuries will be discussed.

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Poster

137. Stroke, Damage, or Disease: Assessment and Treatment II

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Topic: C.09.Stroke

Support: NINDS R01 NS093057
AHA 17POST33660421

Title: Characterization of neuropathologic gene expression in degenerative thalamic injury after stroke

Authors: *Z. CAO, S. HARVEY, T. C. CHIANG, M. Y. CHENG, G. K. STEINBERG
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Abstract: Background: Secondary degenerative thalamic injury results in the disruption of the thalamocortical projection, which is caused by cortical injury after stroke. Thalamic injury progressively develops after stroke and impedes stroke recovery. The cellular and molecular mechanisms mediating the secondary thalamic injury are unclear. Our time course analysis revealed that the thalamic injury begins at post-stroke day 7 and worsened by day 28. In this study we performed a targeted neuropathological transcriptome analysis to investigate the molecular changes in both cortex and thalamus after stroke. **Material and Methods**: Male C57BL6J mice (10-12 weeks) were subjected to permanent occlusion of the left middle cerebral artery to generate cortical ischemic injury. Three experimental groups were used: naïve, post-stroke day 7 (PD7) and post-stroke day 28 (PD28) mice (n=3, 4 and 4 respectively). Ipsilesional somatosensory cortex (iS1) and thalamus (iTH) were dissected and processed for NanoString neuropathology panel. A total of 780 genes were screened and these genes were involved in neurotransmission, neuron-glia interaction, neuroplasticity, cell structure integrity, neuroinflammation and metabolism. NanoString results were analyzed by nCounter Advance Analysis software. Network analysis was performed by Ingenuity Pathway Analysis (IPA). Quantitative PCR was used to validate candidate genes. **Results**: NanoString analysis revealed significant changes in iTH transcriptome after stroke, with 50 differentially expressed genes (DEGs) at PD7 and 191 DEGs at PD28, when compared to naive mice ($P<0.05$ and Benjamini-Hochberg adjusted $P<0.2$). In contrast, iS1 exhibited 296 DEGs in PD7 and 38 DEGs in PD28, when compared to naïve mice. IPA indicates neuroinflammation as the top ranked pathway activated in both iS1 and iTH at both time points. Comparison analysis between PD7 and PD28 showed that 37 common DEGs were expressed in both time points in iTH, including CD14, CCL12, SLC11A1, and GFAP. In iS1, there were 26 common DEGs, including C3, GFAP, CD14, and Itgax. **Conclusion**: Our study revealed the molecular changes between naïve, post-stroke day 7 and day 28 mice, in both primary cortical injury and secondary thalamic injury. Our

data suggest that neuroinflammation is the major event that occurs after stroke in both iS1 and iTH, and highlights candidates involved in microglia/astrocyte activation. Future study will elucidate the role of candidate genes in mediating secondary thalamic injury and stroke outcome using genetic approaches.

Disclosures: Z. Cao: None. **S. Harvey:** None. **T.C. Chiang:** None. **M.Y. Cheng:** None. **G.K. Steinberg:** None.

Poster

137. Stroke, Damage, or Disease: Assessment and Treatment II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 137.15/W13

Topic: C.09.Stroke

Title: Predicting stroke outcomes using dynamic functional connectivity

Authors: *J. Y. NASHED, Y. CHEN, D. J. COOK
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Abstract: Stroke is a leading cause of death and disability worldwide. There is a high degree of variability in the recovery of stroke survivors. The reasons for the disparate recovery amongst similar patients are unclear. One possibility is that cortical remapping is linked to coupling dynamics prior to stroke. Here, we use thirty cynomolgus macaques and a novel functional neuroimaging approach to reveal dynamic changes in coupling strength between networks and the expression of discrete brain configurations both pre- and post-middle cerebral artery occlusion. Although the brain’s collection of functional states was generally preserved, state-specific temporal features, such as the frequency of expression and the amount of time spent in select states varied before and after stroke. Our preliminary results illustrate that pre-stroke dynamic coupling characteristics such as state frequency and amount of time spent in each state could predict recovery with nearly 75% accuracy, which suggests that pre-stroke brain configurations play an important role in dictating outcomes. We identified significant differences in key sensorimotor nodes, between animals that recovered well as compared to those animals that recovered poorly. The results suggest that recovery is strongly linked to the characteristics of the brain’s dynamic coupling prior to stroke, such as the frequency of expression and the amount of time spent in select states.

Disclosures: J.Y. Nashed: None. **Y. Chen:** None. **D.J. Cook:** None.

Poster

137. Stroke, Damage, or Disease: Assessment and Treatment II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 137.16/W14

Topic: C.10. Brain Injury and Trauma

Support: Dedicated Health Research Funds from the University of New Mexico

Title: Damage to ependymal motile cilia contributes to posthemorrhagic hydrocephalus of prematurity in rats

Authors: *J. NEWVILLE[1], T. R. YELLOWHAIR[2], C. SHROCK[3], F. CONTEH[3], A. Y. OPPONG[3], T. A. HOWARD[4], J. R. MAXWELL[2], S. ROBINSON[3], L. L. JANTZIE[2]
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Abstract: Posthemorrhagic hydrocephalus of prematurity (PHHP) results from intraventricular hemorrhage (IVH) in very preterm infants. Genetic mutations that effect the ependymal motile cilia (EMC), responsible for propelling cerebrospinal fluid through the ventricles, cause hydrocephalus with ventriculomegaly and elevated intracranial pressure. Maturation of EMC takes place over the first three postnatal weeks in rats. We hypothesized that early damage to EMC contributes to hydrocephalus in PHHP, and sought to test if EMC function could be modulated with the neonatal neuroreparative agents erythropoietin (EPO) and melatonin (MLT). On embryonic day 18 pregnant Sprague Dawley rats underwent laparotomy for transient uterine artery occlusion and intra-amniotic sac injection of lipopolysaccharide to induce chorioamnionitis (CAM). On postnatal day 1, rats were randomized to either bilateral intraventricular injection of lysed littermate red blood cells (IVH) or vehicle (sterile phosphate buffered saline). On P2 CAM-IVH rats were randomized to EPO+MLT treatment or vehicle. Intra-aural distance was measured daily and all experiments were performed by observers blinded to treatment. Cilia function was assayed by measuring fluorescent bead flow on ependymal wall whole mounts at P9/10 (mid-EMC development) and at P23/24 (EMC maturation), and data was processed in IMARIS for bead speed and path angle deviation. Whole mounts underwent immunohistochemistry and confocal imaging. Additionally, scanning electron microscopy was employed to assess EMC ultrastructure at P21. Comparisons were made with t test or two-way ANOVA with Bonferroni correction, with significance defined as $p<0.05$. In shams mean bead speed increased 3-fold from P10 to P24 (n=4/group, $p<0.01$). At P10 and P24, bead speed of veh-treated CAM-IVH rats was <50% of shams (n=4-6, both ages $p<0.002$). With neonatal EPO+MLT treatment, bead speed normalized at both ages (n=2-6). Likewise, bead angle distribution was significantly more diffuse after CAM-IVH compared to shams, an effect

that was prevented with EPO+MLT treatment at P10 (n=3-5) and at P24 (n=2-4). Imaging of immunohistochemically processed ependymas for γ-tubulin on EMC showed that the planar polarity and ependymal rosettes were present and organized in shams, but disorganized and missing in veh-treated CAM-IVH rats. SEM showed that EMC formed tufts in shams and EPO+MLT-treated CAM-IVH rats, compared to matted, bloated and missing EMC in veh-treated CAM-IVH rats. Together, these results suggest that the structure and function of developing EMC are damaged by IVH in the setting of CAM, and that early EPO+MLT can modulate this injury.

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Poster

137. Stroke, Damage, or Disease: Assessment and Treatment II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 137.17/W15

Topic: C.09.Stroke

Support: NIH Grant NS085272

Title: Phytoestrogen isoflavone biochanin a induces glutamate oxaloacetate transaminase expression and increases glutamate metabolism during ischemic stroke

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Abstract: Glutamate Oxaloacetate Transaminase (GOT) metabolizes glutamate by transferring the amino group from glutamate (Glu) to oxaloacetate, forming aspartate (Asp) and the 5-carbon TCA cycle intermediate alpha-ketoglutarate in the process. We previously reported that transamination of Glu by GOT protects against ischemic stroke-induced injury by (1) clearing neurotoxic extracellular Glu from the ischemic site and (2) generating TCA cycle intermediates that sustain cellular respiration for neural cells in the absence of glucose. Therapeutic strategies that up-regulate GOT expression for protection against ischemic stroke are therefore of interest. In the 5' promoter region of the GOT gene, there are two Estrogen Related Receptor Element (ERRE) sequences. As ERRE sites are known to bind phytoestrogen isoflavones, we screened a panel of isoflavones to identify targets that induce transcriptional activation of GOT. Biochanin A (BCA), a naturally occurring estrogenic isoflavone found in red clover, was identified as the most potent inducer of GOT mRNA in neuronal cells. To determine the significance of BCA for protection against stroke *in vivo*, C57Bl/6 mice were intraperitoneally injected with either

vehicle control (75% DMSO in water) or BCA (up to 10 mg/kg body weight) daily for 4 weeks. Following delivery, mice were subjected to ischemic stroke using the intraluminal thread method of middle cerebral artery occlusion (MCAO). BCA levels were significantly increased in blood and brain of IP-injected mice as measured by HPLC. Immunohistochemical analysis confirmed increased expression of GOT in brain. Furthermore, 9.4T 1H MRS acquired during cerebral ischemia identified lower Glu and higher Asp levels in stroke-affected cerebral cortex of mice treated with BCA. This outcome is consistent with increased GOT activity in brain tissue. Finally, BCA treatment significantly improved post-stroke sensorimotor function and attenuated stroke-induced lesion volume as measured by MRI. Taken together, outcomes support BCA as a potent small molecule inducer of GOT for protection against stroke-induced injury. As a safe, naturally occurring phytoestrogen isoflavone, BCA represents a stroke therapeutic that can be quickly translated to clinical study.

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Poster

138. Spinal Cord Injury I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 138.01/W16

Topic: C.11. Spinal Cord Injury and Plasticity

Support: CIHR
MSsociety

Title: New oligodendrocyte myelin does not contribute to functional recovery after moderate thoracic spinal contusion in mice

Authors: *S. B. MANESH[1], G. DUNCAN[1], B. HILTON[1,2], P. ASSINCK[1], J. LIU[1], A. MOULSON[1], J. PLEMEL[3], W. TETZLAFF[1]
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Abstract: Contusive spinal cord injury (SCI) leads to oligodendrocyte death and demyelination, affecting impulse conduction and leaving axons vulnerable to degeneration. This provides the rationale for clinical trials with myelinating cells humans. However, remyelination in rodents is quite effective and while it may correlate temporally with spontaneous functional improvement, a causal link between remyelination and spontaneous motor recovery has not been determined. Here we use a conditional knockout of the gene coding for the myelin regulatory factor (Myrf) from oligodendrocyte precursor cells using a PDGFRa-CreERT2::Myrf$^{fl/fl}$ mouse line activated by tamoxifen. MYRF is a transcription factor critically involved in the regulation of several

genes coding for myelin proteins and has been shown to be required for developmental CNS myelination. This impairs process outgrowth, ensheathment and subsequent remyelination, while leaving oligodendrocyte precursor (OPC) recruitment and undamaged myelin remain intact. Both Myrf inducible conditional knockout mice (ICKO) and littermate controls (n=23, each cohort) received a 70 Kdyne thoracic contusion at T9/T10. Motor behavioural assessments using the Basso Mouse Scale (BMS), footprint analysis and horizontal ladder revealed no significant behavioural differences between Myrf ICKO and control mice. Histological analyses showed OPC production remained normal (determined by EdU labeling), but oligodendrocyte formation (CC1/EdU cells) was indeed impaired. Additionally, Myrf ICKO mice and controls were crossed with an inducible membrane-tethered GFP reporter allowing for the visualization of new myelin following injury. Very little new myelin (mGFP-positive ensheathment) was observed leaving a large percentage of axons denuded. These data where corroborated by plastic embedding where toluidine blue staining showed a 44% reduction of myelin in Myrf ICKO and g-ratio analysis with electron microscopy showed new myelin only in the control animals. We conclude that oligodendrocyte remyelination following a moderate thoracic spinal contusion in not a major contributor to spontaneous locomotor recovery.

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Poster

138. Spinal Cord Injury I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 138.02/W17

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Patient organization “Verticale” [to GSM, CMB, HNN and FEP]
Patient organization “Demain Debout Aquitaine” [to HNN, YNG, CMB and FEP]
LabeX NUMEV [to GPSM and CGB]

Title: Reduction of microglia proliferation through CSFR1 targeting improves motor recovery following spinal cord injury

Authors: F. E. PERRIN[1], Y. GERBER[2], G. P. SAINT-MARTIN[3], C. BRINGUIER[2], S. BARTOLAMI[2], C. GOZE-BAC[4], *H. NORISTANI[5]
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Abstract: Spinal cord injury (SCI) induces a pronounced neuroinflammation driven by activation and proliferation of resident microglia as well as infiltrating peripheral monocyte-derived macrophages. Depending on time post-lesion, positive and detrimental influences of microglia/macrophages on axonal regeneration had been reported after SCI, raising the issue whether their modulation may represent an attractive therapeutic strategy. Colony-stimulating factor 1 (CSF1) regulates microglia/macrophages proliferation, differentiation and survival thus, pharmacological treatments using CSF1 receptor (CSF1R) inhibitors had been used to ablate microglia.
We analyzed the effect of chronic (10 weeks) food diet containing GW2580 (a CSF1R inhibitor) in mice that underwent spinal cord hemisection at vertebral thoracic level 9. Treatment started 4 weeks prior to SCI and continued until 6 weeks post-lesion.
We first demonstrate that GW2580 treatment did not modify microglial response in non-injured animals. Conversely, a strong decrease in proliferating microglia was observed following SCI. Second, we showed that GW2580 treatment improved several parameters of motor recovery in lesioned animals through better paw placement. Using in and ex vivo magnetic resonance imaging (MRI) we then established that GW2580 treatment had no effect on lesion extension and volume. However, histological analyses revealed that GW2580-treated animals had reduced gliosis and microcavity formation following SCI.
In conclusion, CSF1R blockade using GW2580 specifically inhibits SCI-induced microglia/macrophages proliferation. Moreover, GW2580 treatment reduced gliosis and microcavity formations associated with improved fine motor recovery after incomplete SCI. Preventing microglial proliferation may offer therapeutic approach to limit neuroinflammation, promote tissue preservation and motor recovery following SCI.

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Poster

138. Spinal Cord Injury I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 138.03/W18

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH R01 NS052741
The Minnesota SCI and TBI Research Grant Program
Mayo Clinic Center for Biomedical Discovery

Title: Western Diet impedes recovery after experimental spinal cord injury

Authors: *H. KIM[1,2], H. YOON[1,2], M. R. LANGLEY[1,2], L. KLEPPE[1], I. R. LANZA[2], N. K. LEBRASSEUR[1,2], A. MATVEYENKO[2], I. A. SCARISBRICK[1,2,3]

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Abstract: The cardinal signs of systemic metabolic dysfunction, including insulin resistance, hypertension and a systemic pro-inflammatory state are risk factors for the development of cardiovascular disease, type 2 diabetes, and neurodegenerative conditions, but there is limited information regarding the impact on recovery after spinal cord injury (SCI). To address this gap, we investigated the impact of systemic insulin resistance generated by consumption of a diet high in fat and sucrose (HFHS), often referred to as a Western Diet on neuropathophysiological outcomes in a murine compression model of incomplete SCI. Ten-week-old female C57BL6 mice were provided a regular diet (RD) or a diet high in fat and sucrose (HFHS) for 7 weeks prior to experimental compression SCI.

Mice consuming a Western diet showed impairments in motor recovery evaluated by BMS score and subscore at 14 and 30 dpi, but not at earlier time points examined. Consumption of a diet rich in fat and sucrose also impaired the mean maximal angle achieved on the incline plane test at 14, 21 and 30 dpi and resulted in significant impairments in bladder release. Immunochemical analysis of the spinal cord revealed a number of differences in markers of neural injury between the two groups. First, mice consuming HFHS exhibited significant increases in astrogliosis measured by glial fibrillary acidic protein (GFAP) prior to injury, compared to mice consuming a RD. In fact, the prominent increases in GFAP observed in the intact spinal cord of mice consuming HFHS were equivalent to those observed after SCI in mice consuming a regular chow. In the subacute period, markers of microglial/monocyte activation were elevated in all mice with SCI, however the increase was substantially greater in the spinal cord of mice consuming HFHS. The spinal cord white matter of mice consuming HFHS also showed reductions in the number of oligodendrocyte progenitor and mature myelinating cells prior to SCI and HFHS-associated reductions in myelin producing cells persisted at 14 and 30 dpi. In addition, loss of serotonergic axons in the spinal cord after SCI was exacerbated in mice consuming HFHS. Growth associated protein 43 (GAP43), a marker of axonal growth cones was elevated after SCI in the spinal cord of mice consuming a RD or a HFHS diet, however the magnitude of the increase in growth cones was severely impaired in mice consuming HFHS. These findings suggest that consumption of a Western diet increases the risk for impairments in neurobehavioral recovery at subacute and chronic time points after SCI and suggests the need to identify the mechanisms involved as potential targets for therapies to improve functional outcomes.

Disclosures: H. Kim: A. Employment/Salary (full or part-time):; The Minnesota SCI and TBI Research Grant Program, Mayo Clinic. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH R01 NS052741, Mayo Clinic Center for Biomedical Discovery. **H. Yoon:** None. **M.R. Langley:** None. **L. Kleppe:** None. **I.R. Lanza:** None. **N.K. LeBrasseur:** None. **A. Matveyenko:** None. **I.A. Scarisbrick:** None.

Poster

138. Spinal Cord Injury I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 138.04/X1

Topic: C.11. Spinal Cord Injury and Plasticity

Support: São Paulo Research Foundation – FAPESP - São Paulo - Brazil

Title: Neuroprotection by tauroursodeoxycholic acid (TUDCA) after unilateral sciatic nerve axotomy in neonatal Wistar rats

Authors: *M. V. DA SILVA[1], L. P. CARTAROZZI[2], M. PEREZ[3], M. V. DE CASTRO[4], J. F. VETTORAZZI[1], E. M. CARNEIRO[1], A. L. OLIVEIRA[5]
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Abstract: Peripheral nervous system (PNS) injuries are related to loss of motor and sensory function resulting in poor quality of life and disability in the productive age. Thus, new therapeutic alternatives are necessary to improve PNS regeneration. The tauroursodeoxycholic acid (TUDCA) is a hydrophilic, non-toxic bile acid that is the result of the conjugation of ursodeoxycholic acid and the amino acid taurine. TUDCA is one of the main components of biliary secretion in bear (about 76% in Asian black bear)**.** Since ancient times, TUDCA has been used in the treatment of liver diseases. However, several studies point to its therapeutic efficacy following damage to the nervous system, especially in neurodegenerative diseases. According to the literature, TUDCA acts in the intrinsic apoptosis pathway, in the control of inflammation and reduction reactive oxygen species (ROS). The present study intended to evaluate the effects of treatment with TUDCA on nerve regeneration and neuroprotection in 2 days old (P2) Wistar rats after unilateral sciatic nerve axotomy. The drug was administrated, intraperitoneally, for five days, until P7. Thus, the animals were divided into four experimental groups: Axotomy + PBS (V); Axotomy + 250 mg/kg/day of TUDCA (T250); Axotomy + 500 mg/kg/day of TUDCA (T500); Axotomy + 750 mg/kg/day of TUDCA (T750). The animals were euthanized, and the spinal cord and the dorsal root ganglia (DRG), at L4-L6 levels, were analyzed by Nissl staining. The astroglial and microglial reaction in the spinal cord were analyzed by immunofluorescence (anti-GFAP and anti-Iba1 antisera, respectively). Neuronal counting in the DRG revealed no significant statistical differences, but the T250 group was associated with better preservation of motoneurons in the spinal cord (V: 0.31 ± 0.04; T250: 0.55 ± 0.004; T500: 0.47 ± 0.01; T750: 0.48 ± 0.07 - $\bar{x}$ ± SD; ipsi/contralateral ratio). Astrocytic and microglial/macrophage reactivity revealed no statistically significant differences between the experimental groups and the vehicle treated counterpart. However, there was a trend of reduction in astrocytic reactivity in the T500

and T750 groups. The present results show that TUDCA is neuroprotective to axotomized motoneurons, emerging as a promising therapeutic alternative for the treatment of PNS lesions.

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Poster

138. Spinal Cord Injury I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 138.05/X2

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Analysis of transduction efficiency and tropism of AAV serotypes in chronic spinal cord injury

Authors: *Y. HOSHINO[1,2], K. NISHIDE[2], J. KOHYAMA[2], N. NAGOSHI[1], O. TSUJI[1], K. KOJIMA[1,2], M. MATSUMOTO[1], H. OKANO[2], M. NAKAMURA[1]
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Abstract: Introduction:
A combination of several treatment options, including gene therapy, is thought to be necessary for the management of chronic spinal cord injury (SCI). Optimizing the transportation efficacy of these therapeutic genes can potentially improve the performance, and hence the outcome. In this study, we focused on the potential use of adeno associated virus (AAV) vectors as a therapeutic gene transporter. AAV vectors are known to have a safe and long lasting gene expression in vivo, and are widely utilized in gene therapy nowadays. It is well known that AAV serotypes, with different capsids, have different cell tropisms. However, a comprehensive study investigating the efficiency of the different serotypes in chronic SCI has never been performed. The aim of this study is to elucidate the character of AAV serotypes in chronic SCI.
Method:
We produced AAV serotypes 1, 2, 3, 4, 5, 6, 7, 8, 9, rh10, DJ and DJ/8 with CMV promoter. Firefly luciferase and VENUS were used as an *in vivo* reporter system. First, intraparenchymal injections of each serotype was given to the intact spinal cord (Th10) C57BL6 albino mice and bioluminescence images (BLI) were measured weekly for three weeks. Second, the three most promising serotypes, based on the results of the BLI, were injected into the spinal cord of chronic contusion SCI model C57BL6 mice. BLI were measured and immunohistlogical evaluation was performed 6 weeks after the injections.
Result:

In the first study using intact spinal cords, successful gene expression was observed in all 12 serotypes. Of these, AAV5, AAV6 and AAVrh10 had the highest photon count measurements with BLI three weeks after injection. Therefore, we selected these serotypes for injecting into chronic SCI models (n=4 each). AAVrh10 had the highest photon counts five weeks after injection ($p<0.05$). Immunohistological analysis was used to identify the efficiency/character of the cell tropism. AAVrh10 transduced neurons and astrocytes more effectively than AAV5 and AAV6. As for oligodendrocytes, effective transduction was seen with AAV6 and AAVrh10 compared to AAV5 ($p<0.05$). Transduction to microglia/macrophages and blood vessels could not be observed with all three serotypes. A higher infiltration of the injury epicenter was achieved using AAVrh10.
Conclusions:
We showed that AAVrh10 was the most effective in transducing the spinal cord of chronic SCI model mice. AAVrh10 could be a good candidate for gene transfer and as a tool for research of chronic SCI. In the future, we will perform a therapeutic intervention with this promising AAV vector.

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Poster

138. Spinal Cord Injury I

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Program #/Poster #: 138.06/X3

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH Grant MH101525
NSF Grant 1464686
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NIH Grant EY026427
NIH Grant NS099709
NSF Grant 1707352

Title: Combining bioluminescence driven optogenetic stimulation with swim training for treatment following spinal cord injury in rats

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Abstract: The ability to manipulate specific neuronal populations of the spinal cord following spinal cord injury (SCI) could potentially prove highly beneficial for rehabilitation in patients through maintaining and strengthening still existing neuronal connections and/or facilitating the formation of new connections. A non-invasive and highly specific approach to neuronal stimulation is bioluminescent-optogenetics, where genetically expressed light emitting luciferases are tethered to light sensitive channelrhodopsins (luminopsins, LMO); neurons are activated by the addition of the luciferase substrate coelenterazine (CTZ). This approach takes advantage of utilizing ion channels for current conduction while activating the channels through application of a small chemical compound, thus allowing non-invasive stimulation and recruitment of all targeted neurons. Rats were transduced in the lumbar spinal cord with AAV2/9 expressing the excitatory LMO3 under control of the synapsin promoter. This approach has been effective at promoting locomotor recovery following severe spinal cord injury in rats. We are now working to combine this approach with swim training to re-enforce activity dependent neural network changes induced by stimulation with the excitatory LMO3.

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Poster

138. Spinal Cord Injury I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 138.07/X4

Topic: C.11. Spinal Cord Injury and Plasticity

Support: University of Utah Neuroscience Initiative Pilot Funds

Title: Multi-modal imaging of spinal cord injury and response to therapy in rats

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Abstract: Spinal cord injury (SCI) affects over 7,000 individuals in the US each year. Upon injury, reactive astrocytes form a 'glial scar' that actively stabilizes the injury and seals the blood spinal cord barrier (BSCB) from infiltrating substances. However, over time, the scar acts as a barrier to regeneration of the cord. Our research aims to measure the ability of MRI-Guided High Intensity Focused Ultrasound (HIFU) as a method of breaching the glial scar/BSCB to create opportunity for the delivery of therapeutic drug. **Methods:** Subjects were 200g female Sprague Dawley rats, N=43. The injury model was a laminectomy at T8-T10, then spinal cord compression with a 23g clip at the location of T9 for one minute. Shams received laminectomy only. HIFU setup: Pre-clinical HIFU system from Image Guided Therapy, Inc. (f#=0.8, 940 kHz), Siemens 3T PrismaFIT MRI, custom rat holder with integrated radiofrequency MRI coil.

N=11 sham SCI: laminectomy, no compression or HIFU procedures. N=11 SCI+HIFU: laminectomy + compression, HIFU procedures + sonications. N=10 SCI+HIFU+TAX: laminectomy + compression, HIFU procedures + sonications + 1 mg/kg *paclitaxel* i.p. N=11 SCI+HIFU sham: laminectomy + compression, HIFU procedures, no sonications or drug. For each animal, ImageJ was used to load CT, MRI, and PET scans as stacks of images showing the transverse section. For each respective slice, the CT and MRI scans were used to define a region of interest (ROI). Location of the ROI was mapped to the PET scan and average pixel intensity was sampled to quantify FDG uptake within that slice of the cord. Microsoft Excel was used to plot uptake in the cord against its length. **Results:** FDG-PET imaging detects injury region and decreased caudal uptake. Representative ROI FDG-uptake curves from Sham and SCI subjects can clearly distinguish injury. Group-wise analysis showed significantly decreased uptake in the injury area and caudal regions in SCI compared to shams ($p \leq 0.05$), but effect of drug + HIFU was not significant. *Paclitaxel* + HIFU shows improvement trend in neurological function. *Paclitaxel* group was 33% increased over non-treated SCI subjects (9.3 ± 2.4 vs. 7.0 ± 4.6, $p = 0.08$). In the spinal cord injury model, a modest, sub-threshold effect was seen with combination therapy of HIFU + paclitaxel to open the BSCB and deliver drug. Multi-modal imaging evidenced decreased uptake in injury and caudal regions. With our ability to successfully model traumatic SCI, breach the BSCB/glial scar, and measure changes in uptake along the cord via multi-modal image analysis techniques, this research could have a significant impact on future treatment options following SCI.

Disclosures: R.A. Cheeniyil: None. **A.J. Stump:** None. **M. Ostlie:** None. **A.H. Payne:** None. **G.W.J. Hawryluk:** None. **C.G. Cross:** None. **Y. Anzai:** None. **S. Minoshima:** None. **D.J. Cross:** None.

Poster

138. Spinal Cord Injury I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 138.08/X5

Topic: C.11. Spinal Cord Injury and Plasticity

Support: University of Utah neuroscience initiative pilot funding

Title: MRI-guided high intensity focused ultrasound delivery of paclitaxel in rat model of spinal cord injury

Authors: *M. OSTLIE[1], A. J. STUMP[1], A. H. PAYNE[1], G. W. HAWRYLUK[2], Y. ANZAI[1], S. MINOSHIMA[1], D. J. CROSS[1]
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Abstract: Introduction Spinal cord injury (SCI) affects more than 7,000 people annually. Treatment options for SCI are limited, invasive, and only provide a modest outcome. MRI-guided high intensity focused ultrasound (MRgFUS) is currently FDA approved for non-invasive ablation of some cancerous tumors and more. Previously, we have shown that MRgFUS applied with microbubbles to produce sonoporation results in transient blood spinal cord barrier (BSCB) permeability in uninjured rats. The goal of this study is to use MRgFUS to open the BSCB to facilitate direct delivery of *paclitaxel* to the injury site in rats with SCI. Methods All rats (n=43) received a T8-T10 laminectomy. 11 rats received a laminectomy only, and 32 rats received a laminectomy and compression SCI (23g weighted clip applied at T9 for 1 minute). Rats were divided into *paclitaxel* (1mg/kg, n=10), vehicle only (saline, n=11), and no drug (n=11) treatment groups and administered respective treatments IP at day 3 post-injury. At 30 days post-injury, 21 rats received MRgFUS and 11 rats received sham MRgFUS (all anesthesia and treatment events with no sonications). Laminectomy only rats did not receive MRgFUS or sham MRgFUS. Rats were administered paclitaxel or saline treatment IP immediately after MRgFUS. All groups underwent neurological testing using the Basso, Beattie, Breshnahan (BBB) scale on days 14, 37, and 49 after injury. 30 days after MRgFUS treatment, animals were perfused and spinal cords were removed for histological evaluation. Results ANOVA between group single factor analysis revealed animals treated with HIFU and *paclitaxel* had significant longitudinal improvement in BBB scores from week 2 to week 7 post-surgery (5.45±1.06 vs. 8.81±0.84, $p \leq 0.0222$). Both HIFU and saline treatment and Sham HIFU and saline treatment did not have significant longitudinal improvement in BBB scores from weeks 2 to 7 post-surgery (HIFU plus saline: 5.46±1.15 vs. 7.77±1.35, $p \leq 0.2050$, sham HIFU plus saline: 5.45±1.07 vs. 7.00±1.40, $p \leq 0.1648$). Comparison between the *paclitaxel* and saline treated groups showed a 33% increase that trended toward significance (9.3±2.4 vs. 7.0±4.6, p=0.08). Histological evaluation is currently underway. Conclusion This preliminary study shows a modest, sub-threshold effect of combining MRgFUS with I.P. administered *paclitaxel*. Subsequent studies will include multiple MRgFUS and drug treatments while surviving the animals longer to observe possible continued improvement of motor function and to see if the *paclitaxel* trend turns into a significant improvement over saline treated animals. Results from this research could have a significant impact on treatment options for SCI.

Disclosures: M. Ostlie: None. **A.J. Stump:** None. **A.H. Payne:** None. **G.W. Hawryluk:** None. **Y. Anzai:** None. **S. Minoshima:** None. **D.J. Cross:** None.

Poster

138. Spinal Cord Injury I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 138.09/X6

Topic: C.11. Spinal Cord Injury and Plasticity

Support: National Research Foundation of Korea 2016R1D1A1B03933986

Title: The changes in the pattern of blood vessel destruction over time after spinal cord injury in rats

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Abstract: Spinal Cord Injury (SCI) results in the destruction of neural and vascular structures, which causes the several pathological changes after SCI. The vascular response to SCI, focusing on early event including hemorrhage and disruption of the blood-spinal cord barrier, influences the evolution of secondary injury, but its underlying mechanisms remain unclear. In the present study, we investigated changes in pattern and degree of blood vessel destruction over time after SCI.
Spinal contusion was made at T11 in adult 6w male Sprague Dawley rats (n = 3) by using NYU impactor. To examine the degree of blood vessel damage, a 1% Evans Blue Dye (EBD) solution (1ml/100g) was administered intraperitoneally (IP) 24h before the tissue sampling. Immunostaining with Hoechst was conducted at spinal segment (T10-T12) to confirm the morphology of cell in the spinal cord.
The blood vessel destruction was clearly observed in the EBD with Hoechst staining. The EBD signal was rarely observed in normal spinal cord. However, immediately after SCI, the EBD signal was observed at the injured site (epicenter) and the EBD signal was significantly increased and the area showing the EBD signal was extended over time after SCI in the gray matter. At 24h following SCI, the EBD signal was observed overall the gray matter of spinal cord and the barrier of central canal was almost destroyed. In addition, this blood vessel destruction extended to remote area; epicenter, rostral and caudal spinal segment, from 6h after SCI. The present results showed that the blood vessel destruction starts immediately after SCI and is extended to remote area: rostral and caudal to epicenter from 6h after SCI. This results suggest the importance of the early therapeutic approaches for neuroprotection focused on vascular response to SCI within few hour after injury

Disclosures: M. Kwon: None. **J. Hong:** None. **Y. Kim:** None. **Y.W. Yoon:** None. **J. Kim:** None.

Poster

138. Spinal Cord Injury I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 138.10/X7

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH Grant MH101525
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NIH Grant EY026427
NIH Grant NS099709
NSF Grant 1707352

Title: Restoring function after severe spinal cord injury through bioluminescence-driven optogenetic stimulation of spinal circuitry

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Abstract: The ability to manipulate specific neuronal populations of the spinal cord following spinal cord injury (SCI) could potentially prove highly beneficial for rehabilitation in patients through maintaining and strengthening still existing neuronal connections and/or facilitating the formation of new connections. A non-invasive and highly specific approach to neuronal stimulation is bioluminescent-optogenetics, where genetically expressed light emitting luciferases are tethered to light sensitive channelrhodopsins (luminopsins, LMO); neurons are activated by the addition of the luciferase substrate coelenterazine (CTZ). This approach takes advantage of utilizing ion channels for current conduction while activating the channels through application of a small chemical compound, thus allowing non-invasive stimulation and recruitment of all targeted neurons. Rats were transduced in the lumbar spinal cord with AAV2/9 expressing the excitatory LMO3 under control of the synapsin or the Hb9 promoter. A day after contusion injury of the thoracic spine, rats received either CTZ or vehicle every other day for 2 weeks. We found activation of either interneuron or motor neuron populations below the level of injury to significantly improve locomotor recovery. This is the first example of non-invasive activation of an optogenetic component as a potential therapy following spinal cord injury. We are utilizing morphological and histological methods to identify mechanisms underlying improvements in locomotion. The findings will provide a foundation for a rational approach to spinal cord injury, thereby advancing approaches for functional recovery after SCI in the preclinical arena.

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Poster

138. Spinal Cord Injury I

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Program #/Poster #: 138.11/X8

Topic: C.11. Spinal Cord Injury and Plasticity

Support: VA IP50RX001045 RR&D B7332R
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Craig H Neilsen Foundation
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Bernard and Anne Spitzer Charitable Trust
DOD CDMRP SC170233

Title: Continued development of human neural stem cell grafts into non-human primate spinal cord contusion or hemisection lesions

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Abstract: We previously demonstrated that human neural stem cells (hNSCs) and multipotent neural progenitor cells (hNPCs) grafted into sites of rodent spinal cord injury (SCI) survive, extend axons, form synapses, support host axon regeneration, and improve functional recovery (Lu et al., *Cell* 2012; Lu et al., *Neuron* 2014; Kadoya et al., *Nat Med* 2016). Recently, we published the first steps in translation of this approach to non-human primates using hNSCs derived from fetal tissue (Rosenzweig et al., *Nat Med* 2018).
We now discuss continued development of this approach, specifically addressing issues that will enable potential translation to human clinical trials. We have developed a human embryonic stem cell-derived neural stem cell line driven to a *spinal cord* identity (**H9-scNSC**; Kumamaru et al., *Nat Methods* 2018) as a candidate optimal cell type for human translation. In the present work, we have grafted this lead candidate translational cell line to rhesus monkeys that have undergone either C7 unilateral spinal cord hemisection (Rosenzweig et al., *Nat Neurosci* 2010) or C7

unilateral spinal cord contusion (Salegio et al., *J Neurotrauma* 2016). We find that:
1) H9-scNSC grafts placed into sites of C7 hemisection SCI survive, extend axons, form synapses, and support host axon regeneration. Analysis of possible graft-associated functional improvement is ongoing.
2) H9-scNSC grafts placed into sites of C7 unilateral contusion SCI survive, extend axons, form synapses, and support host axon regeneration. Analysis of possible graft-associated functional improvement is ongoing.
3) In the first subject maintained for 1.5 years after grafting, the graft survived, extended axons, and supported host axon regeneration. Critically, there was no sign of excessive graft growth or other safety problems.

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Poster

138. Spinal Cord Injury I

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Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 138.12/X9

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Wings for Life Individual Research Grant
UCSD Frontiers of Innovation Scholars Program
The Veterans Administration
Nakajima Foundation

Title: Calcium imaging of synaptic connectivity between host and neural progenitor cell graft-derived neurons after spinal cord injury

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Abstract: Neural stem cells (NSCs) grafted into sites of spinal cord injury (SCI) may act as new electrophysiological relays between host neurons above and below the lesion. Host axons regenerate robustly into NSC grafts and form synapses; in turn, graft axons extend long distances into host white and gray matter caudal to the injury and form synapses. To investigate potential functionality of these new synaptic pathways, we performed calcium imaging and whole-cell

patch clamp recordings in mice with NSC grafts after SCI. We placed T12 dorsal column lesions and acutely grafted embryonic day twelve (E12)-derived spinal cord neural progenitor cells (NPCs) expressing the calcium indicator GCaMP6f into the lesion site. From 6 to 8 weeks later, we imaged the activity of populations of neurons within NPC grafts in acute spinal cord slices, anesthetized, or awake behaving animals. After grafting NPCs into acute spinal cord injuries in mice, we imaged the simultaneous activity of populations of neurons within grafts both in vivo and in ex vivo slice preparations. We observed spontaneous activity in both neurons and glia, as well as hindpaw pinch- and cold air puff-evoked responses. Furthermore, optogenetic stimulation of corticospinal tract axons regenerating into grafts evoked robust responses throughout grafts. Activity patterns included large-scale events and independent, single-neuron activity. Spontaneous activity and stimulus-evoked responses were significantly enhanced by bath application of the potassium channel blocker 4-aminopyridine (4-AP). We are currently optimizing methods to interrogate graft-graft and graft-to-host connectivity. These studies will reveal the extent to which, at the cellular level, current NSC grafts are capable of forming functional neuronal relays across spinal cord injuries.

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Poster

138. Spinal Cord Injury I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 138.13/X10

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Patient organization, “Demain Debout Aquitaine” [to HNN and LT]
Patient organization, “Verticale” [to FEP and HNN]

Title: C57BL/6 and Swiss Webster mice display differences in mobility, gliosis, microcavity formation and lesion volume after severe spinal cord injury

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Abstract: Spinal cord injuries (SCI) are neuropathologies causing enormous physical and emotional anguish as well as irreversibly disabilities with great socio/economic burdens to our society. The availability of multiple mouse strains is important for studying the underlying pathophysiological responses after SCI. Although strain differences have been shown to directly affect spontaneous functional recovery following incomplete SCI, its influence after complete

lesion of the spinal cord is unclear. To study the influence of mouse strain on recovery after severe SCI, we first carried out behavioral analyses up to 6 weeks following transection of the spinal cord at T9 level in mice with two different genetic backgrounds namely, C57BL/6 and Swiss Webster. Using immunohistochemistry, we then analyzed glial cell reactivity not only at different time-points after injury but also at different distances from the lesion epicenter. Behavioral assessments using CatWalk and open field analyses revealed increased mobility (measured using average speed) and differential forelimb gross sensory response in Swiss Webster compared to C57BL/6 mice after transection. Comprehensive histological assessment revealed elevated microglia/macrophage reactivity and a moderate increase in astrogliosis in Swiss Webster that was associated with reduced microcavity formation and reduced lesion volume after spinal cord transection compared to C57BL/6 mice. Our results thus suggest that increased mobility correlates with enhanced gliosis and better tissue protection after complete section of the spinal cord.
Support: This work was supported by the patient organizations “Demain Debout Aquitaine” [to HNN and LT] and “Verticale” [to FEP and HNN]. The funding sources were not involved in study design, collection, analysis and interpretation of data as well as in the writing of the report and in the decision to submit the article for publication.

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Poster

138. Spinal Cord Injury I

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Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 138.14/X11

Topic: C.11. Spinal Cord Injury and Plasticity

Support: VA Gordon Mansfield Spinal Cord Injury Collaborative Consortium IP50RX001045
RR&D B7332R
NIHR01EB014986-05

Title: 3D Printed Spinal Cord Scaffolds for Spinal Cord Injury

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Abstract: There exists a great unmet medical need to develop novel therapies that promote axonal regeneration after spinal cord injury. While bioengineered scaffolds have been reported to support axon regeneration into spinal cord lesion sites, these technologies have been limited by

foreign body responses at implantation sites, lack of linear axon guidance through the lesion, and limitations in scaling to human size injuries. We now report the first use of advanced microscale 3D bioprinting to fabricate a scaffold mimicking the complex fascicular architecture of the spinal cord. The printed scaffold is simply and rapidly produced, reduces foreign body responses, and supports linear, aligned host axonal regeneration in the most challenging model of rat spinal cord injury, complete transection. F344 rats underwent T3 complete spinal cord transection, and then received into the lesion site (N=11 per group) either: 1) 3D printed biomimetic multichannel scaffolds made of PEG ("empty" scaffolds), 2) grafts of rat E14-derived multipotent neural progenitor cells(NPCs) without a scaffold, or 3) 3D printed biomimetic multichannel scaffolds that were loaded with NPCs. Scaffolds or grafts were placed into the acute lesion site, and outcomes were assessed 6 mo later. We find that grafting of NSCs within the protected environment of scaffolds enables graft survival and fill of the acute lesion site; dissociated grafts into the acute lesion site did not survival completely or fill the lesion site. Functional testing demonstrated partial functional recovery of animals that received NSCs in scaffolds: BBB score 6.6 $\pm$ 0.5 in scaffold + NSCs, vs. 1.6 + 0.8 in NSCs only and 0.3 $\pm$ 0.2 in empty scaffolds ($p<0.01$, repeated measures ANOVA). Host axons regenerated into stem cell loaded scaffolds to a degree superior to empty scaffolds, and axon growth was aligned in linear fascicles from the rostral to caudal end of the lesion site. We conclude that biomimetic 3D printed scaffolds enable survival of NSCs to *acute* spinal cord lesion sites, likely by providing a neuroprotective environment, and support strikingly linear regeneration of host axons through sites of severe SCI.

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Poster

138. Spinal Cord Injury I

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Program #/Poster #: 138.15/X12

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Federal Ministry for Education and Research (BMBF), Grant Nos. 03VP01121, 03VP01122, 03VP01123

Title: Combination of microconnector implantation and somatic stem cell (USSC) transplantation in the minipig: A preclinical translational model of complete thoracic spinal cord transection - Histological outcome

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WIEDERMANN[8], J. SCHIRA[1], A. LINK[9], A. VOGEL[9], M. HOEHN[8], C. JUERGENS[6], H. TRIEU[4], K. SEIDE[6], H. W. MUELLER[1]
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Abstract: Background: Spinal cord injury (SCI) interrupts the electrophysiological signal transduction and results in various neurological disabilities from damage of the spinal pathways. We have previously described the implantation of a mechanical microconnector system (mMS) composed of polymethylmethacrylate, which was developed for the purpose of bringing together the separated stumps after spinal cord transection (Estrada et al, 2011, SfN Abstract 892.17; Brazda et al, 2013, Biomaterials 34: 10056-10064). We could show that mMS implantation alone results in enhanced regenerative axon growth and improved functional outcome in a rat model of complete thoracic spinal cord transection. Since the pathophysiology of SCI is multifactorial and multi-phasic, it is likely that effective treatments will require combinations of different strategies. Our previous research provided evidence that transplantation of unrestricted somatic stem cells (USSC) significantly improves axonal regeneration and functional recovery in a rodent model of SCI (Schira et al, 2012, Brain 135: 431-446). Therefore, we had also combined implantation of the mMS, which is an ideal device for combinatorial treatment strategies, with somatic stem cell transplantation in order to further improve the functional outcome in a rat spinal cord transection model. In the rodent model we could demonstrate that the combination of the independently effective approaches led to a further improved locomotor function (Kehl et al., 2012, SfN Abstract 252.13).
Current progress: We have now taken the next step to bring this novel treatment strategy closer to clinical application by translating the above described treatment from the rodent to a large animal model. Aachen minipigs underwent complete thoracic spinal cord transection and received either mMS implantation, or mMS implantation and USSC transplantation, or no additional treatment. Here, we will demonstrate the adaptation of the mMS for the large animal model with high resolution MRI. Using histological and immunohistochemical methods, we will further show the outcome of the first experiments (1-6 weeks post injury and treatment), which include the characterization of the lesion area, the analysis of survival and migration of the USSC and their neuro- and tissue-protective effects, as well as regenerative events with a major focus on axon regeneration into and beyond the mMS implantation site. The results of mMS implantation alone or in combination with USSC transplantation will be discussed.
Outlook: Long-term locomotor behavioral experiments and electrophysiological recordings will be performed.

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Poster

138. Spinal Cord Injury I

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Program #/Poster #: 138.16/X13

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Paralyzed Veterans of America (PVA) Research Foundation Grant #3068
National Institutes of Health (NIH) SBIR GrantR43EB018232
Russian Foundation for Fundamental Research Grant 16-29-08173-ofi-m

Title: Regaining trunk stability after spinal cord injury

Authors: *M. RATH[1], D. G. SAYENKO[1], Y. P. GERASIMENKO[2], V. EDGERTON[3]
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Abstract: Recently we have developed the non-invasive electrical spinal stimulation technology for postural control in SCI subjects during standing. However, the potential of non-invasive spinal stimulation to facilitate trunk postural control during sitting in humans with spinal cord injury (SCI) has not been investigated. We hypothesized that transcutaneous electrical stimulation of the lumbosacral enlargement can improve trunk posture. Six participants with non-progressive SCI, C3-T9, AIS A or C, performed different motor tasks during sitting on a force platform. Electromyography of the trunk muscles, three-dimensional kinematics, and force plate data were acquired. Spinal stimulation improved trunk control during sitting in all tested individuals. Stimulation resulted in elevated activity of the erector spinae, rectus abdominis, and external obliques, contributing to trunk control, more natural anterior pelvic tilt and lordotic curve, and greater multidirectional seated stability. During spinal stimulation prior to any training, the center of pressure (COP) excursion decreased to 112.06 ± 36.00 mm from 143.74 ± 30.79 mm (p=.028, Z=-2.2014) without stimulation and to 93.09 ± 37.42 mm from 123.77 ± 48.44 mm (p=.028, Z=-2.2014) without stimulation in quiet sitting before training and after training respectively. Similarly, the limits of stable displacement increased by 31.40 ± 37.28% (p=.046, Z=1.9917), 19.42 ± 15.83% (p=.046, Z=1.9917), 54.11 ± 54.36% (p=.028, Z=2.2014), and 49.69 ± 32.343% (p=.046, Z=1.9917) before training and 24.06 ± 16.06% (p=.028, Z=2.2014), 20.25 ± 22.01% (p=.075, Z= 1.7821), 27.87 ± 11.88% (p=.028, Z=2.2014), and 27.33 ± 44.42% (p=.116, Z= 1.5724) after training in the forward, backward, right, and left directions, respectively. These data demonstrate that the spinal networks can be modulated

transcutaneously with tonic electrical spinal stimulation to physiological states sufficient to generate a more stable, erect sitting posture after chronic paralysis.

Disclosures: M. Rath: None. **D.G. Sayenko:** None. **Y.P. Gerasimenko:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); shareholder interest in NeuroRecovery Technologies. **V. Edgerton:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); shareholder interest in NeuroRecovery Technologies.

Poster

138. Spinal Cord Injury I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 138.17/X14

Topic: C.11. Spinal Cord Injury and Plasticity

Support: UL1TR000124

Title: Automatic segmentation techniques to facilitate quantitative analyses of spinal locomotor networks reconstructed in 3D using tissue clearing

Authors: *B. N. PHAM, JR, B. GOANKAR, N. TILLAKARATNE, H. ZHONG, V. EDGERTON
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Abstract: Segmentation of active neurons expressing immediate early genes (IEG's) using immunohistochemistry (IHC) is still mostly done manually. Tissue-clearing techniques like passive clarity technique (PACT) make it possible to reconstruct whole neural networks in 3D. However, these methods generate large image data sets that make manual segmentation infeasible. Using multiple human raters can increase throughput, but this creates variance due to different segmentation criteria between raters. Machine learning techniques for biomedical image analysis have been explored for cell-counting in pathology, but their performance on IHC staining, especially to label activated cells in the spinal cord is unknown.
A new animal model, FosTRAP, has been developed to capture neural activity in different ways. The FosTRAP mouse model allows for two different c-fos activation patterns due to two different events to be seen in the same animal. TRAP utilizes tamoxifen-dependent recombinase, $CreER^{T2}$, expressed in an activity-dependent manner which then expresses tdTomato (tdT) in a Cre-dependent manner. This allows genetic access of neural activation during desired time frames which can then be compared to c-fos expressed during a later event.
In this study, we evaluate the use of convolutional neural networks (CNN) to segment active neurons after 30 minutes of quadrupedal stepping in 1 mm thick spinal cord sections that have

been cleared using PACT. The training set composed of 20 images from 4 different mice with varying degrees of brightness and image quality. The testing set composed of 45 images sampled from 4 different 1 mm thick cleared sections from 2 animals. The testing set was also manually segmented by two other human raters. The CNN was able to achieve a recall of .836 with a precision of .835. The CNN scored a .785 DSI score, a measure of similarity, when compared to the human rater in which the CNN was trained. Most importantly, the CNN segmented on par with other human raters whose DSI score was .675 when compared to the first human rater. The CNN was able to segment PACT data image sets quickly and efficiently. The work presented here, addresses the analysis time bottleneck of large image data sets generated by c-fos IHC staining techniques, a task that would be virturally impossible to do manually. The use of CNN's for segmentation of large image data sets opens the door for the use of spatial statistics to quantitatively analyze whole neural networks and their 3D distribution. Furthermore, these techniques combined with FosTRAP can potentially identify the differences in spinal locomotor circuit architecture and activation due to different locomotor behaviors or injury states.

Disclosures: B.N. Pham: None. **B. Goankar:** None. **N. Tillakaratne:** None. **H. Zhong:** None. **V. Edgerton:** None.

Poster

138. Spinal Cord Injury I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 138.18/Y1

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Fellowship from Uehara Memorial Foundation

Title: Locomotor training increases synaptic structure with high NGL-2 expression after spinal cord hemisection

Authors: *K. KOBAYAKAWA[1,2], K. A. DEPETRO[3], H. ZHONG[1], C. JUANSING[1], N. ZOGHBY[1], V. EDGERTON[1]
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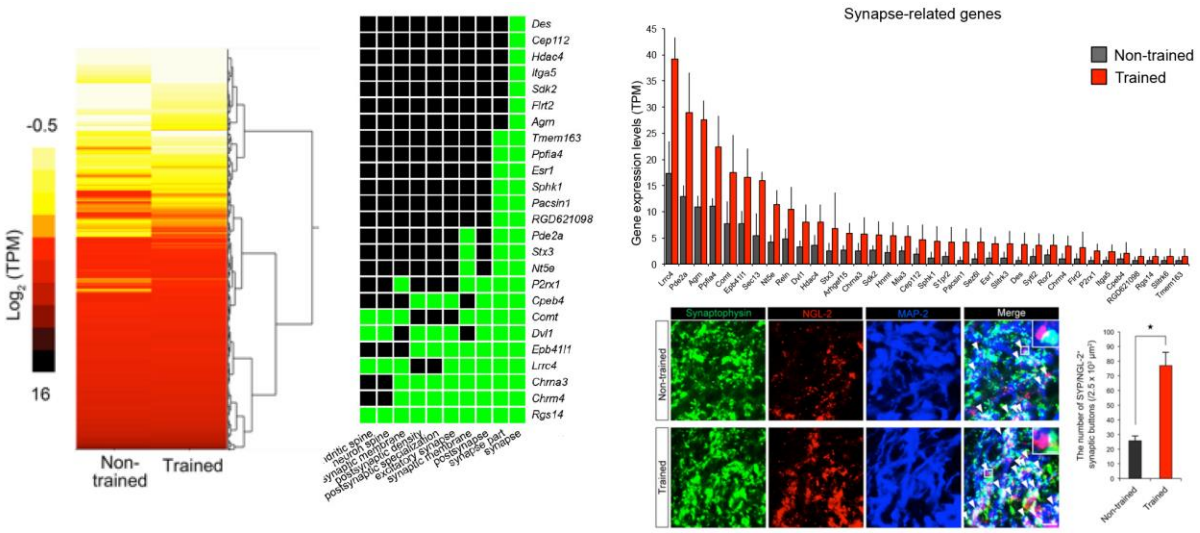
Abstract: Exercise training has beneficial effects on motor improvement after spinal cord injury (SCI). We previously demonstrated that treadmill training of rats after hemisection injury changed interneuronal networks, suggesting increase of synapse in lumbar spinal cord. Here, in order to investigate the training-induced biological changes in lumbar spinal cord after SCI, we performed RNA-Seq analysis of lumbar spinal cord with/without treadmill training after thoracic hemisection injury. GO term clustering demonstrated expression levels of 36 genes related to synapse were increased in trained rats compared to non-trained rats. Among the synaptic genes,

Lrrc4 (cording NGL-2) is the most highly expressed in lumbar spinal cord caudal to the lesion of trained animals. Immunohistochemical analysis demonstrated that treadmill training increased the number of NGL-2/synaptophysin synaptic buttons in lumbar spinal cord after SCI. Our findings reveal that gait training using treadmill changes expression of synapse-related genes, leading to increase of synaptic structures.



Disclosures: **K. Kobayakawa:** A. Employment/Salary (full or part-time):; UCLA full. **K.A. DePetro:** None. **H. Zhong:** None. **C. juansing:** None. **N. Zoghby:** None. **V. Edgerton:** None.

Poster

139. Olfaction: Olfactory Sensory Neuron Development and Function

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 139.01/Y2

Topic: D.05. Olfaction and Taste

Title: Neural and genetic mechanisms of menthol sensation in *C. elegans*

Authors: *E. A. RONAN[1,2], A. R. LAHAIE[2], X. XU[1,2]
[1]Mol. and Integrative Physiol., [2]Life Sci. Inst., Univ. of Michigan, Ann Arbor, MI

Abstract: Menthol is a crystalline, organic alcohol that evokes characteristic sensations such as mint flavor and scent as well as a sense of cool temperature. Popularly known for its culinary applications and analgesic properties, it has widespread use in both the food and drug industries. The mint and cool sensations induced by menthol are thought to be primarily sensed by the transient receptor potential (TRP) channel TRPM8, which is not encoded in the *Caenorhabditis elegans* genome. Despite the *C. elegans* hermaphrodite having a simple and compact nervous

system consisting of only 302 neurons, these animals are capable of eliciting an array of behavioral responses to environmental stimuli. With their short generation time, highly tractable genetics, and completely mapped connectome, *C. elegans* is an ideal model to investigate novel mechanisms of sensory physiology. Here we report in this exploratory study that *C. elegans* robustly sense menthol and elicit an avoidance response. Interestingly, this menthol sensation is independent of TRPA1, another known target of menthol activation. We performed a genetic screen to identify mutant worms lacking behavioral responses to menthol as well as neuronal responses using calcium imaging techniques. Our results reveal that *C. elegans* sense and respond to menthol through a previously uncharacterized neural and genetic pathway which may be evolutionarily conserved and have physiological significance in higher organisms.

Disclosures: E.A. Ronan: None. **A.R. LaHaie:** None. **X. Xu:** None.

Poster

139. Olfaction: Olfactory Sensory Neuron Development and Function

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 139.02/Y3

Topic: D.05. Olfaction and Taste

Support: Barrett Foundation
Mills College

Title: Localization and characterization of odor receptors in *C. elegans*

Authors: I. DIBIANCA[1], A. QUIOGUE[1], C. GHAFFARI[1], O. STAYER-WILBURN[1], V. THAKKER[1], M. HARWOOD[1], S. MAHER[1], L. RESCH[1], S. NATHAN[1], C. DALTON[1], A. COX-HARRIS[1], R. MORTON[1], E. JEROME[1], W. MANKINS[1], L. DAFFEH[1], K. TIRUMALASETTY[1], H. SZENTKUTI[1], J. SULLIVAN[1], G. HERNANDEZ[1], K. FEKRINIA[1], K. GIBBS[1], N. HWANG[1], C. LESTER[1], B. MOSQUEDA[1], N. L'ETOILE[2], *J. J. YOUNG[1]
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Abstract: *Caenorhabditis elegans* uses a small number of sensory neurons to respond to a wide variety of odors. The identities of the odor receptors and the stimuli to which they respond are largely unknown. We are working toward localizing odor receptors and pairing them with the odors they sense, with a focus on AWC-expressed receptors. We are localizing candidate odor receptor proteins using traditional and signal-enhanced translational GFP reporters. We are also generating novel odor receptor gene knockouts using CRISPR, which we are testing in chemotaxis assays for odor response behavior. By helping to illuminate the organization and function of olfactory receptors, we hope to enrich our understanding of the *C. elegans* olfactory system, and the strategies it uses to interpret information.

Disclosures: I. DiBianca: None. **A. Quiogue:** None. **C. Ghaffari:** None. **O. Stayer-Wilburn:** None. **V. Thakker:** None. **M. Harwood:** None. **S. Maher:** None. **L. Resch:** None. **S. Nathan:** None. **C. Dalton:** None. **A. Cox-Harris:** None. **R. Morton:** None. **E. Jerome:** None. **W. Mankins:** None. **L. Daffeh:** None. **K. Tirumalasetty:** None. **H. Szentkuti:** None. **J. Sullivan:** None. **G. Hernandez:** None. **K. Fekrinia:** None. **K. Gibbs:** None. **N. Hwang:** None. **C. Lester:** None. **B. Mosqueda:** None. **N. L'Etoile:** None. **J.J. Young:** None.

Poster

139. Olfaction: Olfactory Sensory Neuron Development and Function

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 139.03/Y4

Topic: D.05. Olfaction and Taste

Support: Grant-in-Aid for Scientific Research (S)17H06113

Title: Understanding how the interneuron AIY mediates salt concentration memory dependent behavior in *C. elegans*

Authors: *L. MABARDI, H. KUNITOMO, H. SATO, Y. TOYOSHIMA, Y. IINO
Univ. of Tokyo, Tokyo, Japan

Abstract: To successfully navigate their environment animals need to receive, encode and store sensory input, and retrieve these memories to make decisions about behavior. Despite the importance of memory storage and retrieval, the underlying mechanisms of these processes remain poorly understood. *C. elegans* is an excellent model organism for researching memory and neural plasticity thanks to its rapid life cycle, ease of genetic manipulation, fully mapped genome and connectome, and translucent body that facilitates imaging. *C. elegans* can memorize experienced salt concentrations; animals chemotax towards salt concentration experienced in the presence of food, and avoid concentrations experienced in the absence of food. Ambient salt concentrations are sensed by the amphid sensory neurons ASE-left and ASE-right, which sense increases and decreases of ambient salt concentration respectively. Downstream of the ASE neuron pair are the interneurons AIA, AIB and AIY which interpret signals from sensory neurons and then signals downstream to other interneurons as well as motor neurons to drive behavior. Animals with AIY intact (but AIA and AIB ablated) chemotax towards all salt concentrations in a manner similar to wild type animals with the exception of decreased chemotaxis towards high concentrations. Here, we seek to uncover the mechanisms of memory formation and learning by using calcium imaging to study plasticity of AIY, specifically focusing on changes that occur between the ASE neuron pair and AIY that occur after experiencing differing salt concentrations in the presence of food. At present, we've found that AIY tends to generate sporadic calcium transients which increase in frequency when the animal is given an increasing salt concentration stimulus. These responses to salt concentration increase

were more prominent in animals cultivated at high salt concentrations. Animals where AIA and AIB were ablated tended to have overall weaker AIY responses to salt stimuli. The work presented here will lay the ground work necessary to characterize the changes that occur to AIY at a molecular level that drive the neural plasticity associated with memory and learning.

Disclosures: L. Mabardi: None. **H. Kunitomo:** None. **H. Sato:** None. **Y. Toyoshima:** None. **Y. Iino:** None.

Poster

139. Olfaction: Olfactory Sensory Neuron Development and Function

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 139.04/Y5

Topic: D.05. Olfaction and Taste

Title: A bioinformatic screen identifies conserved genes highly enriched in the *Drosophila* antenna

Authors: *K. MENUZ, P. MOHAPATRA
Physiol. & Neurobio., Univ. of Connecticut, Storrs, CT

Abstract: Insects rely on their olfactory systems to find food and mates, and repellents that target their olfactory systems are widely used to prevent insect-borne diseases. However, relatively little is known about the molecules supporting odor signaling in the adult antenna beyond the identity of the odor receptors themselves. Here, we used a computational approach to identify candidate olfaction related genes conserved in insects. Using *Drosophila melanogaster*, we first carried out an unbiased comparative analysis of gene expression in antenna versus gene expression in five other tissues and in whole bodies at multiple developmental time points. This analysis revealed 196 candidate olfaction related genes, which are each more than ten times as abundant in antennae compared to other tissues or whole bodies. Antennal enrichment was validated by quantitative PCR for a subset of the genes. Functional annotation and enrichment analysis showed that ciliary genes and biotransformation enzymes are particularly abundant amongst the antennal-enriched genes, in addition to expected categories such as odor receptors and odorant binding proteins (OBPs). Comparisons of RNASeq derived gene expression profiles in wild-type, *amos*, and *atonal* antennae revealed the morphological classes of sensilla in which the antennal-enriched genes are expressed. Finally, we identified orthologues for the antennal-enriched genes in *Harpegnathos saltator, Anopheles gambiae, Tribolium castaneum* and *Apis mellifera*, insect species for which antennal gene expression has been previously characterized by RNASeq. Analysis of these orthologues lead us to identify a small number of genes whose antennal expression is broadly conserved. Together, our data have identified over 100 previously unknown antennal-enriched genes, which are promising candidates for genes that play a role in odor signaling in the periphery.

Disclosures: K. Menuz: None. **P. Mohapatra:** None.

Poster

139. Olfaction: Olfactory Sensory Neuron Development and Function

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 139.05/Y6

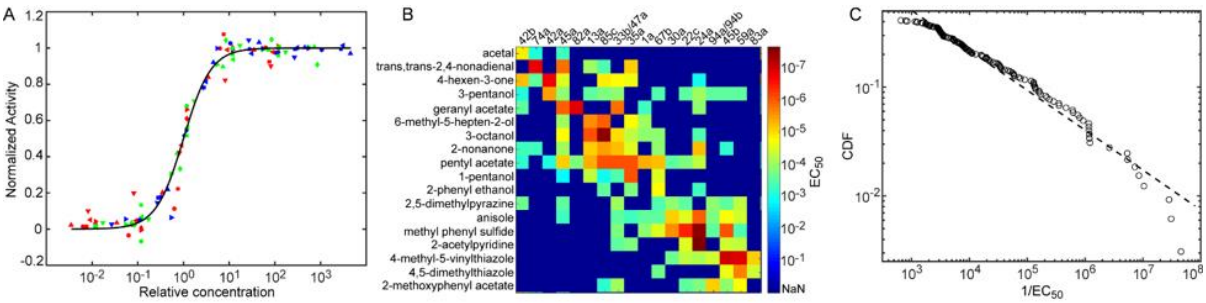
Topic: D.05. Olfaction and Taste

Support: NIH Grant 8DP1GM105383
NIH Grant P01GM103770
NIH Grant F31DC015704

Title: Invariances in a combinatorial olfactory receptor code

Authors: *G. SI, J. KANWAL, Y. HU, C. TABONE, J. BARON, M. BERCK, G. VIGNOUD, A. D. SAMUEL
Harvard Univ., Cambridge, MA

Abstract: Animals can identify an odorant type across a wide range of concentrations, as well as detect changes in concentration for individual odorant type. How olfactory representations are structured to support these functions remains poorly understood. Here, we studied how a full complement of ORNs in the Drosophila larva encodes a broad input space of odorant types and concentrations. We find that dose-response relationships across odorants and ORN types follow the Hill function with shared cooperativity (Figure A) but different activation thresholds (Figure B). These activation thresholds are drawn from a power law statistical distribution (Figure C). A fixed activation function and power law distribution of activation thresholds underlie invariances in the encoding of odorant identity and intensity. Moreover, we find similar temporal response filters of ORNs across odorant types and concentrations. Such uniformity in the temporal filter may allow identity invariant coding in fluctuating or turbulent odor environments. Common patterns in ligand-receptor binding and sensory transduction across olfactory receptors may give rise to these observed invariances in the olfactory combinatorial code. Invariant patterns in the activity responses of individual ORNs and the ORN ensemble may simplify decoding by downstream circuits.



Disclosures: G. Si: None. **J. Kanwal:** None. **Y. Hu:** None. **C. Tabone:** None. **J. Baron:** None. **M. Berck:** None. **G. Vignoud:** None. **A.D. Samuel:** None.

Poster

139. Olfaction: Olfactory Sensory Neuron Development and Function

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 139.06/Y7

Topic: D.05. Olfaction and Taste

Support: SHOU&MSU Marine Joint Research Center Grant A1-0209-15-0806

Title: A single amino acid residue in TM2 determines ligand responsiveness in two highly related sex pheromone receptors of sea lamprey

Authors: *Z. ZHANG[1], Q. ZHANG[3], J. REN[3], T. DEXHEIMER[2], R. R. NEUBIG[2], W. LI[1]
[1]Dept. of Fisheries and Wildlife, [2]Dept. of Pharmacol. and Toxicology, Michigan State Univ., East Lansing, MI; [3]Col. of Fisheries and Life Sci., Shanghai Ocean Univ., Shanghai, China

Abstract: Chemical cues play critical roles in life cycle of sea lamprey (*Petromyzon marinus*), including search of spawning habitats and mates. A bile alcohol, 3-keto petromyzonol sulfate (3kPZS), is a major component of male sex pheromone detected by sea lamprey olfactory system with acute sensitivity and specificity. We identified two highly related odorant receptors, OR320a and OR320b, as cognate receptors for 3kPZS. These two receptors showed 93% identify in amino acids residues and were activated by same set of twenty 3kPZS analogs. However, OR320b displayed weaker responses to all 3kPZS analogues compared to OR320a. By performing functional analysis on a series of site-directed mutants, we identified a single amino acid residue, C79Y in the second transmembrane helix II (TM2) that determines ligand responsiveness. Switching amino acid residue at this position in OR320a and OR320b also switched their ligand responsive properties, indicating that additional residues in TM2, different from the generalized odorant-binding sites in TM3, TM5 and TM6, are also critical for ligand responsiveness in broadly tuned highly related olfactory receptors. Our data identifies receptors

for a steroidal sex pheromone in vertebrates and provides a useful pheromone recognition model in vertebrates.

Disclosures: Z. Zhang: None. **Q. Zhang:** None. **J. Ren:** None. **T. Dexheimer:** None. **R.R. Neubig:** None. **W. Li:** None.

Poster

139. Olfaction: Olfactory Sensory Neuron Development and Function

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 139.07/Y8

Topic: D.05. Olfaction and Taste

Support: NIH/NIDCD: R01DC015784 (JPM)
NIH/NINDS: R21NS104826 (JPM)
Welch Foundation: 1-1934-20170325 (JPM)

Title: Selective and sensitive detection of bile acid information in the mouse vomeronasal organ

Authors: *W. WONG, X. ZHANG, J. CAO, W. I. DOYLE, J. P. MEEKS
Dept. of Neurosci., UT Southwestern, Dallas, TX

Abstract: The rodent accessory olfactory system (AOS) is essential for social and reproductive behaviors, such as mating and territorial aggression. Vomeronasal sensory neurons (VSNs), located in the vomeronasal organ, detect chemosensory cues before relaying the information to the glomerular layer in the accessory olfactory bulb (AOB). Mitral cells (MCs), the main output neuron of the AOB, process this information before relaying it to limbic brain regions. Known ligands for the AOS consist of secretions and excretions from other animals, such as urine, tears, and saliva. We recently discovered that fecal bile acids (BAs) are a novel class of AOS ligands whose potential impact on mammalian social behavior remains largely unexamined. We investigated VSN BA tuning using *ex vivo* volumetric GCaMP6f/s Ca^{2+} imaging in the VNO and the AOB glomerular layer. VSNs showed reliable responses to natural blends of ligands (*e.g.*, mouse feces) and monomolecular BAs. We discovered groups of VSNs that respond to individual BAs at concentrations as low as 300 nM, whereas other VSN groups were broadly tuned to BAs. Furthermore, we identified VSNs that were co-activated by monomolecular BAs and urinary sulfated glucocorticoids, suggesting some VSNs are sensitive to steroid molecules found in different excretions. In the AOB, BA-sensitive VSNs primarily targeted their axons to the anterior AOB, suggesting that BA-sensitive neurons are likely V1R/$G_{\alpha i}$-expressing VSNs. These data collectively illuminate the extent of VSN tuning to novel AOS ligands and improve our understanding of how the AOS discriminates and encodes chemosensory information.

Disclosures: W. Wong: None. **X. Zhang:** None. **J. Cao:** None. **W.I. Doyle:** None. **J.P. Meeks:** None.

Poster

139. Olfaction: Olfactory Sensory Neuron Development and Function

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 139.08/Y9

Topic: D.05. Olfaction and Taste

Support: NSF-IOS-1655113

Title: The chromatographic theory of olfaction a half-century on: Comparisons of simulated odorant sorption patterns with regional electroolfactogram responses in the mouse olfactory epithelium

Authors: E. FITZWATER[1], *D. M. COPPOLA[2], B. A. CRAVEN[3]
[1]Biol., [2]Randolph-Macon Col., Ashland, VA; [3]Mechanical and Nuclear Engin., Pennsylvania State Univ., University Park, PA

Abstract: More than 50 years ago, Mozell drew a comparison between the workings of the nasal mucosa and a chromatography machine, an analogy that crystalized ideas about a mechanism of odor discrimination suggested earlier by his mentor Lord Adrian. This model of olfactory discrimination predicts a relationship between nasal airflow, odor sorption and the distribution of sensory neuron receptive-field types. In the intervening decades, an expansive body of empirical work has accrued supporting the chromatography theory (CT). By contrast, our recent studies in the mouse, comparing computational fluid dynamic (CFD) simulations of airflow with receptor response maps, measured empirically, largely failed to support the most critical predictions of the CT. However, these studies typically employed only one or a few stimulus concentrations and used a limited set of highly-soluble odors, the latter a chemical class about which the CT has its clearest predictions. Here we report results that further test the CT's validity by mapping mouse receptor responses using the electroolfactogram (EOG) and comparing these response maps to CFD simulations of airflow and sorption patterns in the nasal cavity. CFD simulations were performed using airflow velocity estimates comparable to those during active sniffing. The stimulus set used for EOG recordings, unlike previous studies, included a dilution series and odors at the extremes of mucus solubility. Recordings targeted the olfactory epithelium covering the dorsal branch of Endoturbinate II, where CFD simulations reveal marked gradients in the sorption of mucus soluble odors. Results confirm previous observations that, contrary to the CT, EOG response gradients do not correlate with simulated sorption gradients, no matter the concentration or estimated mucus solubility of the stimulus. Importantly, simulations also suggest that above some level of mucus solubility, odor sorption patterns change very little. Thus, our sorption simulation results for the mucus soluble members of our odor set should apply

to any highly mucus soluble odorant. Further, our results reveal a sensitive region on the olfactory epithelium for fatty acids that corresponds to the dorsal-central area, referred to as zone 1, known to contain class I olfactory receptors. Together, our results are incompatible with a key prediction of the CT that there exists an "inherent" pattern of sensory neuron receptive-field types configured to take advantage of odor sorption profiles. However, our results with fatty-acid odorants is consonant with a growing body of evidence that zone 1 is specialized for different functions than the peripheral olfactory epithelium.

Disclosures: E. Fitzwater: None. **D.M. Coppola:** None. **B.A. Craven:** None.

Poster

139. Olfaction: Olfactory Sensory Neuron Development and Function

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 139.09/Y10

Topic: D.05. Olfaction and Taste

Support: NIH Grant DC010381
NIH Grant DC005964

Title: High-throughput optical tools for discovering molecular identity of physiologically-distinct neuronal populations

Authors: *D. LEE[1], T. E. HOLY[2]
[1]Washington Univ. In St. Louis, Saint Louis, MO; [2]Neurosci., Washington Univ. Sch. of Med., Saint Louis, MO

Abstract: Molecular markers have been a powerful tool for labeling and exploring a specific neuronal population. In many cases, however, a single marker corresponds to several, tens, or even hundreds of physiologically-distinguishable cell types. In such cases, there may be incentive to discover more specific markers. One promising approach would be to profile neurons physiologically and then discover genes expressed in a chosen subset of neurons. However, the tools for proceeding from neuronal function to gene expression are less well-developed. In the present study, we report an approach called photoactivated, intersectional physiology sequencing (PIPSeq) to identify genes expressed in physiologically-distinct cell types. In this approach, we record neuronal activity by large-scale calcium imaging, label neurons of interest by photoactivation, and subsequently profile mRNA expression of labeled neurons. We applied PIPseq to the challenge of mapping receptor-ligand pairings among vomeronasal sensory neurons. PIPseq delivered dramatic enrichment of selected neuron types, generating high signal-to-noise sequencing data even of rare (<1%) cell types. Using this approach, we identified the molecular finger prints of the subsets of sensory populations representing overlapping but discriminable chemoreceptive fields.

Disclosures: D. Lee: None. **T.E. Holy:** None.

Poster

139. Olfaction: Olfactory Sensory Neuron Development and Function

Location: SDCC Halls B-H

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Program #/Poster #: 139.10/Y11

Topic: D.05. Olfaction and Taste

Support: Cluster of Excellence and DFG Research Center Nanoscale Microscopy and Molecular Physiology of the Brain (CNMPB); project: B1-9

Title: *In vivo* time-lapse imaging of olfactory sensory neuron birth, differentiation and axogenesis

Authors: *T. OFFNER[1,2], S. J. HAWKINS[3], L. WEISS[3], T. HASSENKLÖVER[3], T. DRESBACH[1,2], I. MANZINI[3,2]
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Abstract: The vertebrate olfactory system has the lifelong capacity to compensate for the loss of olfactory sensory neurons via adult neurogenesis. Additionally to the regular turnover of olfactory sensory neurons for tissue maintenance, the neuronal circuitry is capable of regenerating and rewiring after injury. The bipolar sensory neurons are generated from stem cells in the basal layer of the olfactory mucosa and project their axon via the olfactory nerve to second order neurons in the olfactory bulb. Larval *Xenopus laevis* has proven to be a powerful model to observe olfactory system regeneration after olfactory nerve axotomy on multiple levels of the olfactory system. In this work we present *in vivo* time-lapse imaging of olfactory sensory neuron regeneration after injury, from early progenitor level to the point of axonal pathfinding. Two days after olfactory nerve transection we electroporated progenitor cells of the olfactory epithelium with a genetic probe (LifeAct_P2A_tdTomato) which allowed us to observe cellular morphology and actin cytosceleton dynamics for several days. By expressing LifeAct_P2A_tdTomato under different promotors of genes known to play major roles in vertebrate neuronal development (Pax6, Sox3, NCAM, NbT), we were able to correlate the observed cellular morphologies with the stage in neuronal differentiation. Finally, a combination of sparse cell labelling and immunohistochemistry against those developmental markers complemented our *in vivo* data, providing unprecedented insights into injury-induced neuroregeneration from the single cell to the population level.

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Poster

139. Olfaction: Olfactory Sensory Neuron Development and Function

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 139.11/Y12

Topic: D.05. Olfaction and Taste

Support: Supported by grants from the Stowers Institiute

Title: Regulation of olfactory sensory neuron maturation and axon targeting by non-coding RNA

Authors: *W. XU, Y. WU, A. MORAN, L. MA, R. C. YU
Stowers Inst., Kansas City, MO

Abstract: Noncoding RNAs, including long noncoding RNA (lncRNAs), micro RNAs and circular RNAs, are being recognized to play important roles in regulating nervous system development. We find that in the mammalian main olfactory system, the lncRNA H19 is one of the most abundant transcripts in early embryonic and neonatal olfactory epithelium, but its physiological function is unknown. To examine the role of H19 function in OSN development, we generated transgenic mice that extend the expression of H19 into later stages of development. We find that expression of miR-675, embedded in H19's first exon, is positively correlated with H19 expression in olfactory sensory neurons (OSNs). Ectopic expression of H19 leads to a 10-fold increase of miR-675. Transcriptome analysis has identified the growth-promoting insulin-like growth factor 1 receptor (Igf1r), a known target of miR-675, is downregulated in OSNs from H19 overexpressing mice. We further identify the axon guidance molecules Ephrin-A5 (EfnA5) and Semaphorin-7A (Sema7a) as potential targets for miR675 and both exhibited reduced expression in H19 overexpressing mice. On the other hand, we found that microRNA let-7, which is known to be antagonized by H19, is negatively correlated with H19 expression in OSNs. Interestingly, we observe that overexpression of H19 in mature OSNs results in an increased number of immature OSNs and a reduced number of mature OSNs. Moreover, in mice expressing ectopic H19, OSN axons expressing the same receptor innervate multiple glomeruli in the olfactory bulb. These results suggest that H19 may function through regulating microRNAs during OSN development to influence the development of OSNs and their axon targeting.

Disclosures: W. Xu: None. **Y. Wu:** None. **A. Moran:** None. **L. Ma:** None. **R.C. Yu:** None.

Poster

139. Olfaction: Olfactory Sensory Neuron Development and Function

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Program #/Poster #: 139.12/Y13

Topic: D.05. Olfaction and Taste

Support: JSPS

Title: Differential timing of neurogenesis underlies dorsal-ventral topographic projection of olfactory sensory neurons

Authors: *F. EERDUNFU, N. IHARA, L. BAO, H. TAKEUCHI
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Abstract: Background: The mammalian primary olfactory system has a spatially-ordered projection in which olfactory sensory neurons (OSNs) located in the dorsomedial (DM) and ventrolateral (VL) region of the olfactory epithelium (OE) send their axons to the dorsal and ventral region of the olfactory bulb (OB), respectively. We previously found that OSN axonal projections occur sequentially, from the DM to the VL region of the OE. The differential timing of axonal projections is important for olfactory map formation because early-arriving OSN axons secrete guidance cues at the OB to help navigate late-arriving OSN axons. We hypothesized that the differential timing of axonal projections is regulated by the timing of OSN neurogenesis. To test this idea, we investigated spatiotemporal patterns of OSN neurogenesis during olfactory development. **Methods and Results:** To determine the time of OSN origin, we used two thymidine analogs, BrdU and EdU, which can be incorporated into cells in the S-phase of the cell-cycle. We injected these two analogs at different developmental time points and analyzed distribution patterns of labeled OSNs. We found that OSNs with different dates of origin were differentially distributed in the OE. The majority of OSNs generated at the early stage of development were located in the DM region of the OE, whereas OSNs generated at the later stage of development were preferentially located in the VL region of the OE. **Conclusion:**These results indicate that the number of OSNs is sequentially increased from the DM to the VL axis of the OE. Moreover, the temporal sequence of OSN proliferation correlates with that of axonal extension and emergence of glomerular structures in the OB. Thus, we propose that the timing of OSN neurogenesis regulates that of OSN axonal projection and thereby helps preserve the topographic order of the olfactory glomerular map along the dorsal-ventral axis of the OB.

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Poster

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Program #/Poster #: 139.13/Y14

Topic: D.05. Olfaction and Taste

Support: intramural NIH award to MS

Title: Characterizing odor-elicited response heterogeneity and adaptation in olfactory receptor neurons

Authors: B. KIM, A. KIM, Z. ALDWORTH, *M. A. STOPFER
NICHD, NIH, Bethesda, MD

Abstract: Olfactory receptor neurons (ORNs) have been shown to generate a diverse set of responses to puffs of odors. The goal of this project was to characterize the ORNs' odor-elicited responses to simple, square odor pulses, and to naturalistic plumes of odor, which consist of stochastic sequences of odor pulses. We used a relatively simple model organism, the locust Schistocerca americana. Here, ORNs are found on the antenna within sensilla. We secured a locust to a petri dish, and placed a pulled glass pipette electrode filled with an electrolyte at the base of a sensillum. Because sensilla contain more than one ORN, action potentials detected in the recording were then analyzed using a spike sorting algorithm, allowing us to determine odor-elicited changes in the firing rate of each ORN. We then used hierarchical clustering based on a dissimilarity matrix to categorize the types of responses we observed. We found ORNs generate response motifs of four general types: an excitatory response upon the onset of the odor; an excitatory response delayed from the onset; an inhibitory response during the entire stimulus that recovers back to baseline upon offset; and an inhibitory response during the stimulus followed by an excitatory response upon offset. Furthermore, a given ORN can generate different types of responses to different odors; thus, response type depends upon the odor-ORN combination. We also found that ORNs undergo rapid sensory adaptation to a continuous odor pulse, and to a train of short odor pulses. Responses to a pulsatile stimulus with intervals exceeding 2sec did not show any adaptation, consistent with the antenna's summed activity, measured as an electroantennogram (EAG) by electrodes spanning the antenna. Presented with naturalistic synthetic plumes, ORNs displayed the same response types and time-dependent adaptation elicited by square odor pulses, and showed especially large bursts of activity at the plume's onset and offset. Thus, the timing of the ORN responses appears to play a role in extracting key temporal features in odor information processing. Our results are the first part of a larger project to understand, through experiments and computational models, how the brain extracts information from naturalistic odors.

Disclosures: B. Kim: None. **A. Kim:** None. **Z. Aldworth:** None. **M.A. Stopfer:** None.

Poster

139. Olfaction: Olfactory Sensory Neuron Development and Function

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Program #/Poster #: 139.14/Y15

Topic: D.05. Olfaction and Taste

Support: NIH Grant R01 DC012943

Title: Heterogeneity in olfactory response increases available information in complex odor plumes

Authors: *S. HANEY[1], Z. N. ALDWORTH[3], B. KIM[3], N. RULKOV[2], M. A. STOPFER[3], M. V. BAZHENOV[1]
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Abstract: Animals seeking food or mates often must track odorants through turbulent media that produce large variability in odor concentration over time and space. Thus, natural odor stimuli are often characterized by complex dynamics. Despite the pervasiveness of complex natural stimuli, olfaction is often studied using long, temporally simplistic pulses. How does the olfactory system respond to temporally complex odor plumes, and what components of this system are specialized to process this turbulent input? We address these questions by examining the first stage in the olfactory system, olfactory receptor neurons (ORNs), in the locust - Schistocerca americana. We recorded the responses of ORNs to stimuli that were temporally simple (long odor pulses) or complex (odor plume-like structures), and found that the responses of ORNs clustered into four general groups. We then designed a set of map-based computational models to simulate these response patterns. To test for possible information-processing contributions of this assortment of patterns, we systematically varied the diversity of ORN responses in our computational model from one to many classes, and then determined the response of ORN groups to temporally simple (long pulses) or complex stimuli (plume-like structure). In agreement with previous work we found that ORN diversity allows for better classification of similar odorants. Notably, though, our results also showed that variety in ORN responses was especially helpful for classifying stimuli with temporally complex, plume-like structures. During plume-like stimuli, the variety of adaptation rates observed in ORNs had a synergistic effect with the inter pulse intervals in stimulus structure. We found that this caused different clusters of responsive ORNs were active during different epochs of the complex stimulus and led to greater decorrelation in the response to the initial stimulus and an increase in the available olfactory information from complex plumes. Our study suggests that diversity of the olfactory receptor neuron responses helps animals to respond robustly to specific, salient aspects of a naturalistic stimulus, including temporal features.

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Poster

139. Olfaction: Olfactory Sensory Neuron Development and Function

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 139.15/Y16

Topic: D.05. Olfaction and Taste

Title: Widespread odorant receptor antagonism observed in the peripheral olfactory system

Authors: B. EVANS, J. BRANN, D. RAPS, *B. C. SMITH, M. E. ROGERS
Firmenich, Plainsboro, NJ

Abstract: Antagonism of odorant receptors (ORs) is known to occur and it is tantalizing to suggest that its existence alone might imply a role in olfactory perception. However, little is known about how frequently it occurs and how broadly it acts during the combinatorial encoding of odorant mixtures at the periphery. To begin to investigate this we used a semi-automated Ca^{2+} imaging platform and characterized OR antagonism in populations of dissociated olfactory sensory neurons (OSNs). OSNs were stimulated with binary mixtures of indole and other perfumery ingredients as candidate antagonists and the reduction in indole OSN response due to the other odorant quantified. To compare whether inhibition predominantly occurred with odorants that were structurally similar to indole, the test set included odorants chemically similar to, and diverse from, indole. Strikingly, statistically significant OSN response inhibition was observed in the majority of odor pairs tested; with between 3% and 68% of the indole-responsive OSNs showing >25% reduction in peak height and between 0% and 39% showing >75% reduction. Ingredients from both the set of indole derivatives, and the set of diverse odorants were able to inhibit indole-responsive OSNs with similar strengths and breadths. Furthermore, OSNs were inhibited in a dose-dependent manner and subsets of OSNs affected were highly specific to the odor pair delivered. To confirm that indole was not an unusual odorant in this regard, we further tested 2 other odorants with additional candidate antagonists and found a similar distribution of the breadth and strength of antagonism across odor pairs. We therefore postulate that antagonism is a prevalent feature of peripheral olfactory computation, whose combinatorial nature mirrors that of agonism and which may serve to expand the encoding capacity of the system.

Disclosures: B. Evans: A. Employment/Salary (full or part-time):; Firmenich. **J. Brann:** A. Employment/Salary (full or part-time):; Firmenich. **D. Raps:** A. Employment/Salary (full or part-time):; Firmenich. **B.C. Smith:** A. Employment/Salary (full or part-time):; Firmenich. **M.E. Rogers:** A. Employment/Salary (full or part-time):; Firmenich.

Poster

139. Olfaction: Olfactory Sensory Neuron Development and Function

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 139.16/Y17

Topic: D.05. Olfaction and Taste

Title: Receptor-specific antagonism is prominent in odorant receptor-based combinatorial coding

Authors: *P. PFISTER, B. EVANS, R. ARROYAVE, S. WILLIAMS, M. ROGERS
Cell. Biol., Firmenich, Plainsboro, NJ

Abstract: Little is known about the role of odorant receptor (OR) antagonism in shaping the combinatorial code upon complex odor presentation. Here, we sought to investigate antagonism and its pharmacology using an *in vitro* high-throughput screening platform. We first deorphaned the mouse receptor Olfr740 through single-cell transcriptomics of indole- and skatole-activated olfactory sensory neurons (OSNs). Subsequent phylogenetic analyses revealed the existence of six paralogous receptors sharing over 81% amino acid identity and an additional six members of the closest outgroup ranging between 58% and 63% identity. We first characterized the *in vitro* response profile of a subset of 8 ORs (including all the paralogs and one member of the outgroup (Olfr746) with chemical indole analogs and revealed partially-overlapping but distinct agonist activation profiles. We then used a set of 800 chemically diverse perfumery ingredients to probe their inhibition profiles in the presence of indole and observed distinct receptor-specific inhibition profiles. The number of antagonists per receptor centered around 10% but with highly variable inhibition overlap between receptors. Overall, close to a third of the library exhibited antagonistic properties on at least one receptor. Dose-response IC_{50} curves with select antagonists further confirmed antagonism diversity. In rare cases, compounds displayed opposite pharmacological behavior acting as an antagonist on one receptor and as an agonist on another. Such distinct activation and inhibition profiles between paralogous genes supports the view that OR gene diversification leads to receptive range diversification rather than functional redundancy for both agonism and antagonism. Taken together, we conclude that, even within a family of closely related receptors, antagonism is widespread, likely to play a prominent role in olfactory peripheral computation and may serve to expand the encoding capacity of the system.

Disclosures: P. Pfister: A. Employment/Salary (full or part-time):; Firmenich. **B. Evans:** A. Employment/Salary (full or part-time):; Firmenich. **R. Arroyave:** A. Employment/Salary (full or part-time):; Firmenich. **S. Williams:** A. Employment/Salary (full or part-time):; Firmenich. **M. Rogers:** A. Employment/Salary (full or part-time):; Firmenich.

Poster

139. Olfaction: Olfactory Sensory Neuron Development and Function

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Program #/Poster #: 139.17/Y18

Topic: D.05. Olfaction and Taste

Support: NIH Grant DC014423

Title: Experience-dependent sex-specific variability of olfactory receptor expression in mice

Authors: *A. VIHANI, H. MATSUNAMI
Duke Univ., Durham, NC

Abstract: Using sensory information to guide nervous system development provides an adaptive way to accommodate specific behaviors to specific environments. Here, we investigated this in the context of the olfactory sensory neurons of mice using a combination of RNA-Seq and histology. Comparing the expression of olfactory receptors (ORs) in the olfactory epithelium of virgin male and virgin female mice, we observe a small subset of ORs that are differentially expressed. Examination of the olfactory epithelium *in situ* demonstrates this dimorphism correlates with an alteration in the abundance of olfactory sensory neurons expressing these ORs. Curiously enough, this apparent sexual dimorphism is severely attenuated in breeder mice. Finally, using phosphorylated S6 ribosomal subunit capture combined with RNA-Seq, we have identified potent ligands for some of the sexually dimorphic ORs *in vivo*. Altogether, these observations raise the possibility of an activity-dependent mechanism mediated by sex-specific volatiles to govern the sexually dimorphic expression of specific ORs.

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Poster

140. Auditory Processing: Vocalizations and Natural Sounds

Location: SDCC Halls B-H

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Program #/Poster #: 140.01/Z1

Topic: D.06. Auditory & Vestibular Systems

Support: SNSF PP00P1_157409/1

Title: Hearing noise as voice: How the brain reconstructs voice patterns from acoustic synthetic textures

Authors: *M. STAIB[1,2], S. FRUEHHOLZ[1,2,3]
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Abstract: The perception of human voices activates extended regions of the auditory cortex known as "temporal voice area" (TVA). However, it is unclear whether the observed activity in the TVA represents encoding of the acoustic features that discriminate voices from non-voices, or whether these activation patterns are shared across acoustic domains, such as recorded voices or synthetic sounds. Using a reconstruction model for fMRI data, we demonstrate in 25 human volunteers that the spatial configuration of the activation pattern in primary and higher auditory cortices that discriminates listening to voices from non-voices (target pattern) can be reconstructed with high accuracy from activation patterns elicited by listening to synthetic sounds (reconstruction patterns). The reconstruction model assigns a linear weight to each reconstruction pattern such that the target pattern is fully explained. We found that the linear weights are associated with the acoustic features of the corresponding synthetic sounds across auditory areas. Importantly, in higher auditory areas, the weights can be predicted from each participant's rating of voice-similarity for the synthetic sounds over and above their acoustic features. Using a decoding approach with cross-classification, we then confirm that perceived voice-similarity of recorded and synthetic sounds generalizes across acoustic domains. Our results show that specific activation patterns in the auditory cortex that encode vocal and non-vocal categories represent voice-similarity tracking for sounds across a vast acoustic space. Overall our findings suggest, first, that several auditory areas are involved in estimating voice-similarity across a wide range of acoustic stimuli, and second, that voice-similarity is represented by an invariable activation pattern in higher auditory brain areas.

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Poster

140. Auditory Processing: Vocalizations and Natural Sounds

Location: SDCC Halls B-H

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Program #/Poster #: 140.02/DP06/Z2

Topic: D.06. Auditory & Vestibular Systems

Support: SNSF PP00P1_157409/1

Title: Voice activity detection in the brain: Understanding the spatio-temporal characteristics of voice detection in the auditory cortex during fMRI

Authors: *H. SWANBOROUGH[1,2], M. STAIB[1,2], S. FRUEHHOLZ[1,2,3]
[1]Psychology, Univ. of Zuerich, Zuerich, Switzerland; [2]Neurosci. Ctr. Zuerich, Univ. of Zuerich and ETH Zuerich, Zuerich, Switzerland; [3]3Center for Integrative Human Physiol. (ZIHP), Univ. of Zuerich, Zuerich, Switzerland

Abstract: The ability to perceive speech-in-noise is vital to our ability to effectively communicate. However, despite the substantial body of work regarding speech-in-noise comprehension, there is a lack of understanding of the neurophysiological underpinnings of how the auditory system detects a voice within an auditory scene. This study works to address this gap by investigating the spatio-temporal characteristics of voice-activity detection in the brain, and how the affective quality of a voice may moderate this activity. To this end, the study approaches the auditory cortex(AC) under the assumption that discrete areas are analogous to a computerised voice activity detector, whereby voice-relevant, acoustic features are gradually accumulated from an auditory scene until a binary decision is made. During fMRI acquisition, participants were presented with ten-second samples of speech-in-noise at a continually increasing signal-to-noise ratio, participants responded when they were certain that they had detected voice activity present within the stimulus. Using finite impulse response models, we compared the HRF characteristics in discrete areas of the AC and Amygdala, and how these were moderated by affect. We found evidence that specific populations of neurons in each participant, centred around Heschl's Gyrus, demonstrate a robust response to voice activity detection, but do not show a sustained BOLD response for the duration of voice listening. Additionally, we observed that different affective qualities of vocal stimuli result in differences in the temporal characteristics of the HRF for voice detection, as well observing possible evidence that could be interpreted as accrual of information prior to a perceptual decision being made. The findings from this study possibly indicate that speech-in-noise detection relies on 'voice relevant' acoustics properties reaching a statistical detection threshold, which may then initiate further voice-perception processes.

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Poster

140. Auditory Processing: Vocalizations and Natural Sounds

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Program #/Poster #: 140.03/Z3

Topic: D.06. Auditory & Vestibular Systems

Support: Vontobel Foundation Zurich

Title: Affective whispered voices in the human limbic system: Differences in single-cell firing and local field potentials from the amygdala and hippocampus

Authors: *M. BOBIN[1,3], M. STAIB[1,3], T. FEDELE[4], J. SARNTHEIN[4,3], S. FRUEHHOLZ[1,3,2]
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Abstract: Vocal communication is one of our most versatile and important ways of exchanging a broad variety of information. A speaker's affective state can be reliably decoded by a listener when perceiving the spectral properties of the speech. When perceiving affective vocalizations, a cerebral network including the auditory cortex (AC) and the medial temporal limbic (MTL) system classifies the different acoustic components of affective voices to match stored emotional pattern templates. However, it remains unclear how this neural dynamic identifies the actual emotion when the incoming acoustic signal is degraded. Listening to affective whispered voices is an acoustically sub-optimal situation for a clear comprehension, where the degraded vocal stimuli requires greater functional connectivity between the AC and the MTL. Activity in the MTL, especially when involving the hippocampus (HPC), should reflect an intensive retrieval from long-term memory information (e.g. past experiences, prototypes) in presence of affective voices reduced in acoustical quality. If so, providing this extra contextual memory association would require an acute engagement within the MTL of the bidirectional connected amygdala (AMY) and HPC. We recorded neural activity from nine drug-refractory epileptic patients, implanted with intracranial electrodes in the MTL for clinical purposes, recorded during an emotion identification task on normal and whispered affective voices. Neuronal spikes data and local field potential (LFP) activity corresponding to AMY or HPC channels were extracted and filtered. Spiking analyses revealed a higher proportion of HPC neurons firing following the onset of whispered voices, in comparison to voiced trials, while affective cues elicited significantly more AMY neuronal firing compared to the neutral condition. Furthermore, an increased coupling in the amplitude of LFP for the whispering condition was found between the AMY and HPC. For affective whispered voices, the neuronal firing from certain parts of the MTL was phase-locked to oscillations in the theta and the gamma bands. The combination from LFP and

single-neuron spiking activity thus reflects an increased cooperation between the AMY and the HPC for decoding emotions from whispered voices, where the interpretation of an affective state is more demanding.

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Poster

140. Auditory Processing: Vocalizations and Natural Sounds

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Topic: D.06. Auditory & Vestibular Systems

Support: SNSF PP00P1_157409/1

Title: Neural competition in auditory decoding of own- and other-vocalizations

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Abstract: Listening to the voices of other individuals (other-vocalizations) was reported to elicit increased activity in different neural systems, such as the auditory cortex (AC). Reduced activity in the AC was shown for speakers listening to their own voice while producing vocal utterances (own-vocalizations). Listening to own- and other-vocalizations while speaking could therefore lead to contrary effects on the neural auditory system. Yet little is known about the neural dynamics of this auditory cortical competition between processing own- and other vocalizations. In this experiment we tested how this competition is resolved in the AC.We conducted an fMRI study using human volunteers to investigate brain responses to own- and other-vocalizations being presented, first, in an active speaking task and, second, in a passive listening task. For the active speaking task participants were instructed to vocalize simple vowels that were fed back in real time to one ear (either left or right) while simultaneously being presented with a vocalization of another person to the same (same ear condition) or the other ear (other ear condition). In the passive listening task participants were presented with voice recordings of their own voice and the voice of another person again presented on the same or on separate ears. Our findings did not show that activations by other voices in the auditory cortex are significantly reduced by actively speaking, as overall cortex activation was higher in the active task than the passive task and similar activations were found in the active task in primary and higher auditory areas for own and other vocalizations, regardless of their lateralization. There was, however, a linear decrease

in the passive task when looking at the overall activation in AC in trials where two voices were presented to the same ear. When comparing active and passive tasks, Heschl's gyrus was shown to be persistently more activated during active trials. The findings from this study possibly indicate that cortical competition by own and other vocalizations is not resolved by suppressing the speaker's voice, nor by a simple additive process. Instead, in the active condition the own voice seems to be accounted for before neural competition can occur, which could be explained by a precise forward model modulating the auditory cortical response to self-generated speech.

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Poster

140. Auditory Processing: Vocalizations and Natural Sounds

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Topic: D.06. Auditory & Vestibular Systems

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Title: Decoding of phonemes in continuous speech from fMRI response patterns

Authors: *J. ERB[1,2], D. DÜWEL[1], G. VALENTE[1], F. DE MARTINO[1], E. FORMISANO[1]
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Abstract: Speech comprehension entails the extraction of linguistic features from the acoustic signal. Phonemes are thought to be the first level of abstraction from the acoustic information. How is phonemic information encoded in the cortex? Here, we used an open high-resolution 7T-fMRI data set by Hanke et al. (2014, 'study forrest') where humans listened to a continuous, naturalistic audio movie. We used multivariate regression to reveal the representation of German phoneme classes in the auditory cortex. Results indicate that fMRI responses to the temporal distribution of phoneme classes in continuous speech can be decoded with a mean accuracy of 65% ($p < 0.001$, permutation test). Maps of the most discriminative voxels revealed a distinct spatial distribution across tonotopic fields for different phoneme classes. Thus, we show the feasibility of multivariate linear regression to decode the phonemic information from fMRI responses to continuous, naturalistic speech.

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Poster

140. Auditory Processing: Vocalizations and Natural Sounds

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Topic: D.06. Auditory & Vestibular Systems

Support: ERC Consolidator Grant, LS5, ERC-2014-CoG

Title: The role of the left ventral medial geniculate body in speech recognition

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Abstract: The left human auditory thalamus, i.e. the left medial geniculate body (MGB) is top-down modulated by tasks involving speech recognition in contrast to control tasks on the same stimuli [1, 2]. The amount of this task-dependent modulation positively correlates with speech recognition performance [1]. It is to-date unknown which of the three MGB subdivisions (ventral, medial, dorsal) is modulated by speech tasks. The distinction is important, as these subdivisions have very different physiological response properties. Here we hypothesized, that the task-dependent modulation of the left MGB for speech recognition is present in the ventral part, which belongs to the lemniscal auditory pathway and is regarded as the first order thalamic nucleus. Using ultra-high field 7 T fMRI in 33 human participants, we first identified the ventral MGB based on its tonotopical organization and relative spatial location [3]. Within this region, we found a significant positive correlation of the amount of task-dependent modulation (speech vs. speaker task) and the speech recognition performance across participants. The non-tonotopic subdivisions showed no significant response differences between the two experimental tasks nor a correlation of task-dependent modulation with speech recognition performance. These results show that the task-dependent modulation of the MGB is present in the lemniscal subdivision and that the task-dependent modulation of this subdivision plays a role in speech recognition.
[1] von Kriegstein, K., et al. (2008). Curr Biol.
[2] Díaz, B., et al. (2012). PNAS
[3] Moerel, M., et al. (2015). Sci Rep.

Disclosures: P.G. Mihai: None. **M. Moerel:** None. **F. De Martino:** None. **R. Trampel:** None. **S. Kiebel:** None. **K. von Kriegstein:** None.

Poster

140. Auditory Processing: Vocalizations and Natural Sounds

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 140.07/Z7

Topic: D.06. Auditory & Vestibular Systems

Support: NIDCD, DC014279

Title: Auditory attention decoding: Using ECoG to determine the anatomical locations and neural frequency bands that contribute

Authors: *J. A. O'SULLIVAN[1], J. L. HERRERO[2], E. H. SMITH[3], S. A. SHETH[4], G. M. MCKHANN[3], A. D. MEHTA[2], N. MESGARANI[1]
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Abstract: Decoding an attended speaker from neural recordings (termed auditory attention decoding; AAD) has many applications. The most pertinent is the development of a cognitively controlled hearing aid that can automatically track and amplify an attended speaker. Such devices will likely be limited to either non-invasive or minimally invasive neural recordings. Non-invasive recordings such as electroencephalography (EEG) can typically only record from low frequency (LF; < 50Hz) neural data, and have relatively poor spatial resolution. However, multiple electrodes can be used to target cortical areas using source-localization signal processing strategies. Minimally invasive approaches can place a restricted number of electrodes over specific cortical areas, and can record high frequency (HF; <200Hz) neural data. In both cases, knowledge of the anatomical locations and neural frequency bands that contribute to AAD is crucial.
To investigate, we used an invasive recording methodology known as electrocorticography (ECoG) that can record both LF and HF neural data, and can also localize neural activity to within ~3mm from both deep and surface brain regions, spanning the full extent of auditory cortex. We show that both LF and HF data, as well as deep and surface regions, can be used to decode attention. However, we found a dichotomy between the combination of frequency band and anatomical location that could be used: when using HF data, the anatomical region that produced the most robust encoding of attended speech was superior temporal gyrus (STG; a surface brain region). Conversely, LF data was the best at decoding attention in Heschl's gyrus (HG; a deep brain region). Both of these combinations (LF data in HG, and HF data in STG) provided similar results in terms of decoding speed and accuracy. These results provide the first extensive exploration of the neural frequency bands and anatomical locations that contribute to AAD, and will inform future work on the development of cognitively controlled hearing aids.

Disclosures: J.L. Herrero: None. **E.H. Smith:** None. **S.A. Sheth:** None. **G.M. McKhann:** None. **A.D. Mehta:** None. **N. Mesgarani:** None.

Poster

140. Auditory Processing: Vocalizations and Natural Sounds

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Program #/Poster #: 140.08/Z8

Topic: D.06. Auditory & Vestibular Systems

Support: Wellcome Trust DBT India Alliance Fellowship to SB
IIT Kharagpur SRIC Challenge Grant to SB
DBT JRF fellowship to Y. M S

Title: Altered progress in developmental structure of pup isolation calls in mouse models of ASDs

Authors: *Y. M S[1], S. AGARWALLA[1], S. BANDYOPADHYAY[2]
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Abstract: Autism spectrum disorders (ASDs) are neurodevelopmental disorders affecting social interactions, communication and behavior. Mouse models (genetic and in utero exposure of VPA through the mother) being most commonly used for studying ASDs. Lack or hindered progress and/or onset of vocalizations is the hallmark of ASDs. Mouse pups emit isolation call (PICs) sequences in the ultrasonic range when separated from their nest. PICs and mouse vocalizations are of communicative significance and in certain respects analogous to speech behavior in humans, which is considered to be the key identifier among many other characters manifested in ASDs. Along with many other models, like 16p11.2del, used for autism studies, intra-peritoneal injection of Valproic acid (VPA) in pregnant mice during the critical period of E10.5-E11.5 is also known to cause ASD associated behavior in the pups of the exposed female. In the PICs, there are significant changes in call features like call rate, call duration and peak frequency between the control (WT; C57Bl6) and ASD groups and how they change over age (P5-P13) within groups. The probability distributions of different syllable types in the PICs are different based on Kullback Leibler divergence over ages. While the distributions converge to a particular pattern in case of the control group, they are divergent in case of ASD model mouse pups showing lack of formation of convergent sets and proportions of syllables over age. Further, we observe that the vocalization sequence patterns change over developmental ages from P5 to P13 in the WT group and also in the VPA treated ASD group. To understand the sequence of patterns generated and how they evolve over age, we consider the transition probabilities between pairs of different types of syllables. The above analysis of syllable to syllable transition probabilities is a step towards analyzing structure in the natural sequences of PICs generated. Call structure determined as above, varies over ages within groups and between the groups and the pattern of

changes over P5 to P13 are different between WT and ASD model mice. We further analyze longer term dependence in the PICs beyond single syllable to syllable transitions using mutual information between starting syllables of sequences and subsequent syllables at different positions. Our study paves the way for understanding the typical modification of vocalization patterns with development in the normal mice and their corresponding aberrations in ASD models. Nature of the changes observed between normal and ASD model groups provide pointers to framing hypotheses regarding underlying mechanisms of vocalization production and alteration in ASDs.

Disclosures: Y. M s: None. **S. Agarwalla:** None. **S. Bandyopadhyay:** None.

Poster

140. Auditory Processing: Vocalizations and Natural Sounds

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 140.09/Z9

Topic: D.06. Auditory & Vestibular Systems

Title: Temporal tuning system for acoustic factors in female avian auditory cortex

Authors: *M. INDA, K. HOTTA, K. OKA
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Abstract: Sound discriminability is a key function for many animals to establish sound communication. The zebra finch (*Taeniopygia guttata*) is one of social songbirds, and it uses voices for sound communication in natural environment; males individually sing their own songs, and females select their partners who sing prefer song. The auditory perception and memorization in females are precious factor, the fundamental neural function of encoding for acoustic factors is, however, not well known. Thus we investigated neural encoding mechanism of temporal structures of songs in avian higher auditory regions caudomedial mesopallium (CMM) and caudomedial nidopallium (NCM), which are considered that have a function of song discriminability and memorization of mate song. Because it is little known about neural encoding of temporal acoustic factors in both regions, we tried to investigate the relationship between neural activities and temporal structures of acoustic factors. Auditory neuronal activities from CMM and NCM neurons to song stimuli presentation were recorded and analyzed.First, we measured the latency of neural activities between its onset and song presentation because we assumed that responding time of neurons to song were delayed from actual stimulus onset. Distributions of first spike latencies in both CMM and NCM regions indicated sharp peak in range on 20 to 40 ms. Furthermore, cumulative frequency of first spike latencies in CMM indicated shorter latency than one of NCM, and median value in CMM also shorter than one of NCM. This result suggested that first activating time to sound stimulus in CMM and NCM neurons is slightly different.Second, to evaluate temporal neural coding of these acoustic factors,

we calculated Time Series Correlation (TSC) as a novel index of relationship between neural activities and acoustic factors. TSC could simultaneously evaluate positive and negative correlation intermixing. TSCs of three acoustic factors, Amplitude, Mean Frequency, and Entropy, indicated significantly higher than other four acoustic factors in both CMM and NCM regions. Our results suggested that while responding timing to song stimuli was different between CMM and NCM, these cells encode similar temporal acoustic factors.

Disclosures: M. Inda: None. **K. Hotta:** None. **K. Oka:** None.

Poster

140. Auditory Processing: Vocalizations and Natural Sounds

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Program #/Poster #: 140.10/Z10

Topic: D.06. Auditory & Vestibular Systems

Support: National Coordination of Superior Level Staff Improvement (CAPES)
Ministério da Educação (MEC)
Santos Dumont Institute (ISD)

Title: Processing of simple and complex sounds in the marmoset's (callithrix jacchus) auditory anteroventral pathway

Authors: *J. AVILA-SOUZA[1], E. B. JACOBI[2], F. A. ARAUJO[2], J. F. R. NETO[2], A. S. C. PERES[2], R. C. MOIOLI[2], M. F. P. ARAUJO[2]
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Abstract: The common marmoset (*Callithrix jacchus*) is a small, highly vocal New World monkey, that lives within a relatively complex social structure, placing them as interesting models to study auditory system functioning, communication and prosocial behaviors. In this work, a 32 microelectrode array was implanted in the left hemisphere of a female marmoset targeting the following brain regions: medial geniculate complex of the thalamus (MGCv), primary auditory cortex (A1), rostral-temporal area (RT), rostral parabelt area (RPB), lateral nucleus of the amygdala (AMyLa) and ventrolateral area of prefrontal cortex (VLPC). Spike and local field potentials (LFP) were recorded simultaneously while the animal was fully awake and freely moving on the experimental chamber. During the recording sessions, the animal was submitted to a passive listening protocol with four distinct categories of auditory stimuli: natural (recorded) vocalizations, artificial vocalizations, pure tones and white noise. Regarding spike activity, we recorded 31 single-units, from which 23 (74,2%) were classified as responsive during the presentation of at least one stimuli category. 6 units were modulated by all four stimuli categories, 5 units were responsive to three categories, 6 units were responsive to two

categories, and 6 to only one category. In addition, the presentation of each stimuli category induced specific modulations in the LFP gamma frequency range (30-110 Hz) in all recorded regions. Significant modulations in the beta frequency range (10-30 Hz) were also found in VLPC and AMyLa. Furthermore, the presentation of natural and artificial vocalizations induced a significant decrease in coherence in gamma band oscillations between A1 and RT and between these primary auditory areas and MCGv, RPB, AMyLa and VLPC. On the other hand, the presentation of pure tones and white noise induced significant changes in coherence in theta (4-8 Hz), alpha (8-13 Hz) and beta band oscillations between A1 and MGCv, RT and RPB. For the LFP analysis we considered as significant values that were higher than baseline mean plus two times its standard deviation. The results indicate that the units modulation and oscillations induced by a given stimulus may respond differently in the coding process of simple and complex acoustic characteristics and be involved in the auditory perception and discrimination of the auditory object. In general, this project contributes to the characterization of the neural mechanisms involved in the auditory process and discuss aspects of animal communication and perception in awaken freely behaving primates.

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Poster

140. Auditory Processing: Vocalizations and Natural Sounds

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Program #/Poster #: 140.11/Z11

Topic: D.06. Auditory & Vestibular Systems

Support: SNSF PP00P1_157409/1

Title: Neural voice decoding in the primate auditory cortex is task-dependent

Authors: *S. FRUEHHOLZ[1], P. DIMANOVA[2]
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Abstract: The mammalian and especially the primate brain has an evolved auditory cortical system for voice processing which is located in the superior temporal cortex (STC) and referred to as “temporal voice area” (TVA). The TVA was so far found in human and non-human primates as well as in dogs. The TVA shows higher activity to vocal compared to other nonvocal sounds and has been assumed to be a cortical area with consistent and uniform voice-sensitivity across species. However, given its large cortical extension covering many areas in the primary, secondary, and high-level auditory cortex, we hypothesized that TVA is composed of functional subareas that decode voice information dependent on the current task requirements rather than representing a uniform and state-independent functional area. We, therefore, used functional

magnetic resonance imaging while recording brain response in human volunteers that performed a complex cognitive (i.e. sound classification) or a simple acoustic task (i.e. intensity judgments) while listening to vocal and nonvocal sounds. We hypothesized that different tasks while listening to vocal sounds will elicit differential activity and connectivity of high-level and low-level auditory regions. We found that simple acoustic decisions on vocal compared to nonvocal sounds elicited higher activity in region Te1.0 of the primary auditory cortex (AC), while the complex sounds classification task elicited the highest activity in region Te3 of the higher-level AC. Activity in these regions correlated with the decision drift rate estimated with a standard drift-diffusion model on the behavioral data. Furthermore, while we found functional connectivity between bilateral auditory cortices for the simple decision task, especially left Te3 showed functional connectivity to bilateral inferior frontal cortices during the complex decision task. Taken together, rather than being a uniform functional area the present data highlight the notion that the TVA is a multi-functional and state-dependent cortical area including functional subareas and connectivity patterns that decode important voice and sound information that is most relevant for the current task.

Disclosures: S. Fruehholz: None. **P. Dimanova:** None.

Poster

140. Auditory Processing: Vocalizations and Natural Sounds

Location: SDCC Halls B-H

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Program #/Poster #: 140.12/Z12

Topic: D.06. Auditory & Vestibular Systems

Support: NIH R01 DC012087

Title: Contextual effects on vocal signal processing in primate prefrontal cortex neurons

Authors: *V. JOVANOVIC[1], C. T. MILLER[2]
[1]Neurosciences Grad. Program, [2]Cortical Systems and Behavior Lab., Univ. of California San Diego, La Jolla, CA

Abstract: Vocal communication is characterized by the active, reciprocal exchanges of vocalizations between freely-moving conspecifics . However, most prior studies of vocalization processing have involved traditional, head-restrained subjects that are either passively listening to conspecifics calls or conditioned to respond to these vocal signals. Here we examined the responses of prefrontal cortex neurons in marmoset monkeys to vocalizations across several behavioral contexts - ranging from more traditional head-restrained contexts to freely-moving subjects engaged in active communication. Specifically, we were interested in testing whether data recorded in more traditional contexts was predictive of the pattern of neural responses observed to the same vocalization stimuli during natural communication. Subjects were

presented with vocalizations and various white noise stimuli at a consistent inter-stimulus interval in test sessions comprising head-restrained and freely-moving passive-listening contexts. The same neurons were also recorded in a third active communication context as subjects engaged in reciprocal exchanges of phee calls, a behavior known as antiphonal conversations. Furthermore, we recorded head position of the marmosets in freely-moving conditions and tested head direction in head-restrained contexts in order to determine whether the relative position to the speaker was a key source of variance on neural responses. Analysis have shown that a population of neurons in ventrolateral prefrontal cortex is responsive to acoustic stimuli - including vocalizations - while subjects are head-restrained. However, the same neurons exhibit little to no response to the identical vocalization stimuli while animals are engaged in active communication. This suggests that prefrontal cortex is not only highly affected by behavioral context and that elucidating its role in natural communication likely necessitates studying within that context.

Disclosures: V. Jovanovic: None. **C.T. Miller:** None.

Poster

140. Auditory Processing: Vocalizations and Natural Sounds

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Program #/Poster #: 140.13/Z13

Topic: D.06. Auditory & Vestibular Systems

Support: NSERC Grant

Title: The influence of noise on the spiking activity of inferior colliculus neurons under different stimulus levels and SNRs

Authors: M. HOSSEINI[1], G. RODRIGUEZ[2], H. GUO[2], H. H. LIM[2], *E. PLOURDE[1]
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Abstract: The auditory system has an impressive ability to discriminate relevant information in the presence of a noisy background. However, the processing of noise in the ascending auditory pathway, which leads to this ability, is not well understood. The objective of this research is to study the processing of noisy vocalisations in the central nucleus of the inferior colliculus (ICC). In particular, we are interested in identifying how different stimulus levels, SNRs as well as noise types affect the neural activity of ICC neurons. To do so, we proposed a novel approach that models the influence of noise on the spiking activity of neurons using a modified generalized linear model (GLM). We extracted from this model a metric, CIF_{noise}, which takes a value of 1 if the noise does not affect the spiking activity of a given neuron, and a value higher or lower than 1 if the noise respectively increases or decreases the activity of this neuron.

Multiunit responses from the ICC of three guinea pigs were recorded using a 32-electrode probe following the presentation of 2 different vocalizations corrupted with non-stationary and stationary noises. We used stimulus intensity levels of 55 dB and 65 dB and 2 signal to noise ratios (SNR), 5 dB and 15 dB. Figure 1 presents the distribution of CIF_{noise} values for the different conditions studied. Surprisingly, the difference between the contribution of noise to the spiking activity of a given neuron when using either a 55 dB or a 65 dB intensity level is not statistically significant ($p > 0.1$). Moreover, the contribution of non-stationary noise to the spiking activity of ICC neurons is independent of the SNR. However, the contribution of stationary noise is significantly different between the 5 dB SNR and 15dB SNR cases ($p < 0.009$). In fact, while stationary noise does not contribute to the spiking activity for a SNR of 15 dB, it does so for 5 dB. Therefore, noise influences the spiking activity of ICC neurons, however, this influence is independent of the stimulus intensity for both stationary and non-stationary noises but dependant on the SNR level for the stationary noise case.

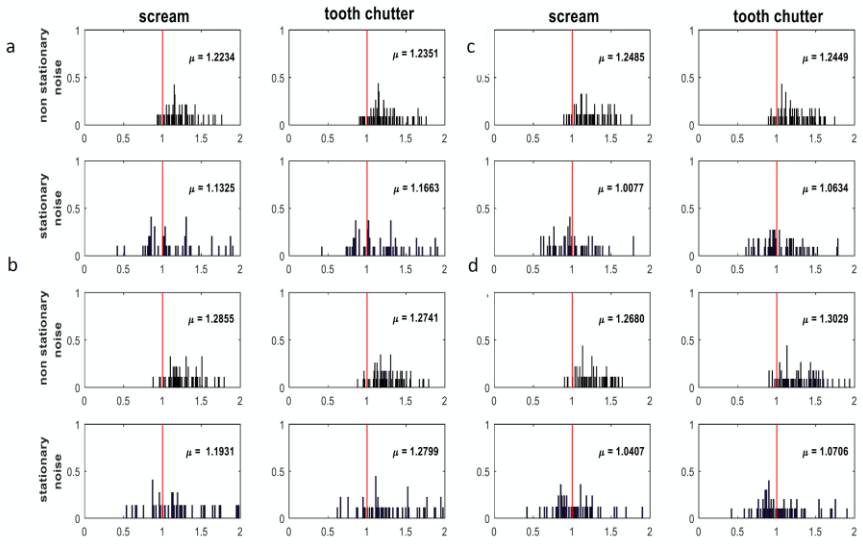

Figure 1. Distribution of CIF_{noise} values for different stimulus intensity levels and SNRs, respectively a) 55 dB and 5 dB, b) 65 dB and 5dB, c) 55 dB and 15 dB, d) 65 dB and 15 dB. For each stimulus level / SNR pair, we present results for both stationary and non-stationary noises as well as two vocalisations, named scream and tooth chutter. Only responsive neurons were considered ($n = 53$ for scream; $n = 69$ for tooth chutter), μ corresponds to the mean CIF_{noise} value for each case. The vertical red line corresponds to a CIF_{noise} value of 1 indicating no contribution of the noise to the spiking activity.

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Poster

140. Auditory Processing: Vocalizations and Natural Sounds

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Program #/Poster #: 140.14/Z14

Topic: D.06. Auditory & Vestibular Systems

Support: Genentech Foundation Fellowship
George A. & Marjorie H. Anderson Fellowship

Title: A candidate pathway for multisensory integration in maternal retrieval behavior

Authors: *A. C. NOWLAN, C. C. KELAHAN, S. D. SHEA
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Abstract: Timed developmental programs and early life experiences interact to define critical periods that result in long-lasting changes to neural circuitry. Landmark life events in adulthood, such as the birth of offspring, can also trigger dramatic brain changes, but the mechanisms of these changes are poorly understood. In mice, the onset of maternal care stimulated by prolonged pup exposure coincides with dramatic changes in circuitry and neuronal responses in the auditory cortex (AC). These changes are suggested to improve encoding and detection of ultrasonic distress vocalizations (USVs) emitted by lonely pups asking to be returned to the safety of their nest. In addition to vocal signals, this behavior depends heavily on olfactory cues from the pups. Surprisingly, these odors modulate auditory processing by mothers, however the functional significance of this modulation is not well understood. One possibility is that pup odors serve as a contextual cue that elevates the behavioral significance of vocalizations, yet little is known about the neural pathways that integrate these two senses. It is also possible that olfaction and audition conspire to trigger long-term synaptic modification in the AC, such that mothers are more attentive to USVs. Previous work from our lab has shown that surrogate mothers, who have acquired maternal behavior through sensory experience, exhibit a transient change in cortical inhibition, which may be indicative of heightened network plasticity. Here we report a novel projection from the basal amygdala (BA) to the AC, which appears to be well positioned to convey pup odors to the AC from the olfactory amygdala. We find that the BA is indeed active during pup retrieval behavior by immunostaining for c-fos as a marker of recent neural activity. We complemented these results by implementing fiber photometry measurement of calcium signals to observe the temporal dynamics of basal amygdala activity during discrete elements of this behavior. Interestingly, the BA exhibited consistently elevated activity during olfactory investigation, suggesting that it may provide the AC with access to odor information parental behavior. We hypothesize that olfactory pup cues may stimulate a window of heightened plasticity within the AC, enhancing cortical representations of USVs. If so, this would constitute a bottom-up mechanism by which olfaction shapes auditory representations to drive appropriate social behavior.

Disclosures: A.C. Nowlan: None. **C.C. Kelahan:** None. **S.D. Shea:** None.

Poster

140. Auditory Processing: Vocalizations and Natural Sounds

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Topic: D.06. Auditory & Vestibular Systems

Support: MEXT KAKENHI 17H05754
MEXT KAKENHI 18H02531

Title: Morphological identification of zebra finch auditory cortical neurons for parallel information processing in song learning

Authors: M. ARAKI, *Y. YAZAKI-SUGIYAMA
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Abstract: Like humans learn to speak, songbirds learn to sing by listening to conspecific adult vocalizations during development. Zebra finch is one type of songbird in which juvenile males develop their own unique song while maintaining species specific characteristics. Recently, we reported that zebra finch juveniles, raised by foster parents of another species, learned the acoustic morphology of song elements, but did not learn the temporal silent gap patterns from the foster fathers' songs. We further found two subsets of neurons in the primary auditory area carrying complementary information about zebra finch songs. One group of neurons respond to song morphology (LF neurons) and the other responds to temporal gap patterns within songs (HF neurons). Those suggest parallel information processing of sound morphology and temporal gap pattern of song via two distinct group of neurons. This enables zebra finches to develop songs that are simultaneously unique yet species-specific (Araki et al., 2016). Here we tried to morphologically identify these two classes of neurons in zebra finch auditory cortex by tissue clearing with X-CLARITY and volumetric imaging. We expressed two different fluorescent proteins in a cell type specific manner by injecting two different adeno-associated virus (AAV) vectors, carrying either pan-neuronal promotor human synapsin (hSyn) or broad range of inhibitory neuron specific promotor, mDlx (Wilson et al., 2017). We found there was little overlap of the two different fluorescent proteins expressed by hSyn and mDlx promotor, suggesting fluorescence protein expressing neurons under hSyn promotor were putative excitatory neurons. These putative excitatory neurons were further classified into three clusters based on morphological characteristics. We also analyzed the morphology of the neurons with intracellular labeling after electrophysiological identification of HF like auditory responses. We found that morphological characteristics of these intracellularly labeled HF neurons fell into one of three clusters, identified with volumetric imaging. Future work aims to determine the neuronal circuit connectivity that enables zebra finches to parallel process information of individual variety and species identity.

Disclosures: M. Araki: None. **Y. Yazaki-Sugiyama:** None.

Poster

140. Auditory Processing: Vocalizations and Natural Sounds

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Program #/Poster #: 140.16/Z16

Topic: D.06. Auditory & Vestibular Systems

Support: LO591506

Title: Ultrasonic vocalizations of the Wistar Rat during its behavioral activity

Authors: *D. PEREZ HERNANDEZ[1], O. LARA GARCIA[2], M. A. LARA GARCIA[1], Y. CRUZ[4], P. PACHECO[5,3]
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Abstract: Several mammalian species produce ultrasonic sounds. Past studies had described that rodents, such as rats produce vocalizations exclusively compound of 22 and/or 50 KHz of frequency, depending of its displayed behavior. However, this is not entirely accurate. In this study, an ultrasonic device model Mini-3 Detector (Ultra Sound Advice, Inc; UK) which tuning range covers frequencies from 15 to 160 KHz was used for recording vocalizations during olfactory response, social interaction and when playing or fighting behaviors. Rats used were young females or males (40-50 days-old) and old females or males (at least 1 year-old) housed in large standard rodents cages with access to food and water ad libitum. Preliminary results show that olfactory response behavior produces vocalizations ranging from 20 to 32 KHz in young, while from 28 to 32 KHz in old rats. Social interaction behavior produces vocalizations from 42 to 52 KHz in young, and from 43 to 55 KHz in old animals. Play or fight behavior produces wide range vocalizations from 60 to 80 KHz in young, and from 60 to 100 KHz in old animals. There were no sex differences in these behaviors. Present observations show that the vocalizations emitted by the rats are in a wider range of frequencies; moreover, ultrasonic vocalizations in old and young rats are more complex than previously described.

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Poster

140. Auditory Processing: Vocalizations and Natural Sounds

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Topic: D.06. Auditory & Vestibular Systems

Support: NIH/NIDCD Grant K08-DC014299.

Title: Front-temporal cortical interactions during vocal production in marmoset monkeys

Authors: *J. TSUNADA, S. ELIADES
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Abstract: Vocal production, including human speech, is a sensory-motor process that requires self-monitoring of produced vocalizations to correct errors between intended and actual outputs. Recent evidence has demonstrated a suppression of auditory cortex during vocal production, a sensory-motor process that is theorized to involve efference copy signals originating in frontal cortical areas. However, whether or not there is communication between frontal or motor areas and auditory cortex during vocalization is unknown. Here, we simultaneously recorded neural activity from both auditory and frontal cortices of marmoset monkeys while marmosets produced self-initiated vocalizations. We found modulations of neural activity in both brain areas immediately before and during vocal production. Interestingly, theta-band activity, thought to be involved in coordinating neural activities, was observed to increase in both brain areas immediately prior to the onset of vocal production. We further tested the timing relationship between activity in auditory and frontal cortex. We found a subset pairs of recording sites with temporally covarying activities, including frontal activation preceding auditory just prior to vocal production, followed by auditory activation that preceded frontal once vocalization began. These results suggest communication between cortical areas during vocal production, with frontal-auditory pre-vocal signals that may reflect preparatory activity, and auditory-frontal signals that may represent self-monitoring of vocal feedback. These different neural interactions between auditory and frontal cortices may underlie mechanisms to calculate and correct for errors between intended and actual vocal outputs during vocal production.

Disclosures: J. Tsunada: None. **S. Eliades:** None.

Poster

140. Auditory Processing: Vocalizations and Natural Sounds

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Topic: D.06. Auditory & Vestibular Systems

Support: NWO Grant 024.001.006

Title: More than motor control: FoxP1 is implicated in feedback based behaviour

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Abstract: Speech and language related phenotypes due to mutations in genes coding for transcription factors of the FoxP family (FoxP1 and FoxP2) have led to studies on these conserved proteins and their functions for vocal learning. In consequence, neural expression of FoxPs has been linked to vocal production learning in humans and birds.Bird song learning is considered the most similar animal analogue to speech acquisition which makes songbirds like the zebra finch (*Taeniopygia guttata*) a well-suited model to study molecular mechanisms of vocal learning. In songbirds, FoxP1 and FoxP2 are expressed in brain regions associated with vocal production and perception.
Female zebra finches do not sing, but establish song preferences and express FoxP 1 and FoxP2 in patterns similar to those seen in male brains. Consistent with prior reports, we confirmed FoxP1 expression in juvenile and adult female zebra finches in HVC and the caudomedial mesopallium (CMM), brain areas which have been linked to perception of auditory stimuli and recognition of previously heard song. We therefore hypothesised that FoxP1 might be involved in memory and auditory perception in these areas.
To test this, we injected lentiviral constructs to knock down FoxP1 expression in HVC or CMM of juvenile and adult female zebra finches. We studied the impact of these knockdowns on behaviours requiring auditory discrimination and memory in preference tests and Go/Nogo tests. Next, we dissected the target nuclei and performed RNA sequencing to identify transcriptional effects of the local FoxP1 knockdown.
Although all treatment groups favoured their tutor's song, adult individuals with a FoxP1 knockdown in HVC did so less than matched controls. Additionally, the knockdown birds requested fewer song stimuli by pushing buttons with their beaks than controls. During Go/Nogo tests, birds were trained to discriminate different song examples and subsequently categorise derived stimuli. Birds which received a FoxP1 knockdown in CMM took longer to respond to

novel stimuli. Preliminary analyses of differential gene expression suggest that glutamatergic receptors like GRIN1 may be regulated by FoxP1.
In summary, our results indicate that FoxP1 is relevant for auditory processing and feedback perception. Given that perception and processing of stimuli are essential for vocal production learning, future studies should inform the link between perception and production in phenotypes caused by FoxP malfunctions to increase our understanding of vocal learning as the base of speech and language acquisition.

Disclosures: F. Heim: None. **S.E. Fisher:** None. **C. Scharff:** None. **C. ten Cate:** None. **K. Riebel:** None.

Poster

140. Auditory Processing: Vocalizations and Natural Sounds

Location: SDCC Halls B-H

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Program #/Poster #: 140.19/AA1

Topic: D.06. Auditory & Vestibular Systems

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Title: Neural activities in marmoset premotor cortex for vocal production during social communication

Authors: *L. ZHAO, X. WANG
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Abstract: The neural mechanism of vocal production has been a long-standing question in non-human primates. While most of previous studies have focused on subcortical regions and the limbic pathway, recent experiments have revealed evidence for the involvement of the frontal cortex during vocal production. Using marmoset monkeys as a model system, studies in our lab and others have found that neurons in the premotor cortex were modulated when marmosets produced phee calls (a long-distance contact call) in vocal exchanges when isolated from their social groups. It has been known that marmosets maintain frequent vocal interactions in rich social contexts using four major types of calls: phee, trill, twitter and trillphee. Given previous findings for phee calls, it is an important question whether neural activities in premotor cortex are correlated with the vocal production of other call types used in social communication by marmosets. Here we investigated this question by recording neural activities from free-roaming marmosets in the colony when the subjects maintained visual contact and engaged in natural vocal interactions with other individuals. We developed a wireless multi-channel neural recording system to enable reliable recording in the marmoset cages and used a targeted acoustic recording setup to isolate subject vocalizations from the background. We found local field potentials and single unit activities in premotor cortex modulated by each type of social

communication calls. Some recording sites and a subset of units showed difference in modulation when marmosets were producing different types of calls, suggesting a representation of call type in the premotor cortex. Our results provided further evidence for the role of the frontal cortex in vocal production and communication.

Disclosures: L. Zhao: None. **X. Wang:** None.

Poster

140. Auditory Processing: Vocalizations and Natural Sounds

Location: SDCC Halls B-H

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Program #/Poster #: 140.20/AA2

Topic: D.06. Auditory & Vestibular Systems

Title: Representational similarity analysis reveals the involvement of supplementary motor area in perceiving speech rhythm

Authors: *S. HIROYA[1], Q. CAI[2], A. SETHI[2], N. LAVAN[3], S. H. CHEN[2], S. MEEKINGS[4], S. K. SCOTT[2]
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Abstract: Suprasegmental features of speech such as intonation, stress and rhythm are essential for understanding speech. We have developed a method that can convert the mora-timed rhythm of an English sentence spoken by a native Japanese speaker into a stress-timed rhythm by a native English speaker. fMRI scans for native English speakers during passive listening have showed that supplementary motor area (SMA) was more activated by mora-timed (less natural) rhythm more than by stress-timed (natural) rhythm. This indicates that SMA may involve in processing of speech rhythm perception. However, it is not clear to which SMA is sensitive, the speech intelligibility or the naturalness of speech rhythm because both intelligibility and naturalness decrease when speech rhythm is altered. In this study, we develop a method for controlling speech rhythm based on articulator velocity and investigate whether SMA activation is correlated with speech intelligibility or rhythm naturalness using representational similarity analysis (RSA) of fMRI data. The velocity was calculated from articulatory data, which were collected using the electromagnetic articulography (EMA) system. Movements of articulators such as the lips and tongue, which reflect bell-shaped velocity profiles, generate speech. The bell-shaped velocity profile of natural speech was converted to emphasized, uniform and reversed velocity profile, without altering sentence duration. Native English participants were asked to listen to the stimuli and repeat them. The result of speech intelligibility (percent keywords correct) showed natural = emphasized > uniform > reversed. Also, they were asked to rate the naturalness of speech rhythm. Results showed natural > emphasized > uniform > reversed. This indicates that pattern of speech intelligibility was different from that of rhythm

naturalness. We scanned fifteen healthy, right-handed English speakers during passive listening to speech with four types of velocity. Region of interest (ROI) analysis showed that emphasized velocity stimuli were most activated in left-lateralized premotor cortex (PMC), inferior frontal gyrus (IFG) and SMA. Representational dissimilarity matrix (RDM) was created from behavioral results of speech intelligibility and rhythm naturalness. RSA showed that activation pattern for rhythm types in left SMA is correlated to naturalness ratings and that in left IFG is correlated to speech intelligibility. This indicates that dorsal pathways of speech perception may not process an intelligibility, but a naturalness. In conclusion, SMA activation during speech perception may be related with rhythm naturalness, not intelligibility.

Disclosures: S. Hiroya: None. **Q. Cai:** None. **A. Sethi:** None. **N. Lavan:** None. **S.H. Chen:** None. **S. Meekings:** None. **S.K. Scott:** None.

Poster

140. Auditory Processing: Vocalizations and Natural Sounds

Location: SDCC Halls B-H

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Topic: D.06. Auditory & Vestibular Systems

Support: NSFC 61621136008/DFG TRR-169
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Title: Temporal tracking of speech periodicity in human auditory cortex

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Abstract: Periodicity is an important temporal feature of natural sound, and it is also the signal basis for the auditory perception such as pitch, semantic tone and intonation (Rosen, 1992). How periodicity of speech is parsed in the auditory system remains to be elucidated. A large number of imaging studies have found that sounds with periodic structure elicits prominent activity in human primary auditory cortex (Zatorre, 2000;Patterson, 2002) . Electrophysiological studies of animal and human brain have found that the temporal pattern of the auditory neural responses also encodes periodic information (Wang, 2012; Nourski,2013). The periodic nature of speech, especially how the fundamental frequency f0 is processed in the human brain, remains controversial(Wang, 2013). In this study, 30 epilepsy patients implanted with intracranial electrodes for clinical diagnosis passively listened to a set of periodic synthethized sounds and speech materials. By analyzing the intracranial EEG (iEEG) responses of 2739 electrodes, we identified 268 electrodes over auditory cortex with responses tracking the fundamental frequency in the sounds. With a set of f0 sweep stimuli, we observed that the tracking response of intracranial EEG is limited to the frequency band of 60-150 Hz, which partially overlaps with the

fundamental frequency distribution of the human speech(English 85-255Hz, Baken 1987; Chinese 110-330Hz, Zhang, 2010), suggesting that temporal following is one of the possible mechanisms for encoding the fundamental frequency of speech in the human auditory cortex. These temporal following responses were mainly located in the Heschl's Gyrus(HG), planum temporale (PT) and the posterior part of the superior temporal gyrus (STG). The strength of temporal following (measured by phase locking value) of iEEG over the PT &HG area on both hemispheres are significantly higher than that of the STG (Wilcoxon rank-sum test; $p<0.05$). PT and STG on both hemisphere tracked the f0 of harmonic stimuli and speech more strongly than that of same frequency pure tone (t-test; $p<0.01$). We also compared the temporal following strength of left and right auditory cortex, but no significant lateralization was observed.

Disclosures: N. Guo: None. **X. Si:** None. **W. Zhou:** None. **B. Hong:** None.

Poster

141. Vision: Visual Cortex: Circuits and Populations

Location: SDCC Halls B-H

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Program #/Poster #: 141.01/AA4

Topic: D.07. Vision

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Title: Correlated noise in visual area v2 of infant monkeys

Authors: *B. ZHANG[1], Y. WANG[2], X. TAO[2], G. SHEN[2], E. L. SMITH, III[2], Y. M. CHINO[2]
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Abstract: Previously we reported that the spiking noise of V2 neurons, e.g., spiking irregularity (CV^2) and trial-to-trial variability (m-FF), for infant monkeys was much *lower*than that for adults. Correlated or shared variability (noise correlation) among nearby neurons can influence the effectiveness of information processing in cortical network and hence, visual functions. Here we compared the noise correlations between infants (4- and 8-week-old) and adults by analyzing responses from pairs of neighboring V2 neurons using a single electrode in anesthetized macaque monkeys. While the use of single electrode, instead of multiple electrodes or arrays, may not be the best approach to study noise correlations, our highly efficient spike sorting method (Tao et al, 2014) similar to the approach used by Martin and Schroder (2013), allowed us to make some credible observations on noise correlation. Specifically, the average noise correlations (r_{sc}) of adult V2 neurons obtained with high contrast gratings was about 0.21-0.22, which is within the range of the previously reported r_{sc}values in primate V1-V2 obtained by

using multi-electrode recording techniques (0.10-0.25). We analyzed correlated noise in 178 pairs in infants and 224 pairs in adult macaque monkeys. We measured noise correlations for stimulus contrast of 0% (spontaneous), 10%, 25%, 50% and 80%. We found that the average noise correlation was much higher for adult V2 compared to that in infants for all stimulus contrasts ($p<0.001$). Moreover, noise correlation in adult V2 was contrast dependent; differences between infants and adults were greater for lower contrasts (10% and 25%) than for higher contrasts (50% and 80%). The data are consistent with similar observations made in adult monkey V1. In infants, noise correlation was also significantly lower for the highest contrast (80%)($p<0.01$). In adult V2, noise correlations during spontaneous activity were higher than that during evoked responses except for 10% contrast ($p<0.01$). This result is consistent with similar observations in V1 (Kohn and Smith, 2005 and Smith and Kohn, 2008). However, for infants, the difference in noise correlations between spontaneous and evoked discharge was found only for 80% contrast ($p<0.05$). The observed lower correlated noise between neighboring neurons in infants may reflect the relative immaturities of long-range intrinsic and/or feedback connections in their V2.

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Poster

141. Vision: Visual Cortex: Circuits and Populations

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Topic: D.07. Vision

Support: NIH Grant NS064033

Title: Four-dimensional map of stimulation-evoked neural responses across the ventral visual areas

Authors: *A. SUGIURA[1], Y. NAKAI[1], H. MOTOI[1], E. ASANO[1,2]
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Abstract: We provided whole-brain level animations showing the dynamics of cortico-cortical evoked potentials (CCEPs) elicited by stimulation of lower- and higher-order visual areas. This study analyzed 26 patients with focal epilepsy who underwent extraoperative electrocorticography (ECoG) recording. Trains of electrical stimuli (biphasic polarity; pulse duration: 0.3 ms; intensity: 5 mA; frequency: 1 Hz; train duration: 40 s) were delivered to a neighboring pair of subdural electrodes as part of presurgical evaluation. ECoG signals were averaged time-locked to the onset of each stimulus to yield CCEPs in electrode sites distant from

the stimulated pair. CCEPs of all individual patients were finally delineated on the FreeSurfer average surface image. Stimulation of the lingual/cuneus region elicited an initial response of negative polarity (also known as N1) in the lateral-occipital region at 10-20 ms. Subsequent responses of negative polarity (N2) involved the lateral-occipital and fusiform regions at 100-120 ms. Stimulation of the lateral-occipital region elicited small N1 and N2 in the lingual/cuneus region but large N1 and N2 in the fusiform region at 10-20 ms and 70-130 ms, respectively. Stimulation of the fusiform region elicited small N1 and N2 in the lingual/cuneus or lateral-occipital region, but large N1 and N2 in the parahippocampal and entorhinal regions at 10-20 ms and 130-150 ms, respectively. N1 responses on CCEPs have clarified the spatio-temporal characteristics of excitatory neural propagation via a single axonal pathway across the ventral visual areas. Both N1 and N2 responses propagate in a direction from lower- to higher-order visual areas more preferentially than vice versa. Our invasive study increases the understanding of the network dynamics of ventral visual pathway.

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Poster

141. Vision: Visual Cortex: Circuits and Populations

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Topic: D.07. Vision

Support: NIH Grant MH93567

Title: Interneuron populations in macaque MT

Authors: *J. J. COPPOLA[1], A. A. DISNEY[2]
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Abstract: Inhibitory interneurons comprise heterogeneous populations in cortex, with considerable structural and functional diversity. Traditionally, these populations have been classified based on their morphologies; more recently, molecular markers have become a prevalent alternative for interneuron classification particularly in non-human primates. Parvalbumin (PV), calbindin-D28k (CB), and calretinin (CR) are calcium-binding proteins whose immunoreactivities are used as population markers for diverse classes of cortical interneurons. Literature suggests these populations differ in their composition and density across the cortical mantle. However, we do not currently have the data to describe these differences across cortex. In macaque middle temporal visual area MT, using both stereological and non-stereological cell counting methods, we find that the composition of interneurons immunoreactive for PV, CB, and CR is different from that found in primate primary visual area V1 and in the prefrontal cortex. Interestingly, however, results from each method differ in their

estimated size and composition of these populations. We also find that similar to other primate cortical areas, but unlike in the rodent cortex, interneurons immunoreactive for PV, CB, and CR in area MT represent distinct, non-overlapping populations. Additionally, our findings provide evidence that caveats exist for the quantification methodologies often used in characterizing these populations. Specifically, we will discuss limitations associated with stereology and non-stereological quantification techniques.

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Poster

141. Vision: Visual Cortex: Circuits and Populations

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Title: Dissecting multiple oscillators in the human alpha rhythm in the resting state

Authors: *C. HAN[1,2], L. SUN[3,4], H. LI[3,4], D. XING[1,2]
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Abstract: Alpha rhythms in the human electroencephalogram (EEG), oscillating at 8-13Hz, are located in parieto-occipital cortex and are strongest when awake people close their eyes. It has been suggested that alpha rhythms are related to attention-related functions. Many studies have suggested that the alpha-band can be divided into two loosely-defined sub-bands (high and low alpha) and that each sub-band might be related to different cognitive functions. But whether or not alpha sub-bands come from multiple oscillatory mechanisms is not clear. If multiple alpha-band oscillations do exist, how can one distinguish them? Here we addressed these questions by studying alpha oscillations in the EEG (128 channel EGI) recorded from 222 adults in a resting state with their eyes closed. We found that the topographic map of alpha power changed with

frequency. The frequency-dependent topographic maps can be explained by a descriptive model that enabled us to dissect three different oscillatory components for each individual. These oscillatory components differed in their frequencies and spatial locations. Our results suggest that resting state alpha oscillations originate from distinct oscillatory mechanisms. Furthermore, different alpha components might support different aspects of attention-related functions. It is necessary to dissect these different alpha components in cognitive studies.

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Poster

141. Vision: Visual Cortex: Circuits and Populations

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Topic: D.07. Vision

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Title: Multivariate analysis of V1 spiking dynamics for ocularity, orientation, and stimulus repetition

Authors: *D. A. TOVAR[1,2], J. A. WESTERBERG[1,3], M. A. COX[1,3], K. DOUGHERTY[3,1], T. A. CARLSON[5], M. T. WALLACE[4], A. V. MAIER[3]
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Abstract: In recent years, linear multielectrode arrays have become increasingly popular to study neuronal activity across isolated cortical (columnar) microcircuits within a given brain area. Laminar array data has been predominantly analyzed using standard univariate methods. Multivariate pattern analysis (MVPA or 'decoding') methods are a powerful approach to study the information represented at the population level. These methods used in conjunction with data from multiple channels across laminae make it possible to study population information represented in individual laminae in the V1 microcircuit. Here, we use time-resolved MVPA to analyze spiking responses from the primary visual cortex (V1) of two awake macaques (*Macaca radiata*). Animals fixated while they were exposed to 200 millisecond-long sequential presentations of high contrast grating stimuli of varying orientation over the receptive field location of the isolated neurons. A linear microelectrode array that spanned all layers of V1 was used to isolate multiunit spiking activity of supragranular, granular, and infragranular origin, respectively. MVPA was employed to decode 1) which eye the stimulus was presented to 2) stimulus orientation and 3) the relative position of the stimulus in sequence (position 1-5) across

laminae. We found significant differences between V1 layers in decoding performance for both ocularity and for decoding initial presentation from stimulus repetitions, but not for stimulus orientation. The time course of decoding was particularly informative, with ocularity being decodable in the retino-geniculo-recipient granular layers before the supragranular layers, followed by the infragranular layers. This temporal sequence is consistent with the canonical microcircuit model of visual cortex that postulates increased intra-cortical processing in a similar order. In line with this idea, peak decoding of ocularity decreased once it reached the infragranular processing stage. None of these differences were apparent when using a univariate approach. Similarly, repeated stimulus presentations led to improved orientation decoding across all layers, despite a reduced univariate response under the same condition. Together, these findings suggest that the spatiotemporal pattern of laminar neuronal responses reveals additional information than what can be gleaned from univariate response magnitudes alone.

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Poster

141. Vision: Visual Cortex: Circuits and Populations

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Title: Efficient mapping of spatial frequency sensitivity in human visual cortex

Authors: *S. AGHAJARI[1], S. LING[2]
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Abstract: Neurons within early visual cortex are selective for basic image statistics, including the spatial frequency content of a retinal image. However, sensitivity is not uniform across all frequencies, peaking at mid-frequencies, and dropping off for both low and high frequencies. How does the window of spatial frequency sensitivity vary across the visual field and across visual areas? Although a handful of previous studies have investigated this using conventional fMRI designs and analysis methods, these measurements are time-consuming and often do not span the entire range of spatial frequencies. In this study, we introduce a model-based fMRI analysis approach that allows for fast and efficient estimation of population spatial frequency tuning (pSFT) for independent voxels. BOLD responses within early visual cortex were acquired while subjects viewed a series of full-field stimuli that swept through a large range of spatial frequency content. Each stimulus was generated by bandpass filtering white noise with a central frequency that changed periodically between a minimum of 0.5 cpd and a maximum of 12 cpd.

To estimate the underlying frequency tuning of each voxel, we assumed a Gaussian pSFT and optimized the parameters of this function by fitting our model output with the measured BOLD time series. With these estimated parameters, we then investigated the relationship between spatial frequency selectivity and other factors, including retinotopic preference and receptive field size. The results show that an increase in eccentricity within each visual area is accompanied with a drop in the peak of the pSFT. Moreover, voxels with larger receptive fields respond more vigorously to lower spatial frequencies.

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Poster

141. Vision: Visual Cortex: Circuits and Populations

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Topic: D.07. Vision

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Title: Activation of a chemogenetic receptor (GluCl/IVM) expressed in primary visual cortex of rhesus monkey produces an impairment in signal detection

Authors: *J. Y. SHIM[1], M. A. ELDRIDGE[1], J. N. TURCHI[1], J. M. FREDERICKS[2], W. LERCHNER[1], B. J. RICHMOND[1]
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Abstract: Artificially introduced ligand-gated ion channels (LGICs) have been used in the mouse to inactivate selected brain regions repeatedly and non-invasively. When the anthelmintic drug ivermectin (IVM) is applied, cells expressing GluCl become hyperpolarized due to its high affinity for IVM. In monkeys, GluCl can be expressed in an area of interest using replication-deficient viral vectors. Thus, when IVM is administered systemically, neurons expressing GluCl become hyperpolarized, causing silencing of the target region.
We expressed GluCl/IVM in the left primary visual cortex of two rhesus monkeys. Using glass syringes driven by an infusion pump mounted on a stereotaxic arm, we injected 10 uL of AAV2 expressing the modified channel under the control of a human synapsin promoter (AAV2-syn::GluCla&b-YFP), at a rate of 0.5 uL/min into each of nine sites in a 3 x 3 grid, covering a region of 4 x 4 mm at a depth of 1.5 mm. We also removed a region of right primary visual cortex symmetrical to the AAV2 injection site by aspiration as a positive control.
After recovery from surgery, monkeys performed a signal detection task. Monkeys maintained fixation on a central target (0.5 x 0.5 degrees) for a variable period of time. On 80% of trials, a high-contrast “target” was presented in one hemifield and a lower-contrast “distractor” was

presented symmetrically about the vertical meridian. The monkey had to saccade to the brighter cue to obtain a liquid reward. To reduce the likelihood of the monkey adopting strategies that relied on patterns/inference, we presented two other trial types: on 10% of trials, a high-contrast target was presented alone (no distractor); on the remaining 10% of trials, monkeys had to maintain fixation on the central point to obtain reward (no cue was presented).
Under baseline conditions, monkeys failed to saccade to the target when it was presented in the scotoma corresponding to the aspiration lesion in the right hemisphere. This was accompanied by an increase in the number of saccades to the distractor presented in the symmetrically opposite site.
To activate GluCl, we administered a subcutaneous bolus injection of IVM (7.5 mg/kg) 24 hours prior to testing. Under this condition, monkeys not only failed to saccade to targets presented in the chronic scotoma, but also failed to detect the distractor in the opposite hemifield (i.e. in the receptive field corresponding to the region of V1 expressing GluCl).
This proof-of-concept study suggests that the GluCl/IVM system may be a valuable tool for systems neuroscience in rhesus monkeys.

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Poster

141. Vision: Visual Cortex: Circuits and Populations

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Topic: D.07. Vision

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Title: A stimulus derived model of gamma oscillations in human visual cortex

Authors: *D. HERMES[1], K. N. KAY[2], J. WINAWER[3]
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Abstract: Viewing spatial contrast patterns can elicit several types of field potential responses in visual cortex. One such response is oscillatory, evident by a spectral peak in the gamma band (~30-80 Hz). A second response is non-oscillatory, evident by a broadband power increase (~50-200 Hz). The broadband response is correlated with multiunit activity, and its stimulus selectivity is well modeled by the same kind of computations used to model spiking and fMRI signals in visual cortex (contrast energy, normalization, spatial pooling, etc.) In contrast, there is no established image-computable model for gamma oscillations. Here we develop such a model

and test it against measurements from implanted surface electrodes (ECoG).
Two subjects implanted with ECoG arrays for clinical purposes viewed 86 different visual patterns. Two signals were extracted from electrodes over V1-V3: high-frequency broadband power, and narrowband gamma power between 30 and 80 Hz. Similar to fMRI measures, broadband power increased with contrast and with spatial irregularity (second-order contrast) of the stimulus. Consistent with a prior fMRI study (Kay et al, 2013), the broadband response was accurately fit by a two-stage model including oriented contrast energy, normalization, spatial pooling, second order contrast, and an output nonlinearity.
The pattern of narrowband gamma responses was strikinging different: gamma peaks were largest for sinusoidal gratings and lower for irregular spatial patterns. We developed a 'narrow-band-Fourier' (NBF) model to explain the pattern of gamma responses. The NBF prediction is proportional to the variance across spatially pooled orientation channels. This value is large for high contrast oriented gratings, lower for plaids, and lowest for noise patterns. The model explained ~60% of the variance in gamma power across stimuli.
The large differences in stimulus selectivity for gamma oscillations on the one hand, and broadband power, fMRI, and spiking on the other, suggests that gamma oscillations reflect a specific circuit property rather than being a general indicator of the level of neuronal responses. We speculate that gamma oscillations are largest for stimuli which happen to evoke just the right balance between excitation and inhibition.

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Poster

141. Vision: Visual Cortex: Circuits and Populations

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Topic: D.07. Vision

Support: Dennis Washington Achievement Grant

Title: Investigating the origin and role of catecholamines in primary visual cortex (V1) of the macaque

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Abstract: The systemic application of drugs that activate dopaminergic receptors influences the processing of visual information, but how endogenous dopamine modulates visual circuits remains unclear. Dopamine receptors can be found in all 6 layers of primate V1, but midbrain dopaminergic innervation is restricted to layer 1. This anatomical organization raises the question as to whether or not dopamine from midbrain nuclei serves as the ligand for these

receptors. One possibility is that the locus coeruleus (LC) co-releases dopamine and norepinephrine into V1. LC terminals marked by dopamine beta-hydroxylase (DBH; the enzyme that converts dopamine into norepinephrine) can be found in all 6 layers of V1. Furthermore, recent studies have demonstrated that dopamine is released into the hippocampus from LC fibers and modulates memory-associated signaling. Another possibility is that norepinephrine itself is the ligand for dopamine receptors in V1. We hypothesize that - if the LC is a source for cortical dopamine - LC terminals might express a dopamine auto-receptor, such as the short isoform of the D2 dopamine receptor, to regulate catecholamine release. We immuno-labeled tissue sections containing V1 for both DBH and the D2 dopamine receptor. Dual-labeled terminals were not observed, suggesting that the a2 adrenoreceptor is likely the sole regulator of catecholamine release into V1. Our results are in agreement with previous studies that found that the local application of D2-receptor antagonists to rodent V1 had little effect on extracellular levels of catecholamines; conversely a2-receptor antagonists dramatically increased the levels of both dopamine and norepinephrine. The lack of dual-labeled terminals observed in our study indicates that if dopamine is released by LC terminals, those terminals are not using that signal as a regulator of their activity. This hints at a model in which norepinephrine may control the release of two functionally distinct neuromodulatory signals in V1.

Disclosures: C. Roach: None. **R. Rios:** None. **A.A. Disney:** None.

Poster

141. Vision: Visual Cortex: Circuits and Populations

Location: SDCC Halls B-H

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Program #/Poster #: 141.10/BB2

Topic: D.07. Vision

Support: ARC DE18010034
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Title: Laminar organization of feedforward input from V1 to MT in marmosets

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Abstract: The laminar architecture of cortex provides a structural basis for organising the synaptic inputs into each brain area. Feedforward inputs are believed to be processed in the superficial layers first. Feedback information is processed initially in the deep layers. Despite this architecture being a ubiquitous feature of cortex, little is known about how this organization

supports the computations carried out in each area. The local field potential (LFP) is thought to reflect the global activity around a recording electrode, including synaptic inputs. Previous studies have found increased gamma-band LFP activity in the superficial compared to deep layers of cortex, suggesting that this may reflect feedforward processing.
The visual cortex provides an ideal testbed for studying feedforward inputs because it is organised hierarchically and the individual areas have been well studied. In particular, the primary visual cortex (V1), the first stage of cortical visual processing, and the middle temporal area (MT), a center for motion processing, are two of the best studied areas of the cortex. MT receives its largest input from V1 and its computational processing is dependent on its V1 input. In order to study the laminar organisation of feedforward processing in MT, we simultaneously recorded from V1 and MT in three anesthetized marmoset monkeys. A 96-channel Utah array (Blackrock microsystems) was placed in V1 while a 32-channel laminar probe (NeuroNexus) was placed in MT, orthogonal to the cortical surface. Receptive fields in each area were mapped, and the MT probe was placed so that the receptive fields overlapped with those recorded on the V1 array. Spiking and LFP data were recorded from each array. Consistent with previous studies, we found significantly higher gamma-band activity (60 Hz) in the superficial layers of MT compared to the deep layers ($p < 0.01$, Rank sum test) during the stimulus period. Furthermore, gamma-band LFPs were tuned for direction of motion, and the tuning matched the spiking activity recorded on the same electrode. However, V1 spike-MT LFP coherence between the two areas was largely limited to the alpha-band (10 Hz). Across the population, alpha-band coherence was significantly greater than gamma-band coherence in both the superficial and deep layers ($p < 0.001$, Rank sum test). Furthermore, the prevalence of gamma-band coherence was sparse (~5% of recording sites).
These results suggest that feedforward information from V1 to MT depends on synchrony in the alpha-, but not the gamma-, band of the LFPs in MT. Instead, gamma-band activity may reflect local processing that reflects to the directional tuning of MT cells.

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Poster

141. Vision: Visual Cortex: Circuits and Populations

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Topic: D.07. Vision

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Irma T. Hirschl Trust

Title: Temporal dynamics of inter-area neuronal population interactions

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Abstract: Most brain functions rely on activity distributed across brain areas. Yet, little is known about how information is selectively routed across neuronal populations in distinct brain areas. First, most previous studies of inter-area interaction have focused on pairwise relationships (e.g., spike-LFP/LFP-LFP coherence), leaving unclear how moment-to-moment fluctuations in neuronal population spiking responses are related across areas. Second, most previous inter-areal studies have neglected the possibility of interaction dynamics, providing a relatively static view of interactions under different stimulus or behavioral conditions.To understand the dynamic relationship between population spiking responses in different cortical areas, we simultaneously recorded neuronal populations in V1 and V2 of 3 anesthetized monkeys, while animals viewed either oriented gratings (evoked activity) or a blank screen (spontaneous activity). We studied how the trial-to-trial variability of V2 responses relates to those in V1 under these two conditions, at multiple time scales.
V1-V2 neuronal interactions were dynamic and different for evoked and spontaneous activity. Analyzing responses on a fine temporal scale, and considering different relative lags between the responses in V1 and V2, we found that soon after stimulus onset, the population-level correlation between these areas was “feedforward dominated”, with V1 activity leading V2 activity in time. This feedforward component decreased throughout the evoked activity period, and was largely absent by the end of the stimulus presentation (1.28 s). For spontaneous activity, on the other hand, the interaction between these areas was “feedback dominated”, with V2 activity leading V1 activity.
We then used coarser time scale analysis to characterize the population-level structure of V1-V2 interactions. In previous work, we showed that evoked V2 population activity is related to a small subset of V1 population activity patterns, which are distinct from the largest shared fluctuations among V1 neurons. Here, we find that inter-area interactions during spontaneous activity are markedly distinct, with the largest shared fluctuations within V1 being most related to V2 activity. Further, for evoked activity, the V1 population patterns related to V2 activity were distinct from those most relevant for relating spontaneous activity in the two areas. Our results thus indicate that the temporal structure of the V1-V2 interaction can quickly change between being feedforward vs. feedback dominated, and that these dynamics are accompanied by a change in the population-level structure of the interactions.

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Poster

141. Vision: Visual Cortex: Circuits and Populations

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Title: Laminar distribution and cellular localization of CB1R system components in the primary visual cortex of vervet monkeys

Authors: *R. KUCERA[1], J. BOUSKILA[2,1,3], M. TOUTOUNGY[1], K. PETERSON[1], R. PALMOUR[2,3], J.-F. BOUCHARD[1], M. PTITO[1,3,4]
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Abstract: Modulating the endocannabinoid (eCB) system effects visual perception by mechanisms which remain not yet fully understood. Past studies on the effects of cannabis consumption on the human body reported visual effects at both low level retinal processing and at high level cortical visual processing. The expression and localization of the eCB system have been well characterized in recent years in the monkey retina and in the dorsal lateral geniculate nucleus (dLGN). However, few data are available on primate cortical visual structures. The goal of this study is to characterize the expression and localization of the cannabinoid receptor type 1 (CB1R), the synthesizing enzyme N-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD), and the degradation enzyme fatty acid amide hydrolase (FAAH) in the vervet monkey primary visual cortex (V1). Using Western blots and immunohistochemistry, we investigated the laminar and cellular expression patterns of CB1R, NAPE-PLD, and FAAH across the rostrocaudal axis of the vervet monkey (*Chlorocebus sabaeus*) area V1. CB1R, NAPE-PLD, and FAAH were expressed in V1 throughout the rostrocaudal axis. CB1R showed very low staining in layer (L) 4, with higher expression in all other layers, especially L1, followed by L2 and L3. NAPE-PLD and FAAH expression patterns were similar, but not quite as low in L4. CB1R, NAPE-PLD, and FAAH were localized in vGlut2-positive cells, representing glutamatergic projection neurons, and in somatostatin (SST)-positive cells, a class of interneurons which

suppress lateral and feedback interactions. The low level of CB1R in L4 indicates less direct endocannabinoid modulation of V1 afferents from the dLGN, but that modulation may occur via the higher expression of CB1R in L2 and L3 on the way to the dorsal and ventral visual streams. This is further supported by the higher expression of NAPE-PLD and FAAH in these layers. Expression in vGlut2-positive and SST-positive cells represents a role at both glutamatergic and GABAergic neurons. These data indicate that CB1R may influence the network of activity patterns in the visual streams after the visual information has reached V1, and thus may influence visual perception.

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Poster

141. Vision: Visual Cortex: Circuits and Populations

Location: SDCC Halls B-H

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Program #/Poster #: 141.13/BB5

Topic: D.07. Vision

Title: Exploring the response patterns induced by electrical stimulation in cat visual cortex

Authors: *J. HU, M. QIAN, H. TANIGAWA, A. W. ROE
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Abstract: Electrical stimulation (estim) has been widely used in neuroscience for research and clinical applications. Compared with other neuromodulation methods such as delivering drug into the cortex, estim can be manipulated more precisely both in time and intensity. Based on known intrinsic connections within visual cortex, we hypothesize that estim of a single functional domain will bias cortical response towards its associated cortical network and away from contrasting/opposing networks. Specifically, by combining focal targeted micro-stimulation and intrinsic optical imaging in cat visual cortex area 18, we hypothesized that stimulation of single orientation domains, during visual presentation of a grating of matched orientation, would bias the cortical network towards the stimulated orientation network and away from the orthogonal orientation network. Our preliminary results show that: (1) stimulating one orientation domain induces enhancement of the local orientation-matched orientation network, (2) in some cases, such stimulation led to relative suppression of the orthogonal orientation network, (3) this stimulation was intensity dependent. These results suggest that focal estim may effect biasing of existing anatomical networks towards selected functional effects and away from opposing effects, something that may be fundamental to neural modulation in brain-machine interface.

Disclosures: J. Hu: None. **M. Qian:** None. **H. Tanigawa:** None. **A.W. Roe:** None.

Poster

141. Vision: Visual Cortex: Circuits and Populations

Location: SDCC Halls B-H

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Program #/Poster #: 141.14/BB6

Topic: D.07. Vision

Title: Two-photon imaging evidence for neuronal responses to circular and dartboard-like stimuli in macaque V1

Authors: *N. JU[1], S. GUAN[2], C. YU[3], S. TANG[3]
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Abstract: V1 neurons respond to oriented stimuli like bars and gratings. Some V1 neurons also respond to more complex stimuli like curvatures and checkerboard patterns. Here we used two-photon calcium (GCaMP5) imaging to further investigate V1 neurons' responses to a series of mathematically well-defined complex stimulus patterns. Two-photon imaging allowed us to record simultaneously the responses of hundreds of neurons at single-neuron precision, and with relatively unbiased neuron sampling.
We recorded >3500 superficial layer neurons (150 and 300-μm depths) at 3-5^{o} eccentricity in two awake, fixating monkeys. The stimuli were Gabor gratings or patterns defined by two-dimensional Hermit functions (Victor et al., 2006, 2009). The Gabors had 9 orientations and 6 SFs. The Hermit patterns ranked from 1-5 (few neurons responded to the 0th rank, a Gaussian pattern), including 15 unique patterns. They can be grouped as 4 one-dimensional Vignetted grating-like patterns, 5 checkerboard patterns, and 6 circular dartboard-like patterns. Each non-circular pattern was presented at 4 orientations. All patterns were presented in 3 sizes. The maximal responses to patterns of various orientations and sizes were used for analysis.
At 150-μm depth, about 60% neurons were orientation and SF tuned and responding to gratings, and 57% neurons responded to Hermit patterns (many responding to both, but 38% to Hermit patterns only). At 300-μm depth, 85% neurons responded to gratings and 25% responded to Hermit patterns (14% responding to Hermit patterns only). Among Hermit-responding neurons, 67% responded to at least one circular/dartboard patterns, 36% to checkerboard patterns, and 24% to Vignetted patterns (some responding to 2 or 3 pattern types). Vignetted patterns resembled gratings, and V1 responses to these patterns, as well as to checker-board patterns, were either expectable or have been reported. However, the large number of V1 neurons responding to circular and dartboard patterns were previously unknown and surprising.
Our results confirm previous reports that V1 neurons respond to Hermit patterns. Moreover, (1) There are V1 neurons responding to Hermit pattern only; (2) Neurons responding to Hermit

patterns at 150-μm are twice as many as at 300-μm, indicating likely emerging responses to these complex patterns in Layers 2/3. (3) There are a disproportionally large number of neurons responding to circular and dartboard patterns, which may carry biological significance for monkey's survival (i.e., finding round-shaped fruits).

Disclosures: N. Ju: None. **S. Guan:** None. **C. Yu:** None. **S. Tang:** None.

Poster

141. Vision: Visual Cortex: Circuits and Populations

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Topic: D.07. Vision

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Title: Suppressive waves disambiguate the representation of long-range apparent motion in awake monkey V1

Authors: *S. CHEMLA[1], L. E. MULLER[2,1], A. REYNAUD[3,1], M. DI VOLO[4], Y. ZERLAUT[5], L. PERRINET[1], A. DESTEXHE[6], F. Y. CHAVANE[1]
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Abstract: The "apparent motion" illusion is evoked when stationary stimuli are successively flashed in spatially separated positions. The illusion depends on the precise spatial and temporal separations of the stimuli, for large spatio-temporal separations it is classified as long-range apparent motion (lrAM). Contrary to the short-range conditions, it remains unclear how the visual system computes direction-selective motion signals in the lrAM conditions. Here we investigate whether cortical interactions could shape a global motion representation at the level of V1 population in response to a two-stroke lrAM sequence. In two fixating monkeys, voltage-sensitive dye imaging reveals the emergence of a spatio-temporal representation of the motion trajectory at the scale of V1 population activity. This representation is shaped by systematic suppressive waves that have similar spatial, temporal and speed properties to those of the evoked activity spread. Our results are compatible with the existence of a global recurrent gain control pooling both the feedforward input and the horizontal intra-cortical network. A conductance-based mean-field model indeed shows that the suppression can be reproduced through

nonlinearity of conductance interactions, combined with the different gain of excitatory and inhibitory cells. To further understand the neural mechanisms at the origin of this suppression, we analyze the waves interactions evoked by the two apparent motion stimuli at the single-trial level. We hypothesize that the function of the suppressive wave is to shape the representation of motion along the apparent-motion trajectory. We develop a decoding model to test this hypothesis and demonstrate that the suppressive wave allows to represent only one stimulus at a time during the apparent motion sequence. To conclude, we propose that such non-linearities explain away ambiguous correspondence problems of the stimulus along the motion path, preformating V1 population response for an optimal read-out by downstream areas.

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Poster

141. Vision: Visual Cortex: Circuits and Populations

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Topic: D.07. Vision

Support: R01 EY026156

Title: Synergistic coding of visual information in cortical networks

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Abstract: One major challenge in visual neuroscience is to understand how neuronal populations efficiently encode natural stimuli. Indeed, during natural viewing sensory neurons are exposed to redundant information every fraction of a second. How visual cortical neurons efficiently extract information from natural stimuli during vision is poorly understood. We recorded simultaneous activity of multiple neurons in primary visual cortex (V1) of awake monkeys using laminar probes while presenting achromatic gratings of various orientations. We used an information theoretic approach to quantify stimulus coding by neuronal responses. Contrary to previous reports in anesthetized primates, the neuronal population consisted not only of redundant pairs and triplets, but a significant number of pairs and triplets encoded stimuli in a synergistic manner. Laminar analysis revealed that synergy in the infra-granular layers was significantly higher compared to that in granular and supra-granular layers. However, cortical layers exhibited no significant differences in the amount of redundancy. To assess the impact of noise correlations in generating synergy and redundancy, we used the Information Breakdown framework to find that only stimulus dependent noise correlations contributed to the observed

synergy while stimulus independent noise correlations contributed to redundancy. Were individual neurons preferential in the kind of interactions (synergy or redundancy) they participated in? We labeled the neurons participating in synergistic/redundant interactions (above chance level) as synergy/redundancy hubs. Each cortical layer had both synergy and redundancy hubs, but we found no preferential layer specific grouping. Do synergy hubs provide an advantage over redundant ones regarding stimulus coding? We decoded stimulus information from sub-populations of neurons containing both synergy and redundancy hubs. We found that a higher fraction of synergy hubs in sub-populations resulted in higher decoding accuracy compared to a similarly sized population with higher redundancy hubs. Our results reveal that stimulus dependent noise correlations are beneficial for stimulus coding across the layers of V1.

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Poster

141. Vision: Visual Cortex: Circuits and Populations

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Topic: D.07. Vision

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Title: Suppressed neural activity in awake monkeys using the anion channelrhodopsin GtACR2

Authors: *S. R. DEBES, A. R. ANDREI, X. LIU, R. JANZ, J. L. SPUDICH, V. DRAGOI
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Abstract: Suppressing neural activity is an extremely powerful design for causal manipulation, however most current anion channelrhodopsins are associated with brief, but strong increase in neural activity during optical stimulation offset. *Gt*ACR2, a naturally occurring anion channelrhodopsin, has been shown to suppress neural activity in multiple preparations without an optical stimulation offset response. Here, we tested this opsin in two macaque monkeys (*macaca mulatta*). The anion opsin *Gt*ACR2 was packaged in a lentiviral construct under the control of the promoter CaMKIIα, and was injected into multiple columns of the primary visual cortex (V1). After allowing 6-8 weeks for viral expression, a 16 or 24 channel laminar probe coupled with an insertable fiber optic cable were used to record and optically suppress single and multiunit activity in V1. Animals completed a contrast detection task, where they maintained fixation on a central point while oriented gratings were presented parafoveally for 300 ms. Animals used a response bar to signal the presence (bar release) or absence (bar held) of a visual stimulus. On half of all trials, optical stimulation (1 Hz, 300 ms) was presented simultaneously with the visual stimulus. We found that neural firing during the visual stimulus presentation was significantly different in trials with optical stimulation compared to trials without optical

stimulation (laser vs control). The magnitude of this change was large, cells were suppressed by an average of 5.7 spikes per second (± 4.1). The largest difference in magnitude was 9.3 spikes per second (± 4.7; *P*=0.0058, Student's t-test), and occurred at 221.8 ms (± 16.3) after laser onset. However, temporal kinetics were slower than expected; Firing rates in optical stimulation trials did not return to baseline levels until 258.8 ms (± 27.6) after laser offset. Additionally, we pioneered a novel biopsy technique to acquire small tissue samples in order to perform immunohistochemical analysis. We found that our construct could reliably target glutamatergic neurons in macaque visual cortex with an efficiency of 97% and specificity of 100%. Overall, our results indicate that *Gt*ACR2 is an adequate tool for optogenetic suppression in vivo in the non-human primate brain. It yields high transfection levels and elicits strong firing rate suppression.

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Poster

141. Vision: Visual Cortex: Circuits and Populations

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Title: Routing information flow by high gamma synchrony allows for 'functionally labeled lines' in higher primate cortex

Authors: *M. ESGHAEI[1,2], M. KHAMECHIAN[3], S. TREUE[1,4,5,6], M. DALIRI[3,2]
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Abstract: The performance of visually-guided behavior involves various neural circuits which underly the use of sensory information for motor actions. The exact neural mechanism underlying these sensory-motor interactions has remained elusive. Here, we trained two macaque monkeys to report a subtle change in the motion direction of a cued moving random dot pattern (RDP), while ignoring another RDP presented on the opposite visual hemifield. Further,

extracellular action potentials and of local field potentials (LFPs) were recorded from the middle temporal area (MT) of the animals, while they performed the visual change detection task. We partitioned the correctly performed trials into fast and slow subsets based on the monkeys' response time (RT) in reporting the direction change. Next, we calculated the degree to which single neuron spikes were coupled to the LFP phase (spike phase coupling (SPC)), and compared it across the fast and slow trial subsets within the period preceding direction change. Performing this comparison across the spectrum of frequencies showed that: 1) single neuron action potentials are coupled to the phase of high-gamma (180-220 Hz) LFPs, irrespective of the spectral leakage of spike waveforms onto the LFP. 2) The strength of neural synchrony within the high-gamma frequency (180-220 Hz) is significantly larger in fast compared to slow trials, an effect not observed in lower frequency bands. This suggests that in fast trials, spikes occur predominantly in a specific phase of high-gamma oscillatory activities. 3) This SPC difference between fast and slow trials exists only when the cued stimulus is presented inside the receptive field. These observations suggest that neural synchrony in frequencies as high as high-gamma helps an efficient transmission of sensory information to motor/associative cortical areas, also enabling higher associative areas to distinguish the information coming via the dorsal from that coming via the ventral visual pathway.

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Poster

141. Vision: Visual Cortex: Circuits and Populations

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Topic: D.07. Vision

Support: NIH BRAIN INITIATIVE R01-MH111417

Title: Simulation and recovery of broadband field potentials

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Abstract: Electrocorticography (ECoG) measures neural activity in the living human brain with millimeter spatial resolution and millisecond temporal resolution. ECoG measurements can be separated into multiple components. One widely used measure is broadband, or 'high-gamma', which reflects an increase in spectral power spanning roughly 50-200 Hz. This component of the ECoG signal is correlated with multi-unit activity near the electrode (Manning et al., 2009) and with the BOLD fMRI response (Hermes et al., 2012; Winawer et al., 2013). Broadband power increases are widely observed in response to sensory stimuli, making it a useful measure of brain

activity. However, computing broadband responses from ECoG measurements involves several processing steps that each influence the broadband estimate, and there is currently no standard procedure for this computation. Here we propose and validate methods for quantifying broadband based on a generative model of how broadband signals emerge from increases in action potentials (Miller et al., 2009). In our simulation, a local field potential time series is created by first rendering a noiseless time course of the idealized time-varying rate of spike arrivals per neuron. Then, noisy samples of this rate are created using a Poisson spike generator followed by a leaky dendritic integrator. After simulating multiple trials with the identical underlying spike rate, we applied different broadband extraction methods to the simulated data and compared how well they recovered the underlying spike rate. The advantage of this simulation is that there exists a ground truth against which to judge the extraction methods. We show that the choice of extraction method can have a large effect on the derived time series, and therefore on the interpretation of the neuronal response. First, for slowly varying spike rates, computing the power of the broadband envelope recovers the underlying rate without systematic bias, whereas amplitude and log power envelopes overestimate low spike rates and underestimate high rates. Second, capturing sharp transients in the input spike rate requires filters with broad bandwidths (at least 10 Hz). Finally, amplifier noise reduces SNR in high-frequency bands, such that signal is best recovered by setting an amplifier-dependent ceiling on the frequency range to include in analysis. These effects are demonstrated with reference to time series with simple temporal features as well as time series resembling empirical data from visual cortex. Based on our results, we provide recommendations and software code for broadband extraction and suggest this can improve the quality of information derived from ECoG data.

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Poster

141. Vision: Visual Cortex: Circuits and Populations

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Title: Dual-gamma oscillations in V1 can be explained by crosstalks within the visuotopic map

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Abstract: In the past decades, gamma synchronization turned into an all-in-one mechanism, responsible for encoding, forming of neuronal representations and control of information flow in the brain. Several experimental studies in awake monkeys and humans found evidence that gamma is key to cognitive functions, such as perceptual binding, attention, and memory. However, many other studies failed to do so. A central point in the debate is how oscillation frequency is mechanistically modulated to entrain phase synchronization. Here, we recorded spiking activity and the local field potential (LFP) simultaneously at different locations in V1, corresponding to distinct eccentricity points in the visuotopic map (N = 150 pairs of recording sites). Data were collected from capuchin (N = 3) and macaque (N = 4) monkeys. Tuning curves were computed for moving gratings of different orientations, sizes, and speeds. Our results show that the oscillation frequency is systematically higher at the central as compared to the peripheral representation of the visual field. This difference cannot be accounted for by attention, as it has been suggested before. Notably, oscillation frequency was rarely stationary along the course of the response. Frequency monotonically decayed, following an exponential function that asymptoted a few hundreds milliseconds after stimulus onset. Overall, these results offer an alternative interpretation to the intriguing findings by Murty et al. (J. Neurosci, 2018) showing that two distinct gamma rhythms appear in V1 depending on the size of the stimulus. For large stimuli (full-screen gratings), the authors report a second slow-gamma component in the LFP and propose a new mechanism by which the two rhythms may boost stimulus encoding. However, our data suggest that the low-frequency component results from responses originating in the periphery of V1 (calcarine, slow gamma), which spread to the central representation region (operculum, fast gamma) by volume conduction. Accordingly, the two gamma rhythms should be conceived, not as playing a complementary role in visual processing, but as a mere epiphenomenon of the topological arrangement of the underlying cortical circuitry.

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Poster

141. Vision: Visual Cortex: Circuits and Populations

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Program #/Poster #: 141.21/BB13

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Title: Recurrent excitatory connectivity in mouse and human cortex

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Abstract: Estimates of connectivity and synaptic properties between distinct cell types is a long sought after goal, yet results vary widely between studies due to differences in experimental approach. The Allen Institute for Brain Science has initiated a large-scale project to systematically characterize synaptic signalling in mouse and human cortex using multipatch electrophysiology supplemented with 2-photon optogenetics. During the system integration test of this pipeline we focused on recurrent connectivity among excitatory neurons within each layer of mouse primary visual cortex and human temporal-frontal cortex. Layer-specific, excitatory cell types were identified either by morphology in L2/3 or through the use of transgenic labeling for layers 4 (Rorb-Cre), 5 (Tlx3-Cre and Sim1-Cre), and 6 (Ntsr1-Cre). The two transgenic types utilized in L5 allowed us to investigate two main projection types, corticocortical projecting (Tlx3) and subcortically projecting (Sim1). Up to eight neurons were recorded simultaneously while trains of electrical stimuli were delivered to each cell in turn. Across layers and Cre-types of mouse V1, 107 connections were found of the 2324 potential connections that were probed (L2/3: 20/255, Rorb: 20/282, Sim1: 34/524, Tlx3: 34/969, Ntsr1:1/318). In human frontal and temporal cortex we probed 332 potential connections across layers 2 through 5 and found 57 connections (L2: 4/38, L3: 42/187, L4: 2/38, L5: 9/69). For both mouse and human connections we quantified single-pulse properties of each connection type including amplitude, coefficient of variation, latency, and rise time and found that the heterogeneity within each layer or Cre-type often exceeded that between types. We went on to describe the short-term dynamics of synapses empirically via stimulus trains of varying frequency and recovery delay. In mouse L2/3, synapses showed a mix of facilitating and depressing responses while Rorb, Sim1, and Tlx3 synapses were dominated by depression. Theoretical modeling of depressing synapses showed that initial release probability and the recovery time constant varied significantly between types. Preliminary analysis of synapses in human L3 indicate a mostly depressing phenotype however, not to the same depth as depressing synapses in mouse. Further analysis across layers of human cortex are likely to show species-related differences in short-term dynamics.

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Poster

141. Vision: Visual Cortex: Circuits and Populations

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Title: Calcium Imaging of population responses from putative inhibitory neurons in macaque visual cortex

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Abstract: Non-human primates (NHPs) are important animal models for exploring human perception, cognition, and motor planning. Selective access to neuronal subclasses can allow for more rigorous investigation of neural circuit-level mechanisms underlying these complex processes. However, utilization of molecular-genetic tools commonly used for circuit dissection, which are based on selective targeting of reporters and actuators to specific cell classes, has been difficult in NHP models. We have therefore focused on the development of virus-based tools to target cell-type specific neural populations in the behaving rhesus macaque.
We have utilized recombinant adeno-associated viral vectors (rAAVs) to access NHP cortical neurons, demonstrating that we can faithfully record from genetically encoded calcium indicators (GECIs) expressed in excitatory neurons via the CaMKII**α** promoter (Seidemann et al., eLife 2016). In addition, we used a new promoter (h56D) to successfully express static reporters in inhibitory neurons with high efficiency and specificity in NHPs (Mehta et al., *submitted*). Here we use the h56D prompter to successfully express GECIs and record functional signal from putative inhibitory neurons in the visual cortex of awake, behaving macaques. Thus far, we have recorded reliable functional signals from 3 injection sites in 2 animals, which have lasted up to 4 months with no clear deterioration of signal within the lifetime of our cortical chambers. Using widefield optical imaging in the primary visual cortex (V1), we can record robust visual-evoked responses and extract reliable orientation maps from these putative inhibitory populations. Additionally, we have observed preliminary quantitative differences when comparing responses from excitatory and putative inhibitory populations.
Although access to responses from inhibitory neurons is a powerful tool, diverse functional subclasses exist within inhibitory neurons, driving us to develop more specific genetic tools to

target these sub-populations. We have observed efficient and selective expression of static reporters within somatostatin positive (SST^+) inhibitory neurons using a novel intersectional viral vector approach in NHPs (Mehta et al., *submitted*) and we are currently testing GECI expression using similar methods. In order to examine the functional contribution of distinct cell classes to visual perception, our ultimate goal is to develop a general genetic toolbox for selective protein expression in all major subclasses of neurons in the NHP brain.

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Poster

141. Vision: Visual Cortex: Circuits and Populations

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Title: Decorrelation in V4 by stimulation outside the receptive field

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Abstract: The spike rate of a neuron to a visual stimulus placed within the classical receptive field (RF) is reduced by stimulation in the RF surround. This phenomenon, termed surround suppression, is maximal when the orientation of the RF stimulus matches the orientation of the surround stimulus. Thus, it is thought to enhance the efficiency of stimulus encoding by removing redundant visual information. Previous research has shown that stimulation in the RF surround also reduces another feature of the responses in primary visual cortex, the correlated trial-to-trial response variability (known as spike count correlation, or r_{sc}). However, what remains unclear is whether or not this decorrelation in trial-to-trial neural variability is orientation-dependent. To address this issue, we simultaneously recorded from neurons in V4 of one macaque monkey using a chronically implanted microelectrode array. The animal performed a visually guided saccade task during which annular and grating stimuli were passively viewed to investigate center-surround interactions. We found that both mean spike rate and r_{sc} were significantly reduced by stimulation outside the RF, consistent with previous results in area V1. However, unlike the decrease in spike rate, the reduction in r_{sc} was not orientation-dependent. Additional analyses revealed that the magnitude of the decorrelation was significantly associated with two key factors: 1) the extent to which the average spiking response of a pair was suppressed by stimulation in the RF surround; and 2) the tuning similarity of a pair. The r_{sc} was

reduced to the greatest extent in pairs of neurons with similar tuning preferences that were strongly suppressed by stimulation outside the RF. These results extend previous findings and point to an additional mechanism through which surround suppression acts to improve the efficiency of stimulus encoding, by reducing correlated variability the most in neurons that have the greatest tuning similarity.

Disclosures: R. Johnston: None. **M.A. Smith:** None.

Poster

141. Vision: Visual Cortex: Circuits and Populations

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Topic: D.07. Vision

Title: Learning pattern invariance and specificity in early visual cortex by temporal association training

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Abstract: Experiencing different views of the same object in close temporal proximity is thought to allow our visual system to associate these views with its successively more abstract representations along the hierarchical visual system, leading to the development of invariance and specificity in object representation. To test this hypothesis, which was first proposed in the slow-feature analysis (Wiskott and Sejnowski, 2002) or memory trace models (Perry et al., 2006), we investigated the development of view invariance and pattern specificity in the neuronal population codes in areas V1, V2 and V4 along the ventral visual streams in two awake behaving monkeys, implanted with semi-chronic multi-electrode Gray-Matter arrays. The monkeys were performing fixation tasks while being exposed to a set of large parameterized prototype patterns (6-8 degrees in diameters), each with variations in scale, orientation, slant or tilt. A total of six experimental courses have been carried out. Each training and testing experimental course lasted for at least 7 days. We found that intense exposure (over 800 trials per day for each prototype) to the variations of a specific set of prototypes produced a contraction or condensation of the neural population representation of the variations of these trained prototypes. This condensation was accompanied by an increase in the discriminability among the variations of the trained prototypes, but not by any significant change in the discriminability among untrained prototypes. The discriminability was better in V2 than V1. This finding suggests that the untangling of the view-manifolds of objects to achieve robustness and specificity in pattern selectivity is local and specific to the parameters of the trained patterns and progresses along the visual hierarchy.

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Poster

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Title: Contextual processing in visual cortex

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Abstract: Context gives meaning to sensations. We process the sensory information according to the context within which it occurs. Psychophysical experiments demonstrate that the perception of a stimulus depends on the context. In the primary visual cortex (V1), pyramidal cells are activated by patches of gratings in their receptive field, and the visual context is given by the stimulus presented outside their receptive field, i.e. in their surround. Contextual information reaches pyramidal cells in V1 both through feedforward and feedback pathways. How these distinct pathways interact remains unclear. Here, we investigate how the response profile of single neurons in V1 is influenced by context. Moreover, we study how contextual information carried by feedforward and feedback pathways interact. Preliminary results show that the information carried by feedforward and feedback pathways can reinforce or cancel each other.

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Poster

141. Vision: Visual Cortex: Circuits and Populations

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Program #/Poster #: 141.26/CC1

Topic: D.07. Vision

Title: Contrast mismatch leads to shifts in interocular balance

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Abstract: Short-term monocular deprivation (STMD, depriving one eye for 1-3 hours, either total occlusion or pattern deprivation) disrupts interocular balance for a period of about one hour after deprivation. This deprived eye (DE)-enhancement has been measured psychophysically in adult humans and in our present studies using anesthetized adult macaques, assessed with intrinsic signal optical imaging and multielectrode recording of V1 activity. The DE boost operates with a decay time constant of approximately 15 minutes. The cells responsible for this effect are not yet known. We observed that the interocular gain shift is bidirectional, moving opposite to the imposed contrast mismatch. A contrast imbalance of between the two eyes (either an increase or decrease) created effects similar to complete deprivation. Elevating (or decreasing) the contrast in one eye, referred to as the manipulated eye (ME), for a period of 1.5-2 hour) elevated (or decrease) responses to an unchanged contrast stimulus in the other, unmanipulated eye (UE). An unequal contrast stimulus, as opposed to full occlusion, allowed for continuous measurement of the ME and UE driven responses. Analyzing responses in the ME during the contrast mismatch period permitted the measurement of the time constant of induction of the STMD-effect as well as the time constant of decay. Establishing the induction and decay time constants will help identify the neural circuit responsible for the STMD-effect. Studying the cortical response to these contrast imbalances provides insight into the dynamics of adult experience-dependent plasticity.

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Poster

141. Vision: Visual Cortex: Circuits and Populations

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Support: Simons Foundation
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Title: Laminar and columnar variation in two-dimensional spatial frequency tuning in macaque V1

Authors: J. PAI, L. E. HALLUM, C. SHOONER, R. T. RAGHAVAN, J. G. KELLY, M. J. HAWKEN, *J. A. MOVSHON
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Abstract: In primates, signals relayed by V1 neurons provide the basis for spatial vision. These neurons are selective for the spatial structure of stimuli presented to their receptive fields, and this selectivity can be captured by measuring their tuning for the orientation and spatial frequency of sinusoidal grating targets, 2-dimensional spatial frequency (2dsf). Previous data show considerable diversity in neuronal 2dsf tuning, and we wanted to know how much of that diversity was attributable to the laminar and columnar location of recordings. We used linear electrode arrays (NeuroNexus, 32 contacts, 0.05 or 0.1 mm spacing) to study the representation of 2dsf throughout the layers of V1 in opiate-anesthetized paralyzed macaques. To sample the laminar and columnar organization of selectivity, we made penetrations at various angles to the pial surface. We measured 2dsf tuning for single- and multiunit activity at each recording site by presenting a pseudorandom sequence of briefly-presented gratings (100 ms) drifting at 10 Hz, moving in 24 directions (15 deg steps) at 13 spatial frequencies from 0.25-16 c/deg (half-octave steps). We measured responses separately in the two eyes, and took the dominant eye's response at each site. We used a combination of histological and electrophysiological criteria to assign each recording site to a cortical layer. To describe responses at each site, we used a polar-separable model in which the 2dsf tuning is taken as the product of an orientation and direction selectivity given by a pair of Von Mises functions separated by 180 deg and a spatial frequency selectivity given by an asymmetric log-Gaussian. We found systematic variations in 2dsf tuning with recording site. Sites in layer 4c often showed broad 2dsf tuning, with little or no orientation tuning and broad spatial frequency tuning. Sites in layers 2 and 3 were the most narrowly tuned on average, with the tightest spatial frequency tuning and relatively narrow orientation tuning. Sites in layers 4a, 4b, 5, and 6 had similar orientation tuning to sites in layers 2 and 3 but somewhat less narrow spatial frequency tuning; most directionally selective sites were in these layers. These variations suggest that much of the diversity in 2dsf tuning is attributable to the laminar location of cells, which is also linked to their anatomical projection targets. V1 may therefore be considered to have multiple parallel representations of image structure, each specialized for the needs of particular downstream areas.

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Poster

141. Vision: Visual Cortex: Circuits and Populations

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NIH Intramural

Title: Characterizing hierarchical computation within V1

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Abstract: The ability to perform complex visual tasks such as object recognition is thought to require a hierarchy of visual areas within the primate cortex. Indeed, this idea is supported by the success of deep neural networks (DNNs), which can be trained to perform such visual tasks with similar performance to humans. However, while such networks might serve as useful qualitative descriptions of visual system function, it is unclear how to validate them using neural data, and more broadly, how to determine how a given stage of processing within the visual cortex contributes to hierarchical computation. Here we introduce a new machine learning approach, the Nonlinear Scaffold Model (NSM), to answer these questions. Responses of hundreds of neurons were recorded over multiple recording sessions from V1 of awake, fixating macaque monkeys using laminar probes -- allowing for the inference of cortical layer location of each neuron -- during presentation of a temporally varying random bar stimulus. We trained the NSM, a multi-level DNN, to predict the responses of all neurons in the dataset. Unlike other DNNs that the predict responses from the output of the last DNN level, the NSM computes responses by sampling outputs across all levels of the DNN. Thus, neurons whose responses represent simpler, "less nonlinear" computations will preferentially connect to earlier DNN levels, and deeper, "more nonlinear" computations can be sampled from the higher DNN levels. We show that the NSM, which fits all neurons simultaneously, on average provides a better description of the recorded neural activity than standard nonlinear cascade models, which are fit to each neuron individually. But more importantly, the NSM provides a "scaffold" for understanding the relationship of individual neurons to each other, in the context of the hierarchical computation being performed. We demonstrate that each successive level in the DNN approximates the computations performed by neurons in different cortical layers: with, for example, layer 4 neurons preferentially connecting to the earlier DNN levels, and layers 2/3 and 5/6 neurons connecting to higher DNN levels. These and related analyses thus provide a novel perspective on the relationship between computations across layers in V1, and more broadly provide a new means to establish the participation of recorded neural data in hierarchical cortical computation.

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Poster

141. Vision: Visual Cortex: Circuits and Populations

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Topic: D.07. Vision

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Title: Dependence of the spatial acuity of V1 neurons on eccentricity across the fovea and parafovea

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Abstract: Visual processing in the fovea (center-of-gaze) is central to human perception, yet most neurophysiological studies of neural processing focus on neurons in the parafovea (>2 degrees eccentricity). This is in part because foveal receptive fields are small relative to errors in conventional eye-tracking hardware, making detailed assessment of receptive field properties unreliable. Previous studies have found that spatial frequency (SF) tuning of V1 neurons increases with eccentricity, which mirrors the increase of overall receptive field sizes. Here, we recorded V1 neurons at eccentricities varying from 0.5 to 16 degrees of visual angle in awake, fixating macaque monkeys during presentation of a temporally varying random bar stimulus. We used a model-based eye-tracking algorithm to accurately correct for fixational eye movements, allowing for the determination of detailed receptive field properties. We fit nonlinear cascade models to the recorded responses, which provided detailed information about their receptive field properties as well has how multiple “subunits” -- each selective to a different spatiotemporal feature -- combine nonlinearly to best predict the observed response. The resulting models demonstrate several clear trends across eccentricity. First, foveal neurons are tuned to high SFs and integrate subunits with different spatial profiles from similar spatial locations, whereas models of cells at high eccentricity required a larger number of subunits that are more spatially dispersed. However, surprisingly, we found that the properties of the subunits themselves - particularly their size and spatial frequency tuning - had little dependence on eccentricity over the ranges tested. We validate these model-based measurements using forward-correlation analysis of spatial frequency tuning, which provides a model-free validation of our, more sensitive, model-based measures. Through the use of model-based eye-tracking and subsequent analyses, this study thus provides the first detailed comparisons of visual processing acuity between foveal versus parafoveal neurons.

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Poster

141. Vision: Visual Cortex: Circuits and Populations

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Support: R01EY024071

Title: Whole cell recording reveals robust anticipatory top-down signal in macaque V1 during visual detection

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Abstract: Early sensory cortical areas receive a large number of feedback projections from higher cortical areas, yet the role of these connections in mediating behavior is not well understood. Such top-down signals could optimize cortical representations for task demands, yet these modulations are likely to be subtle in order to minimize the interference with sensory signals that are useful for multiple concurrent perceptual goals. Consistent with this possibility, top-down modulations are typically small or absent in single unit measurements in macaque V1. It is possible, however, that important top-down modulations are present at the subthreshold level. Indeed, previous (Vm) studies using voltage-sensitive dye imaging, which measures the pooled membrane potential over large populations of neurons, revealed robust top-down modulations in macaque V1 (Chen & Seidemann, 2012), but the effects of these modulations at the single neuron level are unknown. The goal of the current study was to use whole cell recording to measure, for the first time, the effects of top-down modulations at the level of single V1 neurons. Two macaque monkeys were required to report whether a small, low contrast Gabor target appeared at one of two possible locations by making a saccade toward the target location. At a random interval after the animal established fixation, it received a temporal cue 300 ms prior to target onset (tone + fixation point dimming). In a subset of the trials, no temporal cue was given and no visual target appeared. On those 'blank' trials the animal was required to maintain fixation. As expected, when the target appeared in V1 neuron's receptive field, we observed clear contrast-dependent visual response. However, when we compared Vm in detection trials in which no target appeared in the receptive field to Vm in blank trials, we observed reliable depolarization that started shortly prior to stimulus onset. V1 neurons exhibited significant Vm depolarization in the interval between target onset and saccade initiation (Monkey T, mean depolarization =1.84 +/- 0.15 mV, N=11, monkey A, mean depolarization = 1.24 +/- 0.19, N=11). Of the 22 recorded neurons, 18 showed significant Vm depolarization. This small but reliable depolarization has a weak effect on the spiking activity of single neurons, but is likely to have a robust effect at the level of neural population due to the highly

interconnected nature of cortical circuits. These results demonstrate that primary visual cortex is not a purely sensory area, but instead contextual information modulates visual responses in a way that is likely to improve performance in demanding perceptual tasks.

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Poster

142. Vision: Visual System: Response Modulation and Adaptation

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Support: NIH EY04067

Title: Characterization of vip-1 coupling and its modulation by dopamine

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Abstract: Introduction: Amacrine cells consist of a heterogeneous group of inhibitory interneurons that form distinct microcircuits and participate in visual information processing in the inner retina. This study has focused on defining and characterizing the vasoactive intestinal peptide (VIP) type 1 amacrine cell type in the mouse retina using a VIP-Cre recombinase mouse line. VIP-1 amacrine cells are bistratified cells that ramify in strata 1, 4, and 5.
Methods: VIP-tdTomato was generated by crossing a VIP-Cre transgenic mouse line (JAX #10908) with a Cre-dependent tdTomato (JAX #7914) reporter mouse line. Fluorescent cell bodies in retinal whole-mounts were imaged and reconstructed using a Zeiss LSM 710 confocal microscope. VIP-tdTomato fluorescent cell bodies in the inner nuclear layer (INL) were injected with Neurobiotin in retinal whole-mounts to define their general morphology, stratification patterns and to test their gap junction connectivity with other cells in the presence and absence of dopamine.
Results: Tracer injections revealed that VIP-1 amacrine cells are homologously coupled to other VIP-1 amacrine and heterologously to non-VIP amacrine cells in the INL. VIP-1 cells also exhibited coupling in the ganglion cell layer (GCL). Based on RBPMS immunostaining, coupled cells were defined as ganglion cells and displaced amacrine cells.
In order to identify the connexin forming the gap junctions, VIP-1 cells were immunostained against Cx36, the main connexin in the retina. Our data showed that the dendritic field in the ON layer expressed the majority of Cx36 when compared to the OFF layer (t-test ; $p<0.05$), suggesting that the majority of coupling occurs in the ON layer.
The number of coupled cells was significantly reduced in the presence of dopamine in the INL as

well as in the GCL, indicating that dopamine modulates the permeability of the gap junctions to other VIP-1 cells and non-VIP cells.
Conclusion: VIP-1 amacrine cells are homologously and heterologously coupled to cells in the INL and GCL. Electrical synapses/Gap junctions are made up of Cx36, mainly located in the ON layer. The extent of tracer coupling is modulated by dopamine, suggesting that endogenous dopamine might uncoupled VIP circuitry during light adaptation.

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Poster

142. Vision: Visual System: Response Modulation and Adaptation

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Support: Fonds voor Wetenschappelijk Onderzoek Vlaanderen
KU Leuven C1

Title: Probing the mechanisms of adaptation of macaque inferotemporal neurons with optogenetics

Authors: *F. FABBRINI[1], C. VAN DEN HAUTE[2], V. BAEKELANDT[2], W. VANDUFFEL[3], R. VOGELS[1]
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Abstract: Adaptation is the alteration of a neural response to repeated stimuli. This phenomenon has been shown in many brain areas of different species, and can vary from suppression to enhancement of the response. Neurons in macaque inferior temporal (IT) cortex, the end stage of the ventral visual stream, show a decrease in the response with stimulus repetition, known as repetition suppression. Several mechanisms have been proposed to explain adaptation in IT, from firing-rate dependent fatigue to influences of other brain areas, such as inheritance from earlier stages of the ventral visual pathway or top-down expectation effects. In order to bypass the bottom-up and top-down processing stages, we stimulated directly IT neurons using optogenetics and measured the effect of stimulation on their responses. We recorded spiking activity from single units of IT cortex of two monkeys while simultaneously stimulating optically neurons transduced with a depolarizing opsin, ChrimsonR. Optical stimulation could lead to both an increase and a reduction of a neuron's firing rate. Since we used a promoter for excitatory neurons, CamKII, it is likely that indirect inhibitory activation is responsible for the reduced firing rate. Repeated optical stimulation of the cells that showed an excitatory effect lead to a small suppression of the response to subsequent optical stimulation (interstimulus interval 400

ms), in particular when the power of the first stimulation (adapter) was high and the one of the second (test) was small. This suppressive effect was also observed for the response to a visual test stimulus that followed an optical adapter, but only in one subject and at low contrasts of the visual stimulus. This suppression might be the consequence of a long lasting post-photostimulation suppression, which was present in these neurons. Those cells that showed a decrease in the firing rate during optical stimulation showed a strongly reduced response to simultaneously presented visual and optical stimuli, i.e. a compound stimulus. Using this compound stimulus as adapter produced less suppression of the response to a subsequent visual stimulus compared with repetition of the same visual stimulus. However, this decrease of adaptation when the compound stimulus was the adapter was subtle, despite the spiking activity for the adapter being at baseline. Together, these results show that either increasing or decreasing the firing rate of a neuron during the presentation of the adapter stimulus has a minor or no influence on the response to the test stimulus, suggesting only a small if any contribution of spike frequency-dependent mechanisms to repetition suppression in IT.

Disclosures: F. Fabbrini: None. **C. Van Den Haute:** None. **V. Baekelandt:** None. **W. Vanduffel:** None. **R. Vogels:** None.

Poster

142. Vision: Visual System: Response Modulation and Adaptation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 142.03/CC8

Topic: D.07. Vision

Title: Repetition of natural images decreases firing rates and can increase gamma synchronization in V1

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Abstract: Stimulus repetition often results in reduced responses throughout visual cortex. This phenomenon is commonly referred to as “adaptation” or, especially in higher-order cortical areas, as “repetition suppression”. Behavioral performance, however, is often stable or improving, suggesting that neuronal responses do not merely fatigue, but rather change in quality or efficiency. One possible implementation of a more efficient stimulus response may be increased synchronization of spiking (Gotts et al., 2012). Strong increases in gamma synchronization were found with hundreds of repetitions of grating stimuli (Brunet et al., 2014),

but the stimulus-specificity, dynamics within the first few presentations, and generalizability to more naturalistic conditions remained unclear.
We therefore investigated changes in neuronal responses and synchronization during pseudorandomly repeated presentations of colored natural images to four monkeys. Monkeys were engaged in a change detection task on the presented stimuli (20 repetitions/stimulus, ≈2s duration, maximally 4 intervening stimuli between repetitions) while recording neuronal activity from V1. The animals' behavioral performance showed memory effects and thereby likely recognition of the objects.
Responses in multi-unit activity (MUA) decreased for the first few stimulus repetitions, and subsequently remained more stable, resembling results previously observed in IT.
We found that neuronal gamma-band synchronization (power and spike-field locking) was highly specific to individual natural images. Stimulus repetition resulted in stimulus-specific changes that included decreases as observed in the MUA, but frequently also continuous increases. The direction and strength of the repetition effect for a given site was related to the strength of gamma induced at the site. We provide evidence that the repetition effects in V1 gamma are retinotopically specific and therefore likely a local phenomenon. Collectively, we demonstrate dynamic changes in stimulus representation on the timescale of seconds.

Disclosures: A. Peter: None. **J. Dowdall:** None. **L. Klein:** None. **J. Klon-Lipok:** None. **K. Kouroupaki:** None. **M. Schoelvinck:** None. **J. Schmiedt:** None. **K. Shapcott:** None. **W. Singer:** None. **M.C. Schmid:** None. **P. Fries:** None.

Poster

142. Vision: Visual System: Response Modulation and Adaptation

Location: SDCC Halls B-H

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Program #/Poster #: 142.04/CC9

Topic: D.07. Vision

Support: ERC Starting Grant 678286
VIDI grant 452-13-016
James S McDonnell Foundation Grant 220020373

Title: Temporal tuning of repetition suppression across the visual cortex

Authors: *M. FRITSCHE, S. J. D. LAWRENCE, F. P. DE LANGE
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Abstract: The visual system adapts to its recent history. A phenomenon related to this is repetition suppression - a reduction in neural responses to repeated compared to non-repeated visual input. While neural repetition suppression has been extensively studied and employed as a tool to investigate neuronal selectivity using fMRI, the temporal dynamics of repetition

suppression have received less attention. An intriguing possibility is that the timescale over which repetition suppression occurs across the visual hierarchy is tuned to the temporal statistics of visual features. Here, we tested this hypothesis by studying the influence of the temporal lag between successive visual stimuli on repetition suppression along the visual hierarchy using fMRI. Twelve human volunteers each engaged in four fMRI sessions in which we characterized the BOLD response to pairs of repeated and non-repeated natural images with inter-stimulus intervals ranging from 50 to 1000 milliseconds, to quantify both the general temporal tuning of repetition suppression as well as inter-regional differences along the anterior-posterior axis of the visual system. We observed a strong temporal tuning of repetition suppression. While repetition suppression was absent for a very short-inter stimulus interval of 50 ms, it increased and was maximal at 200 ms. Interestingly, the effect turned into repetition enhancement for the longest (1000 ms) inter-stimulus interval. This overall pattern was observed in all visual areas under investigation. Assessing differences between visual areas, in line with our expectation, the rate at which repetition suppression decayed and turned into repetition enhancement was higher for posterior compared to anterior visual areas. Overall, our results provide novel insights into the temporal dynamics of repetition suppression, suggesting that neural responses to repeated versus non-repeated naturalistic visual input are strongly modulated by the amount of intervening time. This may have important implications for our understanding of the relationship between repetition suppression and enhancement and, in general, how humans process visual information over time.

Disclosures: M. Fritsche: None. **S.J.D. Lawrence:** None. **F.P. de Lange:** None.

Poster

142. Vision: Visual System: Response Modulation and Adaptation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 142.05/CC10

Topic: D.07. Vision

Support: NSERC

Title: Effect of visual adaptation on orientation selectivity in cat secondary visual cortex (V2)

Authors: *R. LUSSIEZ, N. CHANAURIA, A. OUELHAZI, S. MOLOTCHNIKOFF
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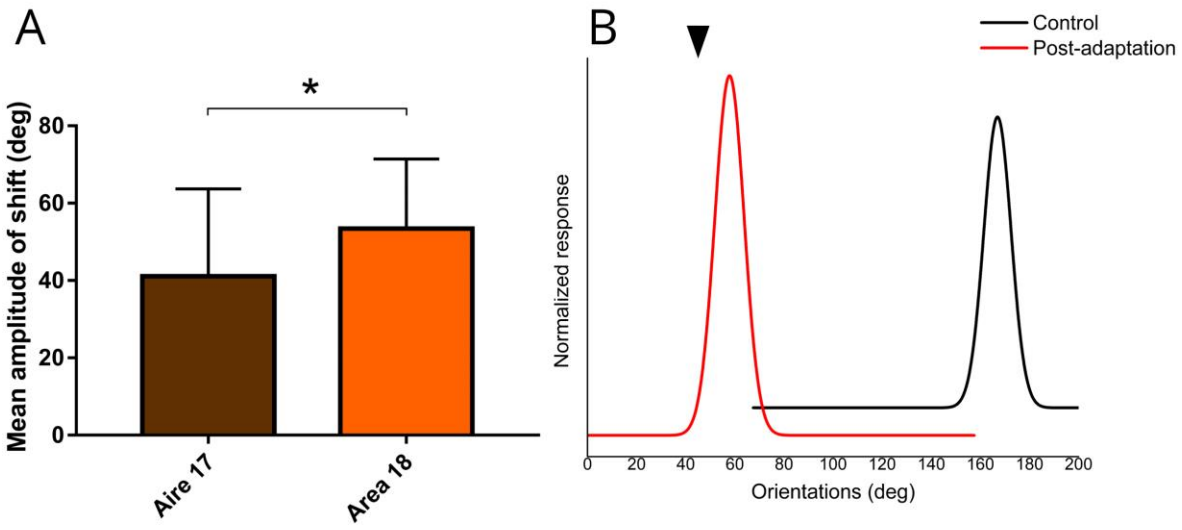
Abstract: The primary visual cortex (V1) is considered to be the main gate to the visual cortex, as it receives visual information from the thalamus, and then relay it to other visual areas, including the secondary visual cortex (V2). As previously demonstrated in plural studies in mammals, orientation selectivity in primary visual area may change, following a period of adaptation. To our knowledge, such change in orientation selectivity has not been shown in V2,

which receives a major input from V1. Our aim in this study is to investigate orientation selectivity changes in V2 cells, following previously established adaptation protocols in V1 studies. Using electrophysiological multi-unit recordings in V2, we recorded the electrical activity of neurons, before and after adaptation, in layers II-III and layer V of an adult anesthetized cat. To better understand the properties of the resulting shift in orientation selectivity, we first quantified the amplitude of the shift. Globally, the amplitude of the shift is bigger with V2 neurons than with V1 neurons. This result then suggests that V2 neurons exhibit a higher plasticity than V1, towards an adaptation protocol. We also studied the orientation selectivity index (OSI) in V1 and V2. At control, the OSI of V2 neurons is significantly lower than that of V1 neurons. The neurons in V2, in spite of responding optimally to one visual orientation, are less selective than V1 neurons. Moreover, OSI decreased in V2 cells after adaptation, suggesting a higher plasticity at the expense of selectivity.

Figure 1. Mean amplitude of shift in area 17 and 18. **(A)** Bar graph of mean amplitude of shit in area 17 and 18 of cat visual cortex. **(B)** Tuning curves of area 18 cell ; black triangle corresponds to adapting orientation.

Sup. : NSERC



Disclosures: R. Lussiez: None. **N. Chanauria:** None. **A. Ouelhazi:** None. **S. Molotchnikoff:** None.

Poster

142. Vision: Visual System: Response Modulation and Adaptation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 142.06/CC11

Topic: D.07. Vision

Support: NSERC

Title: Modulation of cerebral plasticity by drug application: Effect of ketamine on orientation selectivity and variability of neuronal responses

Authors: *A. OUELHAZI, N. CHANAURIA, L. BACHATEN, R. LUSSIEZ, S. MOLOTCHNIKOFF
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Abstract: The plasticity of the adult brain has been one of the most evolving areas of recent neuroscience research. It has been acknowledged that the visual cortex in adulthood can adapt and restructure the neuronal connections in response to a sensory experience or to an imposed input such as in adaptation or ocular deprivation protocols. In order to understand the basic cellular and molecular mechanisms of plasticity in the primary visual cortex (V1), we examined the effects of ketamine, a non-competitive, glutamatergic NMDAR (*N*-methyl-d-aspartate receptor) antagonist, on the orientation of the cortical cells by measuring their response variability and the gaussian tuning curves, in adult anesthetised mice. Neurons were recorded extracellularly using glass electrodes. The ketamine was applied locally by placing a custom-cut filter paper (1x1mm) soaked in the ketamine solution (10 mg /ml) on the cortical surface surrounding the site of electrode penetration. Our results show that the local application of ketamine on V1 modifies the preferred orientation of the visual neurons, established during the critical period of development. Furthermore, ketamine also leads to a decrease in the variability of neuronal evoked responses (measured by Fanofactor) and the orientation selectivity (measured by OSI). These results suggests that ketamine induces plasticity in V1 neurons that might be operated by a different pathway that of NMDA.

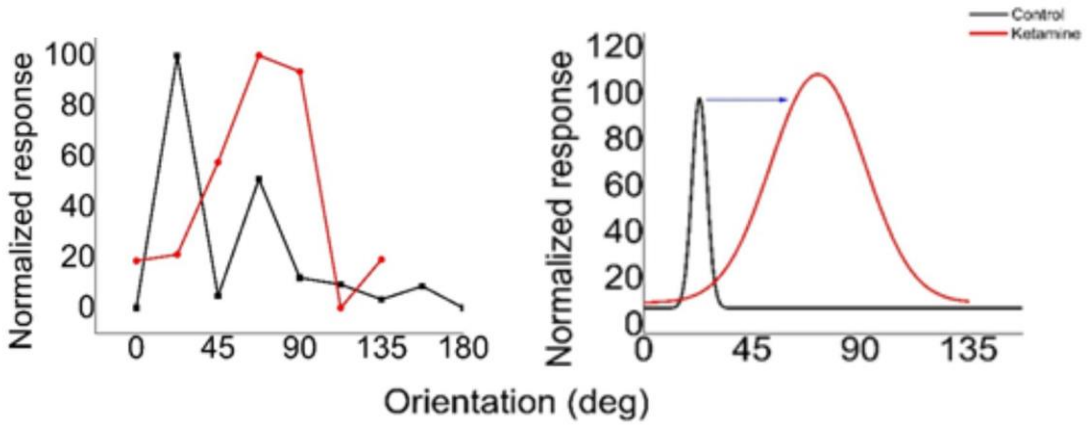

Figure: Typical example of optimal orientation shift. Raw data (left) and Gaussian fit of normalized responses of one neuron for control (black) and after ketamine application (red).

Disclosures: A. Ouelhazi: None. **N. Chanauria:** None. **L. Bachaten:** None. **R. Lussiez:** None. **S. Molotchnikoff:** None.

Poster

142. Vision: Visual System: Response Modulation and Adaptation

Location: SDCC Halls B-H

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Program #/Poster #: 142.07/CC12

Topic: D.07. Vision

Support: MEXT KAKENHI Grant Number JP 18K11485
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Title: Assessing the impacts of correlated V1 activities with dissociated timescales

Authors: *T. TAKAHASHI[1,2], Y. MARUYAMA[4], H. ITO[5], K. MIURA[3]
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Abstract: The problem of correlated fluctuations has been an issue in neuroscience because small correlations in trial-to-trial response variations or noises can decrease coding capacity by sensory neurons dramatically. Although significant noise correlations have been observed in almost all recorded cortical areas, nonstationarity or a gradual drift of baselines might engender artificial correlations even if no actual correlation exists. Therefore, we dissociate the observed noise correlations into short- and long-term components, where the latter is possibly caused by the background trends or fluctuations of the baseline activity. Although attempts to separate them and estimate purely short-term noise correlations under changing environments have been made previously, they were ad hoc and useful only for specific cases. In this study, we propose a information-geometric method to unbiasedly estimate pure short-term noise correlations irrespective of the background brain activities. The accompanying statistical test as well as the existing non-stationarity test enabled us to dissociate short- and long-term noise correlations. When we exclude the spurious noise cor- relations of purely long-term nature, only small fraction of V1 neuron pairs showed significant short-term correlations, possibly reconciling the previous inconsistent observations on existence of significant noise correlations. Finally, with the additional help of the machine learning that classifies stimuli from neural activities, we assessed the impacts on decoding of the presence of short- or long-term noise correlations, separately. We found that the influence of pure short-term correlations was little while the pure long-term correlation significantly increased the classification success rate, possibly with enhanced generalization ability. Thus, our method enables us to elucidate the functions of short- and long-term noise correlations in a dissociated manner.

Disclosures: T. Takahashi: None. **Y. Maruyama:** None. **H. Ito:** None. **K. Miura:** None.

Poster

142. Vision: Visual System: Response Modulation and Adaptation

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Program #/Poster #: 142.08/CC13

Topic: D.07. Vision

Support: KAKENHI16K00220
KAKENHI17K07050

Title: A spiking network model of the rodent visual cortex: What visual information is represented

Authors: *M. MIYASHITA[1], S. TANAKA[2]
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Abstract: In the cat primary visual cortex, almost all neurons selectively respond to specific orientations of image contours presented in the visual field. On the other hand, in the rodent visual cortex, a large number of neurons do not respond to oriented stimuli, although a minor portion of neurons are orientations-selective and sparsely distributed in the visual cortex. This species difference raises a question of what functional roles neurons unresponsive to oriented stimuli play in the visual information processing in rodents. Recently we have reproduced both types of orientation representations in the visual cortices of cats and rodents using the same self-organization model with changing only one parameter: the probability of excitatory cortical lateral connections. Computer simulations have shown that at low connection probability, orientation-unresponsive neurons markedly increased in number and orientation representation became similar to the experimental observations. In the present study, to explore possible functional roles of rodent visual cortical neurons, particularly orientation-unresponsive neurons, we performed computer simulations of spike generation in neural networks composed of LIF neurons with the self-organized afferent inputs, sparse excitatory lateral connections and isotropic inhibitory lateral connections. We mainly examined model neurons' responses to moving oriented sinusoidal gratings and temporally luminance-changing uniform stimuli. A minor portion of orientation-responsive neurons increased firing rate as luminance contrast was elevated in a lower contrast range, but their firing rate was saturated or suppressed in a higher contrast range. On the other hand, orientation-unresponsive neurons responded to temporally luminance-changing uniform stimuli. The average firing rate of excitatory cortical neurons in response to uniform stimuli was maximal at a moderate level of luminance contrasts, whereas the average firing rate of inhibitory neurons increased monotonically as the stimulus contrast was elevated. There was a strong tendency that inhibitory neurons elicited more spikes than excitatory neurons at higher contrasts. These simulation results suggest that orientation-

unresponsive excitatory neurons existing abundantly in the rodent visual cortex are specialized to detect temporal changes in the luminance of the animal's environment, and that their response gain is controlled by inhibitory interneurons.

Disclosures: M. Miyashita: None. **S. Tanaka:** None.

Poster

142. Vision: Visual System: Response Modulation and Adaptation

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Topic: D.07. Vision

Support: NWO VIDI grant 864.10.010

Title: Neuronal adaptation to visual stimuli in the mouse superior colliculus

Authors: *M. AHMADLOU, K. DYL, J. HEIMEL
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Abstract: Efficient detection of novel visual objects in the environment and rapid induction of appropriate behavioral response are crucial for survival. One of the mechanisms responsible for this process, adaptation, has been extensively studied in different visual regions of various species. However, there has been no evidence of visual adaptation in the superior colliculus (SC) of the mouse. As the SC is associated with processing life-threating stimuli, which leads to fear and avoidance behavior, effective detection of unexpected stimuli should constitute a fundamental property of this region. The purpose of this work is to fill this gap by investigating the characteristics, the possible mechanisms and the source of adaptation in the mouse SC. We used electrophysiological multi-unit recordings of the superficial SC (sSC) and the primary visual cortex (V1) in anesthetized mice during visual stimulation. Our results show that sSC neurons adapt to moving objects and drifting gratings. Adaptation is not stimulus-specific, vanishes within 15 minutes and may stem from presentation of surround stimuli. Moreover, we found that the repetition effect in V1 significantly varies from this process in the SC. Our data demonstrate that adaptation in the SC still occurs after silencing V1 or parabigeminal nucleus. Our research suggests that adaptation is generated by two possible mechanisms in the local circuits of the sSC. This study provides new insights into characteristics of visual adaptation in the mouse SC.

Disclosures: M. Ahmadlou: None. **K. Dyl:** None. **J. Heimel:** None.

Poster

142. Vision: Visual System: Response Modulation and Adaptation

Location: SDCC Halls B-H

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Program #/Poster #: 142.10/CC15

Topic: D.07. Vision

Support: DFG: FOR-1847 and IRTG-1901 to F.B.
ERC Consolidator grant 614244 to R.V.

Title: The effects of short-term monocular deprivation on visual perceptual echoes

Authors: J. SCHWENK[1], R. VANRULLEN[2], *F. BREMMER[1]
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Abstract: A specific component in the time-course of visual processing has recently been identified in the EEG, the 'perceptual echoes'. These are long-lasting oscillations in the alpha range (7-13 Hz), induced by random luminance sequences and persisting for a few hundred milliseconds. Although the echoes' functional role for perception has received growing interest, very little is known about the neural processes from which they arise. Here, we aimed to examine the echoes' dynamics in response to homeostatic cortical plasticity. Monocular deprivation (MD) has been established as a paradigm to induce plasticity in primary visual cortex (area V1) in humans. Distinct changes in the processing of visual information to the deprived eye occur after few hours of MD. Among these, an enhancement of early components of the visual evoked potentials (VEPs) was described as well as a reduction of the overall GABA-level in V1, inducing a modulation in cortical excitability.
We employed MD to investigate if and how the perceptual echoes depend on the local state of cortical excitability in early visual areas. Human participants were presented with nonperiodic random luminance sequences (6.25 seconds) before and after 150 min of MD while a 64-channel scalp EEG was recorded. We used a stereoscopic setup in which independent sequences were presented simultaneously to both eyes. Impulse-Response-Functions were derived from the same EEG epochs for the deprived and non-deprived eye by cross-correlating the respective input luminance sequences with the EEG.
Our preliminary results reveal that the amplitude of the perceptual echoes increased from pre- to post-MD measurements in response to stimulation of the deprived eye and decreased for the other eye. This suggests that the late echo components of the visual response are modulated by short-term MD in a similar way as the earlier (VEP) components, and that they may depend directly on the latter's excitatory drive. Interestingly, our results also revealed a decrease of echo peak frequency with deprivation. This stands in contrast with the dynamics of classical alpha oscillations, which tend to increase in frequency with higher states of excitability. Our results

provide further evidence for an active role of the perceptual echoes in visual processing and suggest a dissociation with non-phase-locked alpha oscillations.

Disclosures: J. Schwenk: None. **R. VanRullen:** None. **F. Bremmer:** None.

Poster

142. Vision: Visual System: Response Modulation and Adaptation

Location: SDCC Halls B-H

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Program #/Poster #: 142.11/CC16

Topic: D.07. Vision

Support: NIH Grant EY028163

Title: Population contrast response functions in human visual cortex

Authors: *I. M. BLOEM[1,2], L. VINKE[3,2], S. LING[1,2]
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Abstract: Neurons within early visual cortex exhibit a well-characterized relationship between stimulus contrast and neural response; firing rate initially increases in a relatively linear fashion, but eventually saturates, evoking little-to-no change between higher contrast levels. While this neural contrast response function (CRF) has been demonstrated to have a monotonic relationship with behavioral performance, it remains controversial whether the same holds for population-based CRF measures obtained with human neuroimaging. fMRI BOLD responses reflect the activity of thousands of neurons, exhibiting large variability in their contrast sensitivity within a single voxel. In this study, we utilized an fMRI adaptation paradigm to re-center neurons' dynamic range to the time-averaged mean contrast level, potentially reducing the heterogeneity in contrast sensitivity among neurons within a voxel. We measured BOLD responses in early visual cortex (V1-V3) while participants viewed displays composed of equally-spaced apertures arranged in four concentric ring patterns, each of which increased in size with eccentricity. Participants viewed apertures containing grating stimuli at a fixed spatial frequency (~0.5 cpd), oriented along the radial axis out from fixation, which varied rapidly between contrast intensities throughout a scan (15 contrast levels, spaced between 0%-100% Michelson contrast). Utilizing a novel encoding model to estimate BOLD responses for this wide range of rapidly presented stimuli of various contrast levels, we were able to reconstruct the population contrast response function (pCRF) by assuming that its shape is best reflected as a hyperbolic ratio function (Naka-Rushton function). The results reveal that early visual regions exhibited saturating pCRFs, with its semi-saturation point reflecting the time-averaged mean contrast level. Furthermore, the estimated parameters obtained from modeling the BOLD time courses of individual voxels illustrated the same patterns. In sum, our results demonstrate a method that allows for the

efficient mapping of the pCRF for individual voxels within early visual areas, suggesting that by leveraging adaptation the estimated pCRF seems more comparable to electrophysiological and psychophysical measures.

Disclosures: I.M. Bloem: None. **L. Vinke:** None. **S. Ling:** None.

Poster

142. Vision: Visual System: Response Modulation and Adaptation

Location: SDCC Halls B-H

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Program #/Poster #: 142.12/DD1

Topic: D.07. Vision

Support: EY024858

Title: Neural responses to unexpected stimuli in early and mid-level visual cortex

Authors: *S. S. SOLOMON, E. S. SUSSMAN, A. KOHN
Dept. of Neurosci., Albert Einstein Col. of Med., Bronx, NY

Abstract: Sensory neurons adapt to a prolonged or recurring stimulus, so that responses to rare or different stimuli appear relatively enhanced. This adaptation may explain why recurring or 'expected' stimuli are less salient. However, expectation can also be defined by other stimulus properties than persistence or recurrence. For instance, stimuli may change regularly or smoothly in time, so that there is a clear expectation of what should appear next. Whether sensory cortical networks can adapt to the temporal structure of a stimulus sequence, a more sophisticated form of predictive coding than simple fatigue-based adaptation, is unclear. To test this, we studied neuronal spiking responses and local field potentials (LFPs) recorded in early visual cortex, V1, and mid-level visual cortex, V4, of an awake macaque monkey. We presented three different sequences of gratings, with one sequence presented 80% of the time (standard sequence), and the other two sequences each presented 10% of the time (deviant sequences). The standard sequence generated an expectation of which grating should appear at each time. The deviant sequences allowed us to evaluate the effect of violating this expected pattern. We found little evidence of expectation violation in either V1 or V4, in spiking responses or LFPs. When a grating appeared at an unexpected time during the sequence, responses were either indistinguishable from when that grating was expected, or the different response could be explained by fatigue-like adaptation. We conclude that circuits in V1 and V4 do not signal the violation of an expectation formed by a temporal pattern.

Disclosures: S.S. Solomon: None. **E.S. Sussman:** None. **A. Kohn:** None.

Poster

142. Vision: Visual System: Response Modulation and Adaptation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 142.13/DD2

Topic: D.07. Vision

Support: DFG TE 1182/1-1
R01-EY023581
P30-EY003039
R01-EY023591
NSF IIA-1539034
Eyesight Foundation of Alabama

Title: Rapid adaptation to cone-targeted stimuli during stochastically driven activity in macaque LGN neurons

Authors: *P. TELLERS[1], M. R. HOLLER[2], L. C. SINCICH[1]
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Abstract: Adaptation to fluctuating light levels is an important feature of normal vision. Neural adaptation occurs at many stages of the macaque visual system, beginning with the cone photoreceptors themselves, and appears to involve multiple mechanisms operating on different time scales (Schnapf et al. 1990) and under different luminance conditions (Dunn et al. 2007). A key question is how rapidly such adaptation can take place, especially in the context of everyday vision where incident light can change quickly in time. We have examined this question in a cone-targeted manner in macaque LGN neurons, where we can use a stimulus probe that modulates only one or two cones during otherwise constantly changing stimulation of the remainder of the receptive field. We used a multiwavelength adaptive optics scanning laser ophthalmoscope to simultaneously image the cone mosaic and present movie stimuli over LGN receptive fields in anesthetized macaques undergoing neuromuscular blockade. Imaging was achieved with 840 nm light, while red and green stimulus channels were independently modulated to drive L and M cones equally (543 nm) or L cones preferentially (710 nm). Mean luminance of the combined light sources was 195 cd/m^2. To create ongoing background stimulation via the visible channels, we presented binarized white noise movies at 30 Hz, with spatial resolution of either 100 or 150 pixels/deg and with real-time retinally-stabilized delivery. Spatiotemporal receptive fields were mapped by reverse correlation of spike-triggered average movies and were located 0.4°-4° from the fovea. Because spike activity was tightly phase-locked to the stimulus frame rate, we quantified the change in probability of firing during defined sequences of preferred stimulus modulation in the green channel (either ON or OFF) of a single

stimulus pixel lying within the receptive field center. Stimulus pixel dwell time was 0.1 msec. In 4 ON cells recorded in one macaque, we found that firing probability increased by 37±8% over the background firing rate for the first occurrence of a bright stimulus pixel. By the second occurrence of the same stimulus, the probability of firing dropped to 18±5% over the background rate, indicating that adaptation to a given light level can occur within 33 msec. A similar adapting response profile was found in one OFF cell (53% increased firing over background for the first dark pixel, dropping to half as much for subsequent dark pixels). Because a stimulus pixel impinged on one or two cones in these recordings, our data suggest cone driven activity can adapt rapidly in the LGN in the presence of ongoing visual stimulation in vivo.

Disclosures: P. Tellers: None. **M.R. Holler:** None. **L.C. Sincich:** None.

Poster

142. Vision: Visual System: Response Modulation and Adaptation

Location: SDCC Halls B-H

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Program #/Poster #: 142.14/DD3

Topic: D.07. Vision

Support: NIH Grant EY028163

Title: Luxotonic responses within human visual cortex depend on stimulus contrast

Authors: *L. VINKE[1,3], S. LING[2,3]
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Abstract: The mean luminance in a visual scene is often thought to be discounted by the visual system almost immediately, and is rarely studied beyond the retina. This has been driven largely by models built upon the center-surround organization of visual receptive fields, which emphasize the coding of relative features, such as contrast. That said, there is mixed evidence from animal single-unit studies suggesting potential luminance-driven (luxotonic) activity within visual cortex, with a few studies reporting a nonlinear interaction between luminance and contrast. Is human visual cortex sensitive to changes in the mean luminance of a visual input? To test this, we used fMRI to measure the degree to which different mean luminance levels drive visuocortical responses. We measured BOLD responses in early visual cortex (V1-V3) while participants viewed checkerboard stimuli that varied in contrast and mean luminance. Specifically, our experimental paradigm allowed us to measure luxotonic response functions between 49 and 1278 cd/m^2 at 6 different contrast levels (4% to 96% Michelson Contrast), and at multiple spatial scales (voxel-wise and retinotopic). In order to control for changes in pupil diameter with varying luminance levels, participants viewed the stimuli monocularly through an artificial pupil. Our results reveal reliable luxotonic modulation of responses within primary

visual cortex, increasing nonlinearly as a function of mean luminance. However, the emergence of luxotonic responsivity was contingent on stimulus contrast: luxotonic response functions failed to emerge with low contrast stimuli, but as contrast levels increased, mean luminance changes evoked stronger nonlinear increases in BOLD response. These results reveal that the visuocortical neural code includes information corresponding to changes in the mean luminance of a visual signal, and increases in prominence as contrast level is increased. The mean luminance of a visual signal may act in a modulatory manner, potentially interacting with the center-surround organization of visual receptive fields by altering the balance between excitation and inhibition.

Disclosures: L. Vinke: None. **S. Ling:** None.

Poster

142. Vision: Visual System: Response Modulation and Adaptation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 142.15/DD4

Topic: D.07. Vision

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Title: Intrinsic neural oscillations modulate feature selectivity in human visual cortex

Authors: *N. RUNGRATSAMEETAWEEMANA[1,2], J. M. VETTEL[2,3,4], J. B. OLIVA[5], T. VERSTYNEN[6], J. T. SERENCES[1,7,8], J. O. GARCIA[2,3]
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Abstract: Oscillatory electroencephalography (EEG) is thought to represent the rhythmic process by which individual neurons cohere into ensembles and consequently impact human behavior. Recently, these on-going neural oscillations have been shown to influence the efficiency of information processing (Busch et al., 2009; Mathewson et al., 2009), but the precision of this influence is still unknown. In the present study, we examine the feature specificity of oscillatory modulations in the human brain, as measured via EEG, by having participants view a flickering orientation with a central letter stream and performed an orientation shift, a contrast change, or a letter detection task designed to engage different

perceptual strategies. This contrast-reversing grating created a stereotypical narrowband response restricted to posterior occipital electrodes (steady state visual evoked potential; SSVEP). A forward encoding model was used to convert the SSVEP response to orientation response profiles, creating dynamic tuning functions (DTFs; Garcia et al., 2013). The Functional Shrinkage and Selection Operator (FuSSO; Oliva et al., 2014) was then used to perform sparse functional regression on the tuning functions. Together, these techniques allow us to estimate the responsiveness of neural populations tuned to particular features (i.e., orientations) and to examine relationships between these internal representations and intrinsic neural oscillations (e.g., alpha and beta). We found that populations tuned to particular features of the stimulus are not only oscillating, but that these oscillations follow the optimal strategy of human participants. In addition, we found that oscillations in the alpha and beta band are predictive of the information encoded in the tuning function responses, demonstrating oscillatory modulations of feature specificity in human visual cortex.

Disclosures: N. Rungratsameetaweemana: None. **J.M. Vettel:** None. **J.B. Oliva:** None. **T. Verstynen:** None. **J.T. Serences:** None. **J.O. Garcia:** None.

Poster

142. Vision: Visual System: Response Modulation and Adaptation

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Australian Research Council DP0984919
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Australian Research Council DP0984494

Title: Perceived elongation of visual stimuli into the physiological blind spot can be explained by lateral V1 connections

Authors: *M. A. WILLIAMS[1], F. SMITH[1], S. GRAHAM[2], K. BROOKS[3], P. DELISSA[4], A. RICH[1]
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Abstract: Visual experience is underpinned by compensation for missing information by the brain. The physiological blind spot is a classic example of this; it is perceptually ‘filled-in’ via extrapolation of information from the surround, such that we perceive a seamless visual world. The mechanism for this has long been debated. The blind spot could be filled-in through

feedback from higher cortical regions, sending activity back to the blind spot representation in primary visual cortex (V1), or through lateral spread within V1, propagating activity inward from the borders of the blind spot representation. We tested the lateral spread hypothesis using a width judgement task to measure the perceived elongation of stimuli into the blind spot. After replicating the partial filling-in phenomenon by presenting stimuli in cardinal orientations at blind spot borders, we then presented stimuli oriented either radially or tangentially to the fovea so as to align or misalign stimuli with the arrangement of lateral connections in V1. We find that perceived elongation toward the centre of the blind spot is dramatically affected by stimulus orientation relative to the fovea. When stimuli are presented radially, filling-in is maximised; when stimuli are presented tangentially, filling-in is all but extinguished. These findings reveal the importance of V1 lateral connections for partial filling-in of the blind spot, consistent with the lateral spread hypothesis.

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Poster

142. Vision: Visual System: Response Modulation and Adaptation

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Topic: D.07. Vision

Support: DFG Research Fellowship 318826974

Title: Timescales of adaptation in the primary visual cortex of awake mice

Authors: *A. PAPANIKOLAOU, G. DE FRANCESCHI, S. G. SOLOMON
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Abstract: Sensory pathways in the brain adapt to the current environment by adjusting neuronal responses to the recent history of stimulation. Adaptation effects occur over a wide range of timescales, but how these timescales interact, and the neuronal mechanisms that support them remain unclear. We recorded well-tuned single (n=81) and multiunit (n=101) activity from the primary visual cortex of four awake mice (C57BL/6) in response to a vertical bar (width=5 degrees) that randomly varied in contrast polarity and horizontal location (update rate=20Hz). The ensemble of bar locations was either uniform, or biased to one location. After initial exposure to a uniform ensemble, we presented a biased ensemble for 5 minutes. Biasing the stimulus ensemble decreased the gain of neurons with receptive fields near the adaptor, and repulsed receptive fields away from the adaptor. The reduction in gain was rapid, appearing complete within 10 seconds. The repulsion of the receptive fields was slower, accumulating over at least 3 minutes. This suggests dissociation of adaptation-induced changes in gain and receptive

field organisation. To assess the persistence of adaptation-induced changes, following the initial adaptation period we returned to the uniform stimulus ensemble for 1 minute, then repeated the biased ensemble for another 5 minutes. The magnitude and timecourse of gain changes in this second adaptation period was similar to that in the first. By contrast, we saw stronger and quicker repulsion of receptive field profiles in the second biased ensemble. This suggests that traces of the previously adapted state were retained in the neuronal population. Our observations therefore suggest that changes in environmental statistics lead to rapid recalibration in the sensitivity of neuronal responses and slower, potentially long-lasting changes in receptive field organisation, suggesting at least two distinct mechanisms of adaptation that operate over different timescales.

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Poster

142. Vision: Visual System: Response Modulation and Adaptation

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Title: Frequency and phase-specific direct interaction between visually evoked and tACS induced neural signals

Authors: *Z. SUN[1], L. SHI[1], P. ZHANG[1], S. HE[1,2]
[1]Inst. of Biophysics, Chinese Acad. of Sci., Beijing City, China; [2]Univ. of Minnesota, Minneapolis, MN

Abstract: Transcranial alternating current stimulation (tACS) as a non-invasive approach has been widely applied to investigate the casual relationship between neural oscillations and cognition. However, it remains unclear whether there is frequency and phase-specific direct interaction between sensory stimulus evoked and tACS induced neuronal activities in the human brain. To investigate this question, we performed a series of psychophysical and EEG experiments. Behavioral experiments showed that not only subjects could consistently detect visual beats generated by the interaction between visual flickers and tACS induced signals, but also subjects' flicker detection threshold was elevated when tACS was applied at the same compared to different frequency as the visual flicker. EEG experiments showed that flicker detection performance was modulated by tACS in a phase dependent fashion, higher when the tACS modulation was out-of-phase, compared to in-phase, with the flicker induced SSVEP signals. Furthermore, this phase-specific modulation was highly dependent on the individual

difference of temporal dynamics of SSVEP signals. In addition, we recovered visually evoked SSVEP from tACS and visual stimulation, and found that the amplitude of the visually evoked SSVEP was higher when the tACS and visual SSVEP were in-phase compared to the out-of-phase condition, which explained the phase-specific masking effect of tACS on flicker detection performance. These findings provided strong evidence that there is frequency and phase-specific direct interaction between visually evoked and tACS induced neural signals in the visual cortex.

Disclosures: Z. Sun: None. **L. Shi:** None. **P. Zhang:** None. **S. He:** None.

Poster

142. Vision: Visual System: Response Modulation and Adaptation

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Program #/Poster #: 142.19/DD8

Topic: D.07. Vision

Title: Early visual responses to unexpected stimuli in human participants are inconsistent with predictive coding models of visual processing

Authors: *K. WALSH[1], D. P. MCGOVERN[1], E. MCNICKLE[1], S. KELLY[2], R. O'CONNELL[1]
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Abstract: The visual system has traditionally been viewed as a hierarchy of processing stages, where neurons respond to bottom-up sensory inputs and encode increasingly complex features at progressively higher cortical stages. In contrast, predictive coding models of visual processing suggest that perception arises through an iterative process where top-down predictions are revised based on feedforward error signals. While predictive coding frameworks offer a number of computational advantages, the precise manner in which they may be implemented in the brain is debated. In particular, there is conflicting evidence as to whether it accounts for neural activity at all processing levels. In the present study, we measured early visual responses, via steady state visual evoked potentials (SSVEPs), to contrast changes in a checkerboard stimulus that were expected or unexpected and exploited the fact that traditional and predictive coding models make divergent predictions regarding the neurophysiological response to surprising stimuli. While traditional feedforward models of visual processing predict that early visual responses to checkerboard stimuli should be driven solely by the contrast changes, predictive coding suggests that the neural response should be augmented by the prediction error associated with deviant stimulus patterns, leading to a larger overall response to unexpected, relative to expected, stimuli. In line with the predictions of feedfoward models of visual processing, the SSVEP reliably tracked the contrast changes associated with both the expected and unexpected sequences, with no increase in amplitude for surprising contrast changes. These findings are

inconsistent with predictive coding frameworks in which the effects of stimulus expectation extend to early visual cortex.

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Poster

142. Vision: Visual System: Response Modulation and Adaptation

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Program #/Poster #: 142.20/DD9

Topic: D.07. Vision

Title: Pre-stimulus neural oscillations modulate sensory evoked responses via distinct mechanisms of functional inhibition and baseline shift

Authors: *L. IEMI[1,2,3,4], N. A. BUSCH[5], A. LAUDINI[1], J. SAMAHA[6], A. VILLRINGER[7], V. V. NIKULIN[7]
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Abstract: Sensory evoked responses vary across repeated presentations of the same stimulus. A possible reason for this response variability is spontaneous fluctuations of pre-stimulus brain states, reflected in the power of low-frequency oscillations (8-30 Hz). It is currently hypothesized that states of strong pre-stimulus power attenuate sensory evoked responses (ER) via a mechanism of functional inhibition. This hypothesis has however received only mixed support, with some studies showing that states of strong pre-stimulus power also amplify ER. We addressed this contradiction by considering alternative mechanisms for ER generation which may depend on pre-stimulus power. Most previous studies assumed that neural oscillations are sinusoidal and, consequently, that trial averaging yields a baseline signal with a zero mean. However, because neural oscillations can be non-sinusoidal (e.g. when troughs are stronger than peaks), trial averaging may yield a baseline signal with a non-zero mean. Crucially, event-related desynchronization (ERD) of non-zero-mean oscillations may shift the signal baseline, thereby generating a late deflection of the ER (baseline-shift mechanism). Specifically, an ERD of oscillations with a negative or positive mean is expected to generate an upward or downward shift of the late ER, respectively. Accordingly, we predicted that strong pre-stimulus power of non-zero-mean oscillations would lead to a greater ERD, which would manifest as a stronger shift of the late ER. Moreover, we predicted the direction of this ER modulation to have opposite

polarity relative to the oscillatory mean. To test these mechanisms, we recorded EEG in 27 human participants during rest and during stimulation with identical high-contrast checkerboard stimuli. The analysis of resting-state oscillations revealed that alpha- and beta-band oscillations were indeed non-sinusoidal with a negative mean. We found that strong pre-stimulus power was followed by a reduction of the amplitude of the early visual ER component generated in primary visual cortex (C1, before 90 ms), consistent with a mechanism of functional inhibition. Furthermore, strong pre-stimulus power was followed by a greater ERD and, in turn, by a stronger upward shift of the late visual ER (after 350 ms), consistent with a mechanism of baseline shift. This study demonstrates that spontaneous fluctuations of oscillatory brain activity modulate visual ER via two distinct mechanisms: i) functional inhibition of the early sensory ER component and ii) baseline shift affecting the late ER.

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Poster

142. Vision: Visual System: Response Modulation and Adaptation

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Topic: D.07. Vision

Support: Hundred-Talent Program of Chinese Academy of Sciences Y4CBR11001
Beijing Science and Technology Project Z171100000117003

Title: BOLD signal modulated with perception in the superficial layer of human V1 during binocular rivalry

Authors: *C. QIAN[1], C. LIU[1,2], J. ZOU[1,2], Y. ZHUO[1], S. HE[1,3], P. ZHANG[1]
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Abstract: During binocular rivalry, the alternation of the two eyes' percept correlates with the fluctuation of neural activities throughout the visual pathway, from as early as LGN and V1 to high-level occipital temporal visual cortices. However, it is not clear how feedforward and feedback processes interact to resolve the rivalrous inputs, and in particular, whether the observed neural fluctuations reflect feedforward, local or feedback modulations of resolved rivalry competition. To answer this question, we used ultra-high field fMRI at 7T with submillimeter resolution to measure rivalry-related signals from different layers of the human primary visual cortex, with the assumption that the middle layer is dominated by feedforward signals while feedback signals modulate the superficial and deep layers. BOLD signals in early visual cortex were acquired with T2*-weighted gradient echo EPI or T2-weighted balanced-

SSFP pulse sequences. In the rivalry condition, a pair of orthogonal red and green gratings were dichoptically presented, and subjects reported their percept with button presses (red, green or mixed). In the replay condition, the same red/green gratings were monocularly presented in physical alternations to simulate the rivalry percept. Robust ocular dominance column pattern was obtained in separate scans using alternating monocular checkerboard stimulus. Results show that in the replay condition, eye-specific modulation of BOLD signal was strongest in the middle (input) layer of V1; while in the rivalry condition, the rivalrous modulation of BOLD signal was strongest in the superficial layer. A transient signal was also observed, mainly from the superficial layer of V1, at the time points of perceptual transitions in the rivalry but not in the replay condition. These layer-specific fMRI findings support the idea that rivalry-related activity fluctuation in human primary visual cortex reflects feedback modulation from higher cortical areas.

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Poster

142. Vision: Visual System: Response Modulation and Adaptation

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Topic: D.07. Vision

Support: Human Frontiers Science Program

Title: The contribution of thalamic inputs and cortical interneurons to adaptive responses in primary visual cortex

Authors: *D. BARBERA[1], N. J. PRIEBE[2]
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Abstract: Sensory systems adjust their responses based on the history of their inputs, a phenomenon broadly termed adaptation. We have previously shown that adaptive mechanism in cortex share common features across visual and somatosensory modalities: responses attenuate with frequency and change their dynamics in a similar fashion. We now seek to understand the relative roles of the cortex and the thalamus in shaping these adaptive changes. To investigate the role of feedforward inputs we performed in vivo extracellular recordings from the lateral geniculate nucleus (LGN) while presenting full field light flashes at different frequencies and compared the resulting firing rates to the membrane potential dynamics obtained via whole-cell recordings in primary visual cortex (V1). We found matched signatures of the changes in response dynamics and attenuation with stimulus frequency in the LGN and cortex. In response to the 10 Hz stimulus, responses attenuated to 19.57% of the response elicited by the first

stimulus in the train in the LGN and 15.26% in V1. We hypothesized that these responses may be in part due to recurrent feedback from the cortex to the thalamus. We therefore also recorded the responses of LGN neurons to the same visual stimulus while reversibly inactivating visual cortex, which uncovered little change in thalamic responses. To investigate how interneuron responses are affected by repetitive stimulation we also recorded extracellularly from populations of identified inhibitory neurons. We found similar adaptation profiles in both SST and PV+ inhibitory neurons to full-field flashes. These data suggest that the adaptive features to repetitive stimulation are not cortical in origin, but rather inherited from lower levels of processing such as the LGN or retina.

Disclosures: D. Barbera: None. **N.J. Priebe:** None.

Poster

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Topic: D.07. Vision

Support: NIH EY016774

Title: Testing the efficient coding hypothesis of adaptation in primary visual cortex of macaque monkeys

Authors: *A. ASCHNER[1], A. KOHN[1,2,3]
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Abstract: The statistics of the sensory environment vary over time, imposing changing demands on the brain. The responses of sensory neurons are strongly influenced by fluctuations in environmental statistics, and therefore depend on recent experience, a process known as adaptation. Although adaptation is a fundamental and ubiquitous feature of sensory encoding, its function is unclear. One longstanding hypothesis is that the purpose of adaptation-induced changes in neuronal tuning is to maximize representational efficiency. Surprisingly, there has been limited experimental testing of this hypothesis, particularly using neuronal population responses. We tested whether neuronal population responses in primary visual cortex (V1) adapt so as to maximize representational efficiency. We performed multielectrode recordings in V1, and presented continuous sequences of superimposed gratings alternating between two ‘states’ - a structured state in which stimuli were always composed of two orthogonal gratings, and an unstructured state in which the orientations of the two gratings were randomly chosen. To quantify representational efficiency, we measured joint response distributions for all pairs of cells. We compared the measured distributions to an efficient code, defined as a uniform

response distribution in which all joint responses of a given pair were equally probable. The structured state initially produced inefficient response distributions since two gratings were consistently paired. Over time, however, as neuronal responses adapted, the response distributions shifted to better match the efficient coding prediction. Our results offer credence to theories positing that adaptation may function, in part, to improve representational efficiency.

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Poster

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Topic: D.07. Vision

Support: FEMH Innovation Research Grant FEMH-2017-002

Title: Pupillometry as a quantitative tool to assess pupillary light response in healthy Taiwanese

Authors: *K. Y. JUNG, S. C. YI
Far East Mem. Hosp., New Taipei City, Taiwan

Abstract: Automated infrared-video pupillometry is a tool to evaluate the parameters of light reflex that is not visible to the naked eye. While the standardized pupil light response has been extensively investigated in Caucasian, the study is relatively unexplored in East Asia. We enrolled 659 healthy volunteers ranging in age from 14 to 94 years old, obtaining a total of 1056 paired measurements by automated pupillometer. Under controlled ambient lighting (near 0, 50~100, 200 lux), varying luminance (LED 50, 180 lux) and different light stimulation time (0.8, 1 ms), we collected the maximum aperture, minimum aperture, mean constriction velocity, maximal constriction velocity and average dilation velocity. The maximal aperture averaged 5.31 ± 0.92 mm at ambient lighting near 0 lux, and the minimal aperture after light stimulation(0.8ms, LED 50 lux) averaged 3.43 ± 0.67 mm. At the ambient lighting around 200 lux, the maximal aperture averaged 4.06 ± 0.77 mm, and the minimal aperture after light stimulation(0.8 ms, LED 50 lux) averaged 2.72 ± 0.54 mm. Our data showed ambient lighting was a concerning factor to affect the maximal pupil diameter. This finding is compatible with those of previous studies in Caucasian. The correlation between anisocoria and age was not statistically significant. This study served as an original data of healthy Taiwanese and provided essential information to apply to be a baseline for patient research in the future.

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Poster

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Topic: D.07. Vision

Support: NIH Grant EY027390

Title: Stereopsis in amblyopia: Impaired in the fovea, but intact in the periphery

Authors: *P. VERGHESE[1], S. GHAHGHAEI[2]
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Abstract: Amblyopia is associated with suppression around the fovea of the amblyopic eye, with a concomitant impairment of stereopsis. It has been proposed that in cases of anisometropia or small misalignment, there may be peripheral fusion that can serve as the basis for stereopsis (Siretanu & Fronius, 1981; Harrad, 1996). Thus it is possible that the periphery can mediate coarse stereopsis, which is known to be useful for tasks of daily living. Here, we used a novel stereo-perimetry method to test this conjecture. We measured stereopsis in the fovea and the periphery using a method analogous to perimetry. The observer maintained gaze at a fixation point, while keeping nonius lines aligned, and detected whether a target was presented in front of or behind the fixation plane. The target could appear at one of many locations on the cardinal or diagonal axes, at eccentricities of 0, 1.25, 2.5, 5, and 10 degrees from fixation, with size m-scaled for eccentricity (1° X 1° at fovea). Targets were presented at a large disparity step (10 or 15 arc min) for 1 sec. The inter-trial-interval was 3-4 seconds. Viewing distance was 40 cm. The display was made up of full-field dynamic random dots updated every 500 ms to minimize monocular cues. We tested 9 amblyopic participants (4 with anisometropia, 4 with strabismus, 1 mixed), 1 participant with micro- strabismus, and 8 controls. For controls, the stereo perimetry map for depth discrimination showed high accuracy across the visual field. Amblyopes, as a group, had significantly poorer accuracy with a significant reduction in accuracy in the foveal and parafoveal region (less than 5° eccentricity). Anisometric amblyopes were able to judge the sign of depth at eccentricities larger than 5°. This was also true for strabismic amblyopes with small deviation. However strabismic observers with large deviation were unable to detect depth up to the largest eccentricities we tested (20°). Thus, our stereo perimetry technique demonstrates that when stereopsis is present in amblyopia, it is mediated by the periphery. This supports previous studies that suggest that coarser-scale mechanisms may survive small misalignment and the monocular blur associated with anisometropia (Giaschi et al, 2013; McKee et al, 2003).

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Poster

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Title: Stimulus information is isolated from single whole-cell recordings in the visual cortex of awake mice by exploiting high-dimensional representations of dynamics

Authors: *J. K. JOHNSON[1], S. GENG[2], M. W. HOFFMAN[3], H. ADESNIK[4], R. WESSEL[5]
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Abstract: Stimulus responses of single neurons are highly variable, and this raises fundamental questions about stimulus representation. Cortical pyramidal neurons in the visual cortex have rich intrinsic dynamics but also receive both long-range inputs and inputs from the local recurrent network. The combined intrinsic and extrinsic dynamics mediate a high trial-to-trial response variability to repeated sensory stimuli. It would be useful to have a method for separating the dynamics of single neuron whole-cell recordings into components that contain stimulus information and components that do not, and then associate them with either extrinsic or intrinsic dynamics. We employ a recently introduced “dimensionality expansion” technique to access the high-dimensional phase space underlying one-dimensional whole-cell recordings. First, a delay-embedding of very high dimension (100+) is created by taking many tiny time-shifts of the original data. Next singular value decomposition (or similar dimensionality reduction method) is used to project back down to the principal components of the underlying dynamics (~7D). We made whole-cell recordings from L2/3 pyramidal neurons in mouse V1 during presentation of drifting gratings of varying contrast, size, or orientation. To test if high-dimensional phase space contains stimulus information, we assess the ability to predict stimulus from a brief period of the recording. We use two novel approaches: maximum-likelihood trajectory classification applied to the principal components, and a dynamical parameter classification method which looks for stimulus dependence in the coefficients of a best-fit system of ordinary differential equations (ODEs). The form of the differential equations is chosen by a genetic algorithm variant of the recently introduced Sparse Identification of Nonlinear Dynamics (SINDy) algorithm. For a control, we use the deflection of membrane potential or input current induced by stimulus presentation. We found that trajectories in the high dimensional phase space consistently revealed more information about the visual stimulus than a simple comparison of

evoked deflections and that the compact representation of fitted ODE coefficients was superior to both. Results were consistent across recordings of membrane potential, inhibitory current, and excitatory current and across stimulus variables. This proves that input current and subthreshold membrane potential fluctuations contain subtle stimulus and network information while providing a path for attributing this information to a dynamical source.

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Poster

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NIH R00 MH104259

Title: Firing rate, response reliability, and correlated variability distributions are similar in cortical layer 2/3 and 4 of mouse visual cortex

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Abstract: Mouse visual cortex consists of multiple distinct layers with layer-specific connectivity and cell types. Such differentiated and layered cortical structure raises the question as to its functional role. As a first step towards illuminating this fundamental question, here we ask to what extent the layers differ in their ongoing and stimulus-modulated spiking activity. To address this question, we performed two-photon Calcium imaging (GCaMP6s; 10 Hz sampling) of L2/3 (160 ± 10 um) and L4 (400 ± 15 um) pyramidal neurons in primary visual cortex of head-fixed, awake mice during repeated identical natural movie clips. For each mouse and cortical layer, we measured Calcium responses (ΔF/F) from approximately 300 neurons. We recorded neural activity sequentially in vertically registered imaging planes corresponding to L2/3 and L4 from the same mouse. From the single-neuron ΔF/F of ongoing activity and in response to repeated identical visual stimuli we computed three quantities: (i) The “firing rate” is defined as the time- and trial-averaged inferred firing rates from ΔF/F. (ii) The “response reliability” is the cross-correlation coefficient between ΔF/F from pairs of trials, averaged across all trial pairs. (iii) The “correlated variability” is the average cross-correlation between the residuals from pairs of neurons for the same trial, averaged over all trials. The residual for a

neuron and trial is trial-averaged response subtracted ΔF/F for that neuron. These analyses revealed three important results. First, firing rates redistributed among the recorded neurons when comparing ongoing vs stimulus-modulated activity. This redistribution of firing rates resulted in overall distributions of firing rates that were largely indistinguishable for ongoing and stimulus-modulated activity. Both L2/3 and L4 displayed a qualitatively similar redistribution of firing rates. Second, response reliability in both layers had a long-tail broad distribution with L4 having a lower response reliability compared with L2/3. Third, correlated variability was largely insignificant in both layers. We obtained qualitatively similar results for firing rate and response reliability but different correlated variability from another data set (Allen Brain Observatory), obtained under different experimental conditions. In conclusion, despite the differences in connectivity and cell types, we found much similarity in the statistical properties of neural activity in cortical L2/3 and the thalamorecipient cortical L4 of the same mouse, when evaluated with respect to the redistribution of neural activity, the response reliability, and the correlated variability.

Disclosures: J. Xia: None. **P. O'Neill:** None. **M. Goard:** None. **R. Wessel:** None.

Poster

142. Vision: Visual System: Response Modulation and Adaptation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 142.28/DD17

Topic: D.07. Vision

Support: College of Social and Behavioral Sciences Research Competition Internal Grant
Dell Seed Program

Title: Impact of virtual reality headset use on ocular function and subjective discomfort

Authors: *B. HACKNEY, M. F. AWAD, D. A. DEL CID, R. L. MOSHER, A. KANGAVARY, S. A. DREW
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Abstract: With the ever-growing popularity and availability of consumer-grade Virtual Reality ("VR") headsets for both entertainment and scientific purposes, we thought it prudent to investigate the possible physiological impacts of this technology. Research going back to the 1950s has described frequent visual and somatic complaints associated with exposure to artificial environments, commonly referred to as "simulator sickness" (Carnegie & Rhee, 2015). More recent research suggests that new stereoscopic VR displays may induce visual discomfort via the oculomotor mismatch between the physical distance of the screen and the perceived distance of targets. To help describe these issues, we compared subjective reports of discomfort with objective visual measurements. One hundred participants completed a Virtual Reality Symptom

Survey ("VRSS")—an assessment of VR-specific simulator sickness—after engaging in 30 minutes of an immersive rock climbing simulation using an Oculus Rift. We compared their survey results with optometric measurements taken immediately before and after VR. Analyses showed a significant increase in reported symptoms of visual discomfort post-VR, but also a significant increase in performance on the optometric tests. These findings are theoretically at odds with each other, as previous research has shown that visual discomfort is related to decreased optometric performance. Further research is warranted in both domains.

Disclosures: B. Hackney: None. **M.F. Awad:** None. **D.A. Del Cid:** None. **R.L. Mosher:** None. **A. Kangavary:** None. **S.A. Drew:** 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.; College of Social and Behavioral Sciences Research Competition Internal Grant. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Dell Seed Program.

Poster

142. Vision: Visual System: Response Modulation and Adaptation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 142.29/DD18

Topic: D.07. Vision

Support: Dell Seed Program
College of Social and Behavioral Sciences Research Competition Internal Grant

Title: Examining changes in oculomotor function after immersive virtual reality use

Authors: *R. MOSHER[1], S. A. LUNDQVIST[2], B. C. HACKNEY[2], R. MORALES[3], J. A. ARMENDARIZ[2], S. A. DREW[2]
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Abstract: Recent advancements in immersive virtual reality present new opportunities for research, as the technology allows control over sensory stimuli across multiple modalities. This capability may be valuable in improving understanding of underlying processes in multisensory integration (Parise & Ernst, 2017). An anticipated rise in use of immersive VR both in research and entertainment may warrant further investigation of how users' visual systems might be affected with continued use. Head-mounted stereoscopic displays used in current immersive VR may introduce novel demands on oculomotor systems due to a mismatch between accommodation and vergence depth cues (Carnegie & Rhee, 2015). This vergence-accommodation conflict is associated with self-reports of visual discomfort (Park et al., 2014);

however, little research has examined changes in oculomotor function from immersive VR use. In a previous study we noted differences in accommodative posture, as a subset of participants demonstrated increased fluctuation in their ability to maintain focus on a fixed target after using immersive VR. Based on these preliminary findings we expanded our study to include 100 participants, examining accommodative posture, accommodative facility, and vergence facility before and after 30 minutes performing a simulated mountain climbing task using Oculus Rift. We observed significant changes in oculomotor function across all three measures, as participants' performance improved on accommodative and vergence facility tasks, while variance in accommodative posture increased. With an increase in efficiency resolving targets and a decrease in capacity to maintain focus on a target over time, further research is necessary to investigate factors that may contribute to these differences

Disclosures: R. Mosher: None. **S.A. Lundqvist:** None. **B.C. Hackney:** None. **R. Morales:** None. **J.A. Armendariz:** None. **S.A. Drew:** None.

Poster

143. Striate Cortex: Plasticity

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 143.01/EE1

Topic: D.07. Vision

Title: Temporal contiguity learning does not break the tolerance of orientation preference across different spatial frequencies

Authors: *E. CRIJNS, D. KALIUKHOVICH, H. P. OP DE BEECK
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Abstract: The images projected onto the retina can vary widely for a single object. Despite these transformations in size, position, ⋯, primates can quickly and reliably recognize objects. This transformation tolerance could be built (or broken) through temporal contiguity, as shown in monkey inferotemporal (IT) cortex (Li & DiCarlo, Science 2008). The primary visual cortex (V1) is typically seen as a robust feature detector for orientations and spatial frequency (SF), however, experience-dependent plasticity has been observed in adult V1. Therefore, we investigated whether temporal contiguity learning could influence the tolerance of orientation preferences across spatial frequency in rat V1.
32-channel extracellular recordings were performed in eight rats. The rats were repeatedly exposed to two sequential gratings with different spatial frequency, including one grating at a reference SF (0.06cpd), and the other grating at a higher (0.12cpd) or lower (0.03cpd) SF and with different (swap SF) or with the same orientation as the reference grating (control SF). The swap SF (high or low) was alternated between animals, yielding similar amount of single units for both conditions.

Before each session a receptive field mapping task was performed. On average, receptive field size and position did not change after exposure, confirming that similar cortical locations were targeted.
Multi-unit responses were recorded before and after exposure to determine responses to the six individual gratings. Pre-exposure 239 single neurons were isolated, and 234 post-exposure. There was no difference in basic properties of the neurons pre and post-exposure. Baseline activity remained similar as well as average responses to each SF. During exposure a decreased peak response was observed for the second stimulus of the pair, with stronger modulation for control pairs as to swap pairs.
We analyzed whether the single neuron orientation preference at the reference SF, is still found at the control and swap SF. Contrary to earlier results in monkey IT, no change in selectivity to the swap SF was detected after exposure.
This result is consistent with further behavioral results in a touchscreen paradigm, in which we did not find any effect of temporal contiguity exposure upon the ability of rats to generalize a discrimination task learned at a reference spatial frequency to new spatial frequencies.
In sum, Temporal contiguity learning does not appear to affect the tolerance of orientation selectivity in V1. The basic filter mechanisms that characterize V1 processing seem unaffected by temporal contiguity manipulations.

Disclosures: E. Crijns: None. **D. Kaliukhovich:** None. **H.P. Op de Beeck:** None.

Poster

143. Striate Cortex: Plasticity

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 143.02/EE2

Topic: D.07. Vision

Support: Toyota Motor Corporation

Title: Neuronal plasticity in the occipital cortex induced by driving skill acquisition using auditory substitution of vision

Authors: *S. UEDA[1], H. SAKAI[2], T. KUMADA[3]
[1]RIKEN, Nagoya, Japan; [2]Toyota Central R&D Labs., Inc., Nagakute, Japan; [3]Kyoto Univ., Kyoto, Japan

Abstract: People can acquire non-innate skills using sensory substitution devices (e.g., shape discrimination using soundscape). In the present study, an fMRI study exploring the underlying neuronal mechanisms of driving skill acquisition using auditory substitution of vision was conducted. A pretest-training-posttest design was employed to compare brain activation before and after lane-keeping training in a simulated environment. In the training, fourteen participants

were subjected for 1-h lane-keeping training of 8 days. The participants were divided into two groups of seven: in the auditory substitution (AS) group, the lower part of landscape was occluded and the auditory cue for vehicle lateral positions was provided instead as binaural balance of noise volume; in contrast, in the control group, the whole landscape was provided with a noise sound with a constant volume. In the pretest and posttest, activation in response to binaural balance changes of noise volume was measured in both groups. Results showed no significant between-group differences in lane-keeping performance, interestingly suggesting that the AS group could acquire a lane-keeping skill comparable to the control using the auditory cue. In addition, fMRI revealed that visual area (the inferior occipital gyrus) was bilaterally more activated by auditory stimuli corresponding to vehicle lateral positions after the training in the AS compared to the control group. In conclusion, our data demonstrates that plastic reorganization of sensory cortical areas is involved in skill acquisition using sensory substitution.

Disclosures: S. Ueda: None. **H. Sakai:** None. **T. Kumada:** None.

Poster

143. Striate Cortex: Plasticity

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 143.03/EE3

Topic: D.07. Vision

Support: BBSRC BB/M021408/1

Title: Ocular dominance plasticity of higher visual areas in the mouse

Authors: *A. VASALAUSKAITE, A. RANSON, F. SENGPIEL
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Abstract: Ocular dominance (OD) plasticity is a well-established paradigm of experience-dependent brain plasticity, whereby occluding one eye (monocular deprivation, MD) shifts responses of visual cortical neurons towards the open eye, in particular during a critical period early in life. Studies of OD plasticity have for over 50 years focused on the primary visual cortex (V1). Mechanisms of OD plasticity are age dependent and in juvenile mice the OD shift is typically characterised by an initial decrease of V1 responses to the closed eye, while subsequently responses to the open eye are potentiated. In young adult animals, only potentiation of open-eye responses is observed. Once binocular vision is reintroduced, a recovery of closed eye responses and reduction of the open eye responses may occur.
Binocular V1 is considered to be the main site of OD plasticity, but recently MD was shown to also cause an OD shift in neurons of the lateral geniculate nucleus projecting to V1. Conversely, it is unknown whether higher visual areas that are direct downstream targets of binocular V1 and themselves contain binocular neurons express OD plasticity. Our investigation focused on LM, a

key area of the mouse ventral stream.
We performed longitudinal in vivo two-photon imaging in awake mice expressing genetically encoded calcium indicator GCaMP6f in CaMKII positive neurons. We first used intrinsic signal imaging to determine the boundaries of discrete cortical visual areas. We then recorded from cells in layer 2/3 of binocular V1 and secondary visual areas immediately before and after MD and during subsequent recovery. MD was performed for 14 days, starting between postnatal days 45 and 55. Mice were presented drifting gratings of 4 orientations independently to each eye. Magnitude of responses through each eye and ocular dominance index (ODI) were calculated for each cell.
As expected, we observed a significant OD shift among V1 neurons towards the ipsilateral (open) eye; responses recovered to pre-MD baseline values after 7 days of binocular vision following eye reopening. We observed a similar OD shift towards the open eye in LM, but notably the OD shift was greater than in binocular V1. Therefore, we conclude that OD plasticity is not confined to V1 but can be observed in at least one secondary visual area. The observation that the OD shift was greater in the higher visual area additionally suggests that the OD shift is not simply inherited from V1, but instead that additional plasticity has occurred within LM neurons.

Disclosures: A. Vasalauskaite: None. **A. Ranson:** None. **F. Sengpiel:** None.

Poster

143. Striate Cortex: Plasticity

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 143.04/EE4

Topic: D.07. Vision

Title: Shared naturalistic auditory comprehension leads to shared spatial patterns in visual cortex across congenitally blind individuals

Authors: *E. MUSZ, R. E. LOIOTILE, J. CHEN, M. BEDNY
Psychological and Brain Sci., Johns Hopkins Univ., Baltimore, MD

Abstract: Previous work has shown that in congenital blindness, the “visual” cortices respond to a range of nonvisual inputs, including tactile (Sadato et al., 1996), auditory (Weeks et al., 2000), and linguistic stimuli (Roder et al., 2002; Bedny et al., 2011). However, it is currently unclear whether the spatial patterns of activity in visual cortices of blind individuals actually encode information, what type of information is encoded, and whether this information shares a common spatial organization across different individuals. We leverage a naturalistic stimulus paradigm and inter-subject alignment techniques to address these questions.
When sighted people watch naturalistic movies, each meaningful segment of the movie (i.e., event) elicits a stable and discriminable spatial activity pattern that is distinct from other events

patterns and robustly similar across the brains of different individuals (Chen et al., 2016). We presented sighted and blind participants with auditory movies in an fMRI experiment, to test whether the visual cortices of different blind individuals encode similar information in response to naturalistic auditory stimuli.
Congenitally blind (CB; n=18) and sighted subjects (S; n = 17) listened to auditory excerpts from movies (3 excerpts, 6 minutes each), a list of unconnected sentences (6 mins), and a backwards speech control stimulus (6 mins). Each clip was broken into twenty 10-second-long events. The movie stimuli were additionally analyzed according to subject-rated event boundaries. In a within-group, whole-brain searchlight analysis, we measured the spatial patterns evoked by each event in each subject. For each event, we then compared each subject's pattern to the group-average pattern for the remaining subjects from their respective subject group. In regions associated high-level semantic and linguistic processing, including fronto-temporal and parietal areas, pattern similarity was greater for matching versus mismatching movie events across individuals in both the CB and S groups. Crucially, this event-specific, cross-subject pattern similarity effect also emerged in the visual cortex, but only for the CB group. In contrast, for both subject groups, the auditory stimulus that lacks high-level semantic content (i.e., the backward speech) only induced common spatial patterns in early auditory cortex. Taken together, these results suggest that blind visual cortex encodes meaningful, event-level information contained in naturalistic auditory stimuli, and that these representations are spatially organized in a similar fashion across different blind individuals.

Disclosures: E. Musz: None. **R.E. Loiotile:** None. **J. Chen:** None. **M. Bedny:** None.

Poster

143. Striate Cortex: Plasticity

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Program #/Poster #: 143.05/EE5

Topic: D.07. Vision

Support: NIH R01 EY02874
NIH K99 EY029002

Title: Close temporal coupling of locomotion and plasticity in adult visual cortex

Authors: *Y. J. SUN[1], M. P. STRYKER[2]
[2]Physiol., [1]UCSF, San Francisco, CA

Abstract: Sensory cortices in adult animals become stable and maintain only limited plasticity, which is qualitatively different from plasticity during critical periods in early development, as well as being smaller and slower. In mice, locomotion gates an enhancement of responses to the specific stimuli viewed for as few 1 hour/day over 5 days (Kaneko, Fu & Stryker, 2017). The

enhancement of responses by locomotion, as well as stimulus-specific plasticity, requires the activity of a class of interneurons expressing vasoactive intestinal peptide (VIP). However, it remains unclear how tight is the coupling of locomotion and visual stimulation necessary to induce plasticity. The VIP neurons that are critical for locomotion-mediated plasticity release both GABA, which acts within milliseconds, and VIP peptide, the effects of which last for seconds to minutes. Therefore, we devised a close-loop visual exposure procedure to determine the time course of the coupling of locomotion to visual plasticity: one orientation of a drifting grating (termed 'running stimulus') was presented during locomotion, and the orthogonal orientation (termed 'still stimulus') was presented when animals are stationary, with a blank stimulus presented as buffer to ensure similar amount of exposure to the two orientations. During 5 days of exposure, animals alternated between running and standing still several times per minute. Using two-photon calcium imaging of primary visual cortex in alert mice, we measured responses of single neurons to gratings moving in different directions, including the stimuli to which it was exposed in the two different behavioral states. We characterized changes in the responses of excitatory neurons in 8 animals and found that neurons selective for the running stimulus increased their responses by 48.9% during locomotion and 80.2% measured while the mouse was stationary. Responses of neurons selective for the still stimulus changed only by 25.1% and 15.9%, respectively. We conclude that the heightened plasticity of adult visual cortex is tightly coupled in time with the locomotion and is therefore likely to be mediated by fast $GABA_A$ transmission rather than by VIP peptide.

Disclosures: Y.J. Sun: None. **M.P. Stryker:** None.

Poster

143. Striate Cortex: Plasticity

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Program #/Poster #: 143.06/EE6

Topic: D.07. Vision

Support: NIH K01MH101639
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NIH R01NS065856

Title: Rem2 stabilizes intrinsic excitability and spontaneous firing in visual circuits

Authors: *A. R. MOORE[1], S. E. RICHARDS[2], S. D. VAN HOOSER[3], S. PARADIS[3]
[1]Biol., Temple Univ., Philadelphia, PA; [2]Neurosci., [3]Biol., Brandeis Univ., Waltham, MA

Abstract: Sensory experience plays an important role in shaping neural circuitry through activity-dependent regulation of both synaptic connectivity and intrinsic properties of individual

neurons. Identifying the molecular players responsible for converting external stimuli into altered neuronal output remains a crucial step in understanding experience-dependent plasticity and circuit function. Using the mouse visual system as a model, we investigated the role of the activity-regulated, non-canonical Ras-like GTPase Rem2 in ocular dominance plasticity. Our *in vivo* analysis reveals that a primary function of Rem2 signaling is to stabilize the intrinsic excitability of cortical neurons in order to maintain proper levels of network activity. Consistent with these findings, both *in vitro* and *in vivo* recordings reveal increased spontaneous firing rate in the absence of Rem2. In addition, our data establishes a novel, cell-autonomous role for Rem2 in regulation intrinsic excitability of layer 2/3 pyramidal neurons, prior to changes in synaptic function. Taken together, we propose that Rem2 functions as a calcium-sensitive cytoplasmic signal transduction molecule and works to convey changes at the membrane into changes in gene expression in the nucleus to regulate intrinsic excitability. Our molecular studies promise to yield significant insight into the transcriptional program by which a neuron instructs its intrinsic properties.

Disclosures: A.R. Moore: None. **S.E. Richards:** None. **S.D. Van Hooser:** None. **S. Paradis:** None.

Poster

143. Striate Cortex: Plasticity

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Program #/Poster #: 143.07/EE7

Topic: D.07. Vision

Support: NIH Grant EY028212

Title: Development- and sensory experience- dependent expression of neuregulins and ErbBs in mouse visual cortex

Authors: *S. GRIECO, N. GONG, X. XU
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Abstract: Neuregulins (NRGs) are a family of epidermal growth factor (EGF) related proteins, and they have diverse functions in the development of the nervous system. Signaling through their tyrosine kinase receptors (ErbBs) has been implicated in synaptic plasticity associated with long term potentiation (LTP) and GABAergic circuit development. We discovered recently that NRG1/ErbB4 signaling in parvalbumin-expressing (PV) inhibitory neurons is crucial for the initiation of critical period visual cortical plasticity by controlling excitatory synaptic inputs onto PV neurons and thus PV-cell mediated cortical inhibition that occurs following visual deprivation. Considering the multiple types of NRGs and ErbBs and their potentially different contributions to visual cortical plasticity, we performed a detailed analysis of NRG and ErbB

expression in the visual cortex of critical period and young adult mice, as well as normal versus dark reared mice. Cell-type specific translating ribosome affinity purification (TRAP) and qPCR was used to determine and compare the mRNA expression of NRGs and ErbBs in PV and excitatory neurons in visual cortex under different developmental times and with different sensory experience. We found that PV neuronal NRG1 and NRG3 expression is strongly dependent on developmental age. PV NRG1 had a 76% reduction at P56 compared with P28, whereas NRG3 had a 77% increase with age (P28 versus P56). Dark rearing affected PV neuronal NRG1 expression with an 83% reduction compared with normal P28 mice. In excitatory neurons NRG3 but not NRG1, had significant changes, increasing 41% from P28 to P56. Sensory manipulation via dark rearing did not alter NRG expression of excitatory neurons. Interestingly, while ErbB4 expression in PV cells is ~50-100 fold more than that of excitatory cells, ErbB expression in PV cells does not differ significantly with age or sensory experience. Age modulates ErbB expression in excitatory cells with a 73% decrease of ErbB1 and a 76% decrease of ErbB3 from P28 to P56. Overall, expression of NRG2 and NRG4 is low in both PV and excitatory neurons, and does not change with age or dark rearing. Thus, our data indicate cell-type specific expression of NRGs and ErbBs is influenced by developmental times and sensory experience. Likely NRG/ErbB signaling in PV and excitatory neurons differentially contribute to visual cortical circuit development and plasticity.

Disclosures: S. Grieco: None. **N. Gong:** None. **X. Xu:** None.

Poster

143. Striate Cortex: Plasticity

Location: SDCC Halls B-H

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Program #/Poster #: 143.08/EE8

Topic: D.07. Vision

Title: Effect of short-term monocular deprivation on time-dependent binocular responses

Authors: *C. J. NG[1], B. FARELL[2]
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Abstract: Ocular deprivation during the critical period can result in permanent visual deficits. By contrast, short-term deprivation in adulthood can temporarily improve post-deprivation vision (Lunghi *et al*., 2011; Zhou *et al*., 2013; Kim *et al*., 2017). However, it is unclear whether stereo sensitive mechanisms would be affected, and if so, how. Assuming that monocular deprivation increases the gain in the post-deprived eye, the overall response to binocular input would be raised but at the cost of a binocular imbalance. We measured stereo thresholds in stereo-normal adults before and after 2.5h of monocular deprivation and, indeed, the data was inconclusive. We therefore adopted the strategy of measuring pre- and post-deprivation motion in depth using the

Pulfrich effect. This effect is typically explained by the principle that higher amplitude signals are propagated more quickly. Hence, an amplitude mismatch between two otherwise identical moving images induces an interocular delay and an instantaneous disparity, resulting in an illusory perception of motion in depth. Using drifting random-dot displays with a two-alternative forced-choice constant-stimulus procedure, we measured pre-deprivation biases in the reported direction of motion of the observers, which may be attributed to eye dominance. Then we measured the effect of depriving the non-dominant eye, as determined by the baseline PSE. Post-deprivation perceived motion was shifted in a direction opposite to the observer's inherent bias. This result suggests that stereo depth mechanisms are indeed affected by binocular imbalance induced by monocular deprivation, and the effect can be captured by a motion illusion. Our finding is consistent with reports of atypical Pulfrich effects in ambylopes (Tomas *et al*., 1984).

Disclosures: C.J. Ng: None. **B. Farell:** None.

Poster

143. Striate Cortex: Plasticity

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Program #/Poster #: 143.09/EE9

Topic: D.07. Vision

Support: NIH R01EY024678

Title: Role of inhibition in the development of visual acuity

Authors: *S. J. KUHLMAN[1], A. D. SWAIN[2], B. B. JEON[1]
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Abstract: Critical periods are defined developmental time windows during which neural representations are malleable to sensory experiences. Both spatial acuity and ocular dominance exhibit critical period plasticity. The extent to which these two forms of developmental plasticity share similar mechanisms is unclear. There are known differences, for example acuity continues to develop past the closure of the critical period for ocular dominance plasticity. On the other hand, maturation of inhibition, specifically development of parvalbumin (PV) inhibitory interneurons, is implicated in setting the time window of critical period for both processes. Using a mutant mouse model in which the development of PV neurons is disrupted, we identified additional key differences between the development of acuity and ocular dominance. We found that the development of spatial acuity is impaired at the perceptual level in mutant mice, using a Go/No-Go behavioral paradigm to assess spatial acuity in head-fixed mice. However, unexpectedly at the level of individual neuronal responses, development of spatial frequency tuning appears to proceed normally in mutant mice in many aspects. The results are unexpected because these same mutant mice have been shown to have disrupted ocular dominance plasticity.

We are currently developing paradigms to improve perception of spatial frequency in these mutant mice and relate improvement to functional response properties of individual neurons as well as population coding of scene statics.

Disclosures: S.J. Kuhlman: None. **A.D. Swain:** None. **B.B. Jeon:** None.

Poster

143. Striate Cortex: Plasticity

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Topic: D.07. Vision

Support: FWO Flanders
C1 grant Research Council KU Leuven

Title: To DREADD or not: Manipulation of interneuron and astrocyte contributions to cortical plasticity

Authors: *L. H. ARCKENS, M. HENNES, I. SCHEYLTJENS, M. HOLT
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Abstract: Disentangling functional contributions of specific cell types to cortical plasticity in general, and cross modal plasticity in particular, is dependent on validated strategies allowing cell type-specific modulation of activity. Along with optogenetics, chemogenetics is emerging as a promising method and involves expression of the mutant G-protein coupled receptors hM4Di and hM3Dq which can be activated by the exogenous agonist clozapine (CNO); Designer Receptors Exclusively Activated by Designer Drugs (DREADD). We explored using recombinant adeno-associated viral (rAAV) vectors to transduce interneurons and astrocytes within the sensory cortex of adult C57BL/6J mice. These cell types were prioritized as potential plasticity mediators because they modulate synaptic transmission and maintain correct neuronal function. Several rAAV serotype and promotor combinations (each with different transduction efficiencies and tropisms) were tested for cell-specific expression and spreading in visual cortex and somatosensory barrel field cortex. Immunohistochemistry, using specific markers, confirmed cell type-specific DREADD expression. We were able to specifically express DREADDs in neurons and astrocytes and collected evidence for their active presence on the plasma membrane. The impact of CNO administration was analyzed using activity reporter gene expression as a read out and indicated successful cell- and brain-region specific activation or silencing. Using this DREADD toolbox, we explored a model of cross modal brain plasticity, driven by late-onset vision loss induced by monocular enucleation. We report that cell type-specific contributions to cortical plasticity extend beyond those observed for somatostatin interneurons using optogenetic

approaches (Scheyltjens et al, 2018). Our results indicate the potential of the DREADD system for probing complex biological systems.

Disclosures: L.H. Arckens: None. **M. Hennes:** None. **I. Scheyltjens:** None. **M. Holt:** None.

Poster

143. Striate Cortex: Plasticity

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Topic: D.07. Vision

Support: NEI R01EY024918
NEI F31EY028829

Title: α2 nicotinic acetylcholine receptors in deep cortical layer somatostatin interneurons drive juvenile-like plasticity in adulthood

Authors: *M. SADAHIRO[1], M. P. DEMARS[1], P. N. BURMAN[1], P. E. YEVOO[1], Y. GARKUN[1], M. R. SMITH[1], A. ZIMMER[2], H. MORISHITA[1]
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Abstract: The decline of cortical plasticity from adolescence into adulthood poses a major challenge for functional recovery from brain injury or disorders in later life. Cortical somatostatin (SST) interneurons are well positioned to manipulate restoration of cortical plasticity in adulthood as they integrate a variety of inputs from bottom-up sensory signals to neuromodulation mediated by locomotor activity or top-down control. Using ocular dominance plasticity in mouse primary visual cortex as a model, we aimed to identify the neuromodulatory mechanism that enables restoration of cortical plasticity through the SST interneurons. In situ hybridization revealed that in the cortex the α2 subunit-containing nicotinic acetylcholine receptor (nAChRα2) are near-exclusively expressed in the deep layer SST interneurons. Whole-cell patch-clamp recordings showed that these nAChRα2-expressing SST interneurons exhibit ACh induced current increase in the presence of atropine, which was completely blocked by the non-α7 nAChR antagonist Dihydro-β-erythroidine. Activation of nicotinic signaling specifically in the adult visual cortex nAChRα2-expressing SST interneurons through viral over-expression of Lypd6, an endogenous positive nAChR modulator, rapidly increased their visual evoked responsiveness within a day in an experience-dependent manner, and subsequently reinitiated ocular dominance plasticity through the action of nAChRα2. Direct chemogenetic activation of adult visual cortex SST interneurons similarly reinitiated ocular dominance plasticity, suggesting the causal role of SST interneuron activation in restoring cortical plasticity in adulthood. Finally, consistent with the role of locomotion in physiologically increasing visual evoked response of

SST neurons, and enabling restoration of a juvenile-form of visual cortex plasticity in adulthood, genetic deletion of nAChRα2 prevented voluntary physical exercise-induced plasticity in adulthood, implicating a physiological role of nAChRα2 in regulating cortical plasticity. Collectively, our study demonstrates the nAChRα2-expressing deep layer SST inhibitory interneuron as a promising novel target for restoring cortical plasticity in adulthood. This provides potential insights into therapeutic strategies against disorders where recovery in adulthood is limited due to diminished plasticity, such as amblyopia, as well as psychiatric disorders where genome-wide association studies recently implicated nAChRα2 as one of the risk genes.

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Poster

143. Striate Cortex: Plasticity

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 143.12/EE12

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Title: Effects of sight restoration on the dendritic complexity of the primary visual cortex

Authors: *M. Y. LIPIN[1], T. GODBOLE[1], P. COOK[1], A. WILLETT[1], Y. YU[1], G.-S. YING[1], A. MAGUIRE[2], J. BENNETT[1], G. ZHANG[3], M. ASHTARI[1]
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Abstract: Introduction. Much of our knowledge on the effects vision loss or gain may have on the visual cortex comes from animal studies. For example, reduction of dendritic architecture of the primary visual cortex (V1) in animals with unilateral eyelid suture and its subsequent reversal in eyelid unsutured animals. Similar studies examining the modulations of V1 microstructures in response to visual input have not been previously possible in human in vivo due to the invasiveness of the procedures. Current study offers a possibility of drawing a parallel in human using an advanced method of diffusion MRI, called Neurite Orientation Dispersion and Density

Imaging (NODDI) in a group of LCA2 patients who regained their vision through bilateral retinal gene therapy (GT). **Methods**. Ten LCA2 patients before 1, 3, 6, and 12 months after GT and 9 matched controls twice, one year apart, underwent NODDI. All MRI scans were obtained on a 3T system using a 32-channel head coil. Images were processed to correct for subject motion and eddy current compensation and group templates (separately for patients and controls) were constructed to delineate V1 and motor (control brain region) areas. The V1 areas were divided into the superior and inferior gray matter (GM) along the banks of calcarine fissure (CF). The random effect repeated measures analysis was performed to compare changes in main NODDI parameters, the orientation dispersion index (ODI) and content of the dendritic tissue over time. **Results**. Statistical analysis showed a significant increase in dendritic dispersion of the inferior GM tissue of the V1 (P=0.02). The post hoc t-test analysis showed the increase in ODI at 3-month post GT (0.553 to 0.575, P=0.043). The changes in dendritic density were not significant in either superior or inferior CF GM. No changes in the ODI or dendritic tissue content were detected for the motor cortex. Statistical results for sighted controls did not show significant changes in the V1 or motor cortex. **Conclusion**: What was previously only detected in animals following their sight restoration is now shown in human in vivo. Using the advances in diffusion imaging, for the first time, changes in the dendritic complexity of the primary visual cortex are demonstrated in a group of LCA2 patients after regaining their sight through GT. These results show that retinal GT not only recovers retinal function but also induces plasticity in the primary visual cortex. The finding that this complexity maximized at 3 months post GT may indicate a critical window for the plasticity of V1 which may play an essential role for when visual rehabilitation training would be the most effective.

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Poster

143. Striate Cortex: Plasticity

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Program #/Poster #: 143.13/EE13

Topic: D.07. Vision

Support: NSERC

Title: Lifespan changes in expression of inflammatory markers in human visual cortex

Authors: *K. M. MURPHY[1,2], K. ARBABI[1], F. BINOTTO[1], S. MANCINI[2], D. AHUJA[1], K. CLIFFORD[1], I. VELIKOVA[1], D. G. JONES[3], J. L. BALSOR[2]
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Abstract: Recent studies have shown that inflammatory factors are found in the healthy uninfected central nervous system where they modulate a range of complex neural processes. These factors include both pro- and anti-inflammatory cytokines that animal studies have shown are constitutively expressed by oligodendrocytes, microglia, astrocytes, and neurons, and are involved in experience-dependent plasticity. Furthermore, over- or under-expression of cytokines is associated numerous pathological states including infection, autoimmune disease (i.e. multiple sclerosis), neurodegenerative disease (i.e. Alzheimer's disease), trauma, stroke, and neuropsychiatric disorders such as depression and bipolar disease. Thus, cytokine signaling plays an important role in the functioning of the healthy and diseased cortex, and yet we know little about the expression of cytokines in the human cortex or how they change across the lifespan. Here we studied the expression of a large collection of inflammatory markers in post-mortem tissue samples from human visual cortex in cases ranging in age from 20 days to 80 years (n=31, 12 female, 19 male). None of the cases had a neurological or psychiatric disease. We measured the expression of 200 inflammatory proteins using the RayBiotech Quantibody 4000 array. This array is a slide-based quantitative analysis of protein concentration, and 72 of the inflammatory markers had protein expression levels that could be reliably measured. These proteins included cytokine and chemokine ligands and receptors such as tumor necrosis factor (TNF), interleukins (IL), and transforming growth factor (TGF) that have been implicated in neural development, aging, disease, and experience-dependent plasticity. For example, the pro-inflammatory cytokines TNF-a its receptor TNF-R1 as well as the neuroprotective agent TGF-B1 had modest peaks in childhood; the pro-inflammatory receptor IL-1R1 increased rapidly in infancy while the pleiotropic cytokine receptor IL-6R decreased across the lifespan.
These findings provide new insights into the expression of inflammatory factors in the human visual cortex and how the expression of those markers changes across the lifespan. The complex pattern of changes raises questions about the role of inflammatory factors in neural function in health and disease.

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Poster

143. Striate Cortex: Plasticity

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Program #/Poster #: 143.14/EE14

Topic: D.07. Vision

Title: Expression of synaptic and non-neuronal proteins reveal parallel plasticity states across human visual cortex development

Authors: *J. L. BALSOR[1], S. BESHARA[1], K. C. WILLIAMS[1], J. G. A. PINTO[1], C. SIU[1], D. G. JONES[3], K. M. MURPHY[2,1]
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Abstract: Perceptual and cognitive development is typically described as a series of stages, but recent brain imaging studies suggest continuous development of features such as cortical thickness, area, and volume. This discrepancy between structure and function raises questions about how to link human behaviour with brain development. We have been studying developmental changes in synaptic and non-neuronal proteins in human visual cortex and focusing on mechanisms that mediate experience-dependent plasticity. Typically, our lab and others have analyzed these features by either age-binning or scatter plots. However, both approaches assume an underlying pattern of development. Here we removed these *a priori* assumptions and applied a data-driven approach to characterize the pattern of development in the human visual cortex.
We used sparse subspace clustering (R package *RSKC*) to analyze expression of 23 synaptic and non-neuronal proteins in post-mortem tissue samples from human visual cortex (n=31, age range 20 days - 80 years, 13 female, 18 male). These proteins act as triggers or brakes on experience-dependent plasticity and include markers of pre-synaptic function, glutamatergic and GABAergic receptor subunits, receptor anchoring proteins, dendritic spines, myelin, and components of the extracellular matrix. A matrix (23x403) of protein expression was used for the cluster analysis. Importantly, *Age* was not a variable in the matrix. PCA was also run on the protein matrix to identify plasticity features that capture the variance in these data. We visualized developmental changes in the clusters by plotting the plasticity features by age for each sample in a cluster and fitting loess curves to each cluster. Next, we analyzed the plasticity features that identified each cluster by comparing eigenproteins determined by PCA.
We found strong clustering but were surprised to find that clusters were not good fits to either age defined stages or continuous development. Instead, clusters appear as a set of parallel "plasticity states" spread across ages. These findings suggest a new way of thinking about the development of human cortex where there may be a set of "plasticity states" that individuals jump between at different times in development.

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Poster

143. Striate Cortex: Plasticity

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Topic: D.07. Vision

Support: NIH R01-EY023037-05

Title: Distinct requirements for layer 4 NMDA receptors in experience-dependent visual cortical plasticity

Authors: *M.-F. FONG[1], P. S. FINNIE[1], T. KIM[1], A. THOMAZEAU[1], E. S. KAPLAN[2,1], S. F. COOKE[3,1], M. F. BEAR[1]
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Abstract: Within primary visual cortex (V1), numerous forms of plasticity occur as a consequence of deprivation or enhanced experience. Examples of such plasticity include juvenile ocular dominance (OD) plasticity, characterized by reduced cortical response to one eye that is temporarily deprived of vision; adult OD plasticity, manifested as a elevated cortical response to vision through the open eye after monocular deprivation; and stimulus-selective response potentiation (SRP), caused by selective visual experience to a single stimulus orientation. Testing the cortical response to visual stimulation presented to one eye at a time has revealed that these forms of plasticity are input-specific within binocular V1, implying that they occur prior to binocular integration. This psychophysical evidence suggests that modification occurs at thalamocortical synapses onto dendrites within layer 4 (L4), the layer with the densest thalamocortical input. A common feature of many of these forms of plasticity is the requirement for NMDA-type glutamate receptor (NMDAR) activation in excitatory principal neurons of V1. We therefore hypothesized that NMDARs in L4 neurons would be necessary for NMDAR-dependent, input-specific visual cortical plasticity. Here, we tested this hypothesis in awake mice using an intersectional transgenic approach to selectively delete NMDARs from L4 principal cells. We found, unexpectedly, that SRP and adult OD plasticity persist, both of which feature a potentiation of visual cortical response, even when NMDARs are genetically eliminated from principal cells in L4. In contrast, juvenile OD plasticity, characterized by depression of deprived eye responses following monocular deprivation, was impaired in mice lacking L4 NMDARs, as predicted. The impairment in response depression resulting from deprivation could be explained mechanistically by the loss of long-term synaptic depression onto V1 neurons in L4, which *ex vivo* slice experiments confirmed in these animals. Our findings therefore reveal a crucial requirement for L4 NMDARs in visual cortical synaptic depression, and a surprisingly negligible role for L4 NMDARs in visual cortical response potentiation, suggesting that these forms of NMDAR-dependent plasticity occur in other cortical neuronal populations. These results sit at the forefront of emerging evidence that NMDARs on distinct cellular subpopulations mediate different forms of experience-dependent visual cortical plasticity.

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Poster

143. Striate Cortex: Plasticity

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Program #/Poster #: 143.16/FF2

Topic: D.07. Vision

Support: NIH Grant 3R01EY023037-05S1

Title: A laminar dissection of V1 circuitry supporting visual recognition memory

Authors: *P. S. FINNIE[1], A. THOMAZEAU[5], D. J. HAYDEN[2], M.-F. FONG[3], S. F. COOKE[4,6], M. F. BEAR[1]
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Abstract: Neocortex is typically arranged into 6 cell layers possessing relatively stereotyped connectivity. Although it is widely accepted that neocortex undergoes life-long experience-dependent plasticity, the field is only just beginning to understand how specific cortical circuits are modified to store memory in a retrievable form. Our lab has previously shown that a simple form of visual recognition memory, observed when mice are repeatedly exposed to an oriented visual grating stimulus, depends on lasting plasticity in primary visual cortex (V1). This form of recognition memory can be revealed by changes in both behavioral and physiological responses. A stereotyped visually-driven motor behavior undergoes gradual orientation-selective response habituation (OSH) over days. Meanwhile, the magnitude of visually-evoked potentials recoded in V1 undergoes stimulus-selective response potentiation (SRP). N-methyl-D-aspartate (NMDA) receptors in excitatory neurons of V1 are necessary for both OSH and SRP acquisition, but the requisite sites of plasticity within the cortical microcircuit are unknown. We therefore used an intersectional transgenic approach to systematically knock out NMDA receptors from principal cells in specific cortical layers: Cux2-Cre^{ERT2} for L2/3, Scnn1a-Cre for L4, Rbp4-Cre for L5, or Ntsr1-Cre for L6. SRP was impaired only in mice lacking NMDA receptors in L6 Ntsr1-expressing cells - the majority of which have bifurcating axons with both intracortical and corticothalamic branches. We hypothesized that after learning, altered L6 corticothalamic feedback might drive burst firing in the lateral geniculate nucleus while mice view a familiar stimulus. However, knocking out the voltage-gated T-type calcium channels responsible for bursting in thalamus had no effect on the acquisition or expression of SRP and OSH. Ntsr1+ cells are also known to contact Parvalbumin (PV)-expressing V1 interneurons that exert inhibition within layer 4. As PV+ V1 interneurons contribute to expression of both SRP and OSH, we now hypothesize that Ntsr1+ layer 6 neurons are a primary site of plasticity that

influences layer 4 during SRP via PV+ intermediaries. The resultant potentiated response may serve to suppress visual attention and behavioral responding, but the precise mechanisms remain elusive.

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Poster

143. Striate Cortex: Plasticity

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Program #/Poster #: 143.17/FF3

Topic: D.07. Vision

Support: NIH Grant EY012124

Title: Rapid bidirectional changes in ocular dominance induced by neuromodulators after the critical period in the mouse primary visual cortex

Authors: *S. Z. HONG[1], S. HUANG[2], A. KIRKWOOD[1]
[1]Mind Brain Inst., Johns Hopkins Univ., Baltimore, MD; [2]Hussman Inst. for Autism, Baltimore, MD

Abstract: Neuromodulatory signals mediated by G protein-coupled receptors are essential for the induction of experience-dependent cortical plasticity. Neuromodulators can exert a push-pull control of Hebbian plasticity, with receptors coupled to Gs and Gq11, promoting the induction of LTP and LTD, respectively, *in vitro*. Previously, we showed that the activation of β- or α-adrenergic receptors, which is coupled with Gs or Gq11, respectively, promotes lasting bidirectional changes in ocular dominance of visual response in the primary visual cortex of juvenile mice *in vivo*. In the present work, we tested whether the activation of these receptors can also promote the bidirectional ocular dominance change in the adult mice after the critical period (>p130). Using optical imaging of intrinsic cortical signals, we found that pharmacological activation of α-adrenergic receptors, in conjunction with brief visual conditioning of the dominant eye, reduces the ocular dominance of the conditioned eye. Furthermore, the same visual stimulation, paired instead with the activation of β-adrenergic receptors, to the long-term deprived eye promotes the increase of ocular dominance of the conditioned eye, resulting in a partial recovery of the ocular dominance of the long-term monocular deprived mice. These results suggest that neuromodulator signals can promote the experience-dependent cortical plasticity in the fully mature brain.

Disclosures: S.Z. Hong: None. **S. Huang:** None. **A. Kirkwood:** None.

Poster

143. Striate Cortex: Plasticity

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Program #/Poster #: 143.18/FF4

Topic: D.07. Vision

Support: NIH Grant EY025922-01

Title: A selective excitatory input disconnection during the critical period of ocular dominance plasticity

Authors: *D. SEVERIN, A. KIRKWOOD
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Abstract: Sensory deprivation can induce profound re-arrangements of glutamatergic circuitry in sensory cortices. These changes are in turn preceded, and possibly initiated, by rapid decreases in the functional strength of cortical inhibition. Attractive identified candidate mechanisms to mediate cortical disinhibition include a reduced excitability of Parvalbumin-positive interneurons (PV-INs), a reduced synaptic inhibitory output of these cells, and a reduced excitatory synaptic drive onto these cells. We examined how brief monocular deprivation (MD: 1 day) affects different excitatory inputs onto layer 2/3 PV-INs in the mouse primary visual cortex.
Using a combination of standard slice electrophysiology methods, along with optogenetics and whole-cell recordings from PV-INs and pyramidal cell pairs, we evaluated the modifiability of excitatory inputs originating from layer 4 and from layer 2/3. We found that 1 day of MD does not affect the magnitude of the “ascending” excitatory inputs from layer 4 onto layer 2/3 PV-INs, but it severely reduces the magnitude of the “lateral” inputs from layer 2/3. Further studies revealed that 1) the plasticity of these lateral inputs has an extended critical period that lasts more than two months and 2) the reduction in input strength results primarily from the loss of connections from local pyramidal cells.

Disclosures: D. Severin: None. **A. Kirkwood:** None.

Poster

143. Striate Cortex: Plasticity

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Topic: D.07. Vision

Support: NIH Grant EY016431

Title: The stimulus-selectivity of visual response potentiation is gated by fast-spiking interneurons

Authors: *C. L. LANTZ, S. MURASE, E. M. QUINLAN
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Abstract: Repeated presentation of high contrast gratings induces robust stimulus-selective potentiation of visually-evoked potentials (VEPs), recorded in rodent primary visual cortex (V1, Frenkel, 2006). Previously we demonstrated that deletion of the neuronal pentraxin NARP inhibited VEP potentiation to stimuli presented at 1 Hz, but not 10 Hz (Gu, 2013). Here we ask if the temporal frequency of repetitive visual stimulation impacts the stimulus-selectivity of VEP potentiation in C57/b6 adults. Immunohistochemistry for the immediate early gene *c-fos* revealed that 10 Hz visual stimulation activated significantly more neurons in adult V1 (>P90, 19.2±6% of NeuN stained cells, post ANOVA Tukey, $p<0.001$) than 1 Hz (10.6±7%) or 20 Hz visual stimulation (7.0±9%). Similarly, *in vivo* recordings of awake, head-fixed subjects demonstrated that 10 Hz visual stimulation elicited higher spike rates in regular spiking (RS) neurons (2.96±0.18 Hz, post ANOVA Tukey, $p = 0.0001$) than 1 Hz (2.36±0.12 Hz), 5 Hz (1.84±0.18 Hz) or 20 Hz (2.28±0.23 Hz). As reported previously, 1 Hz visual stimulation induced VEP potentiation 24 hours after stimulation, which was restricted to the familiar orientation (pre: 112.7±16.3 mV, post: 130.0±16.0 mV, novel: 97.6±11.5 mV, post RANOVA Tukey $p<0.02$). In contrast, 10 Hz visual stimulation induced VEP potentiation to both familiar and novel orientations (pre: 108.7±15.0 mV, post: 134.6±18.6 mV, novel: 122.5±16.2 mV, post RANOVA Tukey $p<0.02$). 10 Hz, but not 1 Hz, stimulation decreased visually-evoked fast-spiking interneurons (FS INs) spike rates, to both familiar and novel stimuli (pre: 6.91±0.9 Hz, post: 4.65±0.5 Hz, novel: 5.22±0.6 Hz, post RANOVA Tukey $p<0.05$), and spontaneous FS IN spike rates (pre: 5.31±0.5 Hz, post: 3.88±0.5 Hz). Neither 10 Hz nor 1 Hz visual stimulation modified the firing rates of RS neurons 24 hours after stimulation. To ask if FS IN activity regulates the stimulus-selectivity of VEP potentiation we used an inhibitor of erbB4, a receptor tyrosine kinase known to regulate the strength of excitatory synapses onto FS INs (Gu, 2016). ErbB4 inhibition during 1 Hz visual stimuli blocked VEP potentiation at 24 hours after stimulation (pre: 87.04±17.4 mV post: 83.35±16.3 mV). In contrast, erbB4 inhibition at 24 hours after 1 Hz visual stimulation revealed VEP potentiation to familiar and novel stimuli (pre: 79.9±11.6 mV, post: 97.2±13.7 mV, new: 102.4±18.9 mV). This suggests that FS IN activity gates the stimulus-selectivity of VEP potentiation and can be manipulated by the frequency of repetitive visual stimulation and erbB4-dependent changes in excitatory synaptic drive.

Disclosures: C.L. Lantz: None. **S. Murase:** None. **E.M. Quinlan:** None.

Poster

143. Striate Cortex: Plasticity

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Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 143.20/FF6

Topic: D.07. Vision

Title: Influence of visual cortical GABA concentration on perceptual suppression and binocular summation in amblyopia

Authors: *A. MUKERJI, K. N. BYRNE, E. YANG, L. LI, D. M. LEVI, M. A. SILVER
Univ. of California, Berkeley, Berkeley, CA

Abstract: Amblyopia is characterized by reduced visual acuity due to abnormal early visual experience and is often accompanied by suppression of signals from the amblyopic eye. Recent studies indicate a role for intracortical GABAergic inhibition in amblyopic suppression as well as a link between occipital GABA levels and fMRI visual cortical response amplitudes in healthy individuals. To better understand the relationships among behavioral suppression, GABA, and visual responses, we measured several types of perceptual suppression, visual cortical GABA levels with magnetic resonance spectroscopy (MRS), and fMRI response amplitudes in amblyopes and healthy controls.

Specifically, we obtained the magnitude of surround suppression, overlay suppression, and interocular suppression for each participant using well-established psychophysical approaches. Additionally, we recorded fMRI activity in retinotopically-defined early visual cortical areas for four stimulus conditions: binocular, dichoptic (different stimuli in both eyes), and monocular stimulation of either the amblyopic/non-dominant or non-amblyopic/dominant eye. Finally, MRS was performed without visual stimulation to measure resting GABA concentration in bilateral occipital cortex.

As expected, we found increased surround suppression in amblyopes when the target was presented to the amblyopic eye and the surround to the non-amblyopic eye, relative to the reverse stimulus configuration and to interocular surround suppression in controls. We quantified binocular summation of fMRI responses by computing the difference between binocular versus monocular and between binocular versus dichoptic response amplitudes. Amblyopes exhibited less fMRI binocular summation than controls for both metrics, and these measures of binocular summation were inversely correlated with visual cortical GABA concentration. Surprisingly, especially given our findings of increased perceptual suppression in amblyopia, visual cortical GABA levels were significantly lower in amblyopes than controls. Ongoing analyses are focused on correlating behavioral metrics of interocular interactions and perceptual suppression obtained from psychophysical data with visual cortical GABA levels and with fMRI response amplitudes in visual cortex.

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Poster

143. Striate Cortex: Plasticity

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Program #/Poster #: 143.21/FF7

Topic: D.07. Vision

Support: R01EY016431

Title: Activation of MMP-9 at thalamo-cortical synapses via light reintroduction to amblyopic eye

Authors: *S. MURASE, E. M. QUINLAN
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Abstract: Chronic monocular deprivation (cMD) results in severe amblyopia that is increasingly difficult to reverse with age. Recently we reported that light reintroduction (LRx) after dark exposure (DE) induces degradation of extracellular targets by matrix metalloproteinase-9 (MMP-9) which reactivates structural and functional plasticity in adult mice (Murase 2017). To examine the role of MMP-9 in recovery from amblyopia following DE/LRx, we used immunoblot analysis, *in vivo* zymography and immunohistochemistry (IHC), all of which reported a similar increase in MMP-9 activity in both deprived and non-deprived primary visual cortex (V1). Here we test the hypothesis that DE/LRx to the deprived eye is sufficient to activate MMP-9, induce ECM degradation and reactivate plasticity. In the subjects rendered amblyopic by cMD, LRx is delivered after DE with a light-occluding eye patch covering the non-deprived eye. The density of chondroitin sulfate proteoglycans, assessed with FITC-labeled wisteria floribunda agglutinin (WFA), and the density of the specific proteoglycan, aggrecan (measured with IHC) in the binocular region of V1 (V1b) are significantly reduced in the visual cortex contralateral to cMD (deprived cortex) following DE/LRx to the deprived eye (WFA: 48.7±7.0% of ipsilateral, n=5 subjects, $p=8.5e^{-4}$, anti-aggrecan: 50.2±16.1% of ipsilateral, n=5 subjects, p=0.019, Student's T-test). There is no difference across the two hemispheres in the intensity of the calcium-binding protein, parvalbumin (83.3±17.7% of ipsilateral, n=5 subjects, p=0.44, Student's T-test). *In vivo* delivery of a biomarker that reports activity of MMP-2/9, shows an increase in the densities and intensities of biomarker puncta in the visual cortex contralateral to the cMD (deprived cortex) following DE/LRx to the deprived eye (density: 208.8±23.1% of ipsilateral, n=6 subjects, p=0.014; intensity: 161.1±22.9% of ipsilateral, n=6 subjects, p=0.046, Student's T-test), with no change in biomarker puncta size (111.7±14.8% of ipsilateral, n=6 subjects, p=0.56, Student's T-test). Following DE/LRx to the deprived eye, we observe a significant increase in co-localization of the MMP-2/9 biomarker puncta with vesicle glutamate transporter 2 (VGluT2; contra:

54.3±1.6%; ipsi: 31.4±7.8%, n=6 subjects, p=0.017, Student's T-test), demonstrating induction of perisynaptic proteolysis at thalamic inputs to cortical neurons in the chronically deprived V1b. Together this suggests that MMP-2/9 activity can be activated by DE/LRx in the amblyopic visual cortex, where LRx of cMD eye alone is sufficient to induce extracellular proteolysis at thalamic inputs to cortical neurons.

Disclosures: S. Murase: None. **E.M. Quinlan:** None.

Poster

144. Visual Motion I

Location: SDCC Halls B-H

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Topic: D.07. Vision

Support: HHMI support through Michael Reiser

Title: Probing visually-guided behaviors using a fast, modular LED display and virtual reality control system

Authors: *M. ISAACSON[1], M. B. REISER[2]
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Abstract: Simulating visual environments to head-fixed animals is a powerful method for studying the sensory control of complex behaviors in a laboratory setting. Advances in display technology have enabled more precise visual stimulus systems that produce more accurate, dynamic, and ethologically relevant simulations of natural environments. While consumer-based display technologies, such as LCDs and DLP projector systems have been widely adopted for animal displays, we have established a display system based on custom-built modular LED 'panels'. The primary advantages of our LED-based display is the speed, brightness, uniformity, and ability to be configured into a variety of display geometries. In order to make use of this new high-speed display system we have also developed a high-speed control system that can supply the display with synchronous visual stimuli at speeds of up to 1 kHz. Using displays assembled from these LED modules, we have performed a series of behavioral experiments that are enabled by this system's technical improvements. Utilizing faster display refresh rates, we conducted experiments that probed the upper limits of *Drosophila* motion vision. New LED panels present higher resolution scenes with uniform intensity on a variety of display configurations, with greater control over wavelength, intensity, and dynamic range, allowing for multi-color (UV/Green) LED panels which enable experiments on color vision. Our control system is integrated with data acquisition and analog outputs. This integration allows for recording behavioral outputs that are precisely synchronized with displayed stimuli. The analog output channels, also precisely synchronized, enable the control and timing of additional experimental

components such as cameras, LEDs for optogenetic stimulation, or other hardware that can be used to create a multi-sensory experimental setup. We also developed a flexible approach to presenting optic flow scenes for electrophysiology and functional imaging of motion-sensitive neurons. Finally, the system has been optimized for the on-line analysis of behavioral measures, image rendering, and display streaming with high frame rates and low latency, creating a very fast closed-loop interactive virtual visual landscape. To our knowledge, this is the first such system that addresses this full set of important technical challenges that face vision science experiments.

Disclosures: M. Isaacson: None. **M.B. Reiser:** None.

Poster

144. Visual Motion I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 144.02/FF9

Topic: D.07. Vision

Support: NSF Grant DMS-1120952
NIH Grant MH-065339
NEI Core Grant EY-002520-37

Title: Ca^{2+}imaging reveals novel excitatory inputs onto a looming sensitive neuron

Authors: Y. ZHU[1], *R. B. DEWELL[2], F. GABBIANI[3]
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Abstract: The ability to detect and avoid impending collisions is critical to survival for people and other animals. By studying how animals accomplish this feat we can further our understanding of neural processing and develop technologies that can improve on our collision avoidance abilities. Of the neural circuits dedicated to collision avoidance, one of the best understood is in locusts, which have a single neuron called the lobula giant movement detector (LGMD) for each eye that is critical to this process. The LGMD responds preferentially to objects approaching on a collision course toward the animal or their two-dimensional simulations on a screen called looming stimuli. The LGMD has three distinct dendritic fields: A, B and C. Field A receives retinotopic excitatory inputs through nicotinic acetylcholine receptors (nAChR). Fields B and C receive inhibitory inputs, and it has long been believed that their synaptic inputs were limited to non-retinotopic, GABAergic feedforward inhibition. Using calcium imaging of the dendritic fields during looming stimuli, we discovered an additional novel excitation impinging onto field C. Field C calcium fluorescence increased as looming stimuli expanded and was stronger for white looming stimuli on dark background (luminance increment) than for black

looming stimuli on a white background (luminance decrement), opposite of what occurs for the previously described excitation onto field A. Direct application of acetylcholine onto field C increased Ca^{2+} fluorescence and local application of the nAChR blocker mecamylamine removed the looming induced Ca^{2+} influx and decreased LGMD firing, confirming that the calcium fluorescence was generated by excitatory cholinergic inputs. Similar to the excitatory inputs onto field A, these inputs onto field C are modulated by muscarine since application of scopolamine, a muscarinic acetylcholine receptor antagonist, reduced the Ca^{2+} signal in field C. Here, we characterize this novel excitatory pathway of the LGMD and investigate its role in looming detection and collision avoidance.

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

Poster

144. Visual Motion I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 144.03/FF10

Topic: D.07. Vision

Title: Glutamate signalling in the fly visual system

Authors: *F. RICHTER, S. FENDL, J. HAAG, A. BORST
MPI For Neurobio., Planegg, Germany

Abstract: Abstract
A central goal of circuit neuroscience is to understand what signals neurons relay to their downstream partners. With the development of reliable, high-performance genetically encoded calcium sensors, calcium imaging has become the default approach for mapping these response characteristics, with the calcium signal serving as a proxy for transmitter release. How well these measurements indeed represent the signaling properties and the true underlying kinetics of the output of any given cell, however, remains unclear. Newly engineered indicators are able to directly report release of transmitters such as glutamate. Here, we demonstrate the viability of the glutamate sensor iGluSnFR for 2-photon *in-vivo* imaging in the fly *Drosophila melanogaster* and prove its usefulness for estimating spatiotemporal receptive fields in the visual system. We use white noise stimulation and reverse correlation to describe the response properties of visual interneurons in the motion pathway and compare the results obtained with expression of iGluSnFR with the ones obtained with the genetically encoded calcium sensor, GCaMP6f. We find that the spatial aspects of the receptive fields are preserved between indicators. In the temporal domain, however, measurements obtained with the glutamate sensor iGluSnFR reveal much faster visual response properties than those obtained with the calcium sensor GCaMP6f. Our approach thus offers a more accurate perspective on the dynamics of signal processing in visual circuits of the fruit fly.

Disclosures: F. Richter: None. **S. Fendl:** None. **J. Haag:** None. **A. Borst:** None.

Poster

144. Visual Motion I

Location: SDCC Halls B-H

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Program #/Poster #: 144.04/FF11

Topic: D.07. Vision

Support: HU-Max Planck center
Gatsby foundation

Title: Electrotonic separation between feedforward and lateral interactions of neuronal network optimizes prediction

Authors: *S. WANG[1], S. E. PALMER[2], A. BORST[3], I. SEGEV[4]
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Abstract: Although efficient prediction is essential to survival, such that that optimal encoding of predictive information is at work in the early visual system of vertebrates [Palmer et al. 2015], little is known about what mechanisms allow predictions to be instantiated in neural systems. Specifically, how does neuronal network organize themselves to implement efficient prediction? Here, we show that the electrotonic separation of feedforward synaptic inputs and lateral interactions among neurons can enable such optimal encoding of predictive information in the fly visual system. Namely, we explore how effective neuronal network can encode predictive information with respect to evasive maneuvers [Mujires et.al 2014] with and without this electronic separation in the blowfly visual system, which is known for having strikingly strong gap junctions only at the axons between neighboring neurons whereas keeping their synaptic inputs only at the dendrites. By comparing this encoded predictive information (with and without the electronic separation) with the theoretical optimum obtained via an information bottleneck (IB) calculation, we show that the optimal encoding of the predictive information: 1) exists in the fly vertical motion sensitive system (Vertical Sensitive (VS) cells), and 2) only presents at the timescale helpful for the evasive maneuver with the lateral gap junctions locates at axons, separating them from the upstream synaptic inputs that reaches the dendrites of these VS cells. Moreover, we show that the subpopulation readout scheme of this VS network allows the downstream system to receive almost all predictive information about the stimuli, across the entire behavior span. Given the profound role of aggregation and segregation for intracellular processing in neocortical pyramidal neurons, we believe that this work may shed light on a candidate principle to understand even more complicated neuronal networks, like the neocortex.

Disclosures: **S. Wang:** A. Employment/Salary (full or part-time):; Hebrew University of Jerusalem. **S.E. Palmer:** None. **A. Borst:** None. **I. Segev:** None.

Poster

144. Visual Motion I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 144.05/FF12

Topic: D.07. Vision

Support: SFB 870

Title: Electrical synapses in the visual system of *Drosophila*

Authors: *G. AMMER, A. BORST
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Abstract: Electrical synapses are abundant in most nervous systems across diverse phyla. In recent years, a range of studies has shown a particular importance of electrical coupling in neural circuits for visual processing. Here, we exploit *Drosophila's* genetic toolbox and well-described circuit architecture to study the role of electrical synapses in its nervous system with a special focus on the visual system.
We first determined the expression patterns of most innexin proteins in the *Drosophila* central nervous system by immunhistochemical methods. We found only a subset of innexins to be strongly expressed in the CNS of *Drosophila*, with innexin8 (shakB) being most widely expressed in the visual system. The expression patterns of these innexins are non-overlapping and restricted to specific neuropil subregions and layers. Additionally, to identify candidate cell-types that are electrically coupled, we performed co-labelling of innexin proteins together with cell-type specific expression of GFP as well as neurobiotin injections.Subsequently, we focused our functional characterization on shakB. To this end, we performed electrophysiological recordings from cells in the optic lobes of shakB mutant and RNAi knockdown flies. Visual projection neurons from flies that were deficient for shakB displayed altered electrophysiological properties and were severely impaired in processing diverse visual stimuli. To differentiate between cell-intrinsic and network effects, we performed functional calcium imaging of neurons that provide synaptic input to these visual projection neurons.
Taken together, our results show that electrical synapses play a major role in *Drosophila* visual processing. Thus, any connectomic or functional wiring diagram of a *Drosophila* visual circuit that lacks electrical connections is likely incomplete.

Disclosures: A. Borst: None.

Poster

144. Visual Motion I

Location: SDCC Halls B-H

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Program #/Poster #: 144.06/FF13

Topic: D.07. Vision

Support: Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0012276)

Title: The intermittent loss of ERG b-wave correlates with reversal motion in goldfish

Authors: *C.-S. JUNG
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Abstract: The retinal perception of fast-moving objects is closely attributed to behavioral property in motion. ‘Temporal resolution’ or ‘temporal coding’, the ability to process a visual signal in a series of ‘snapshot’ intervals, has been extensively studied in goldfish to explore the retinal pathway in relation to motion perception. A study on the specific role of GABA revealed that the GABAergic mechanism is influenced by the $GABA_A$ rather than the $GABA_C$ receptor, which plays a key role in retinal coding. Moreover, the $GABA_A$ agonist muscimol has recently been shown to induce reversal motion in the goldfish optomotor response (OMR); however, $GABA_A$-induced alteration of the snapshot interval effect on goldfish motion in association with relevant visual signal properties has not yet been fully clarified. Here, we investigated whether the $GABA_A$ -induced effect on goldfish motion results from specific changes in retinal signal coding using a full-field electroretinogram (ERG), and whether ‘snapshot’ intervals modulated via a $GABA_A$ agonist affects visual perception. In goldfish, after intravitreal injection of muscimol, subsequent ERG recordings resulted in no b-wave response to a single light illumination; however, the b-wave was randomly prominent in the flicker-like ERG (2Hz flash).This intermittent b-wave pattern induced via the $GABA_A$ agonist occurred almost simultaneously with the abnormal OMR responses in goldfish. Therefore, we suggest that muscimol induces the alteration of specific snapshot intervals, which enables goldfish to acquire an intact visual signal. Intermittent loss of the ERG b-wave may reflect the altered visual signal activity causing backward rotation.

Disclosures: C. Jung: None.

Poster

144. Visual Motion I

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Program #/Poster #: 144.07/FF14

Topic: D.07. Vision

Support: NSERC Discovery Grant RGPIN- 2016-05381

Title: Spatiotemporal tuning of visual neurons in the nucleus lentiformis mesencephali of zebra finches and hummingbirds

Authors: *D. L. ALTSHULER[1], G. C. SMYTH[1], A. H. GAEDE[1], D. R. WYLIE[2]
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Abstract: Optic flow—global image motion resulting from self-motion—is a visual signal that is critical for controlling locomotion and navigation. It is known that optic flow processing begins with large-field motion detectors in the retina that project to the midbrain, but the mechanisms encoding different aspects of image motion, such as image velocity, are poorly understood. We focus on optic flow processing in birds because their optic flow circuits are extensive, well-described, and highly conserved with mammals. Previous recordings from the pigeon lentiformis mesencepali (LM), a midbrain nucleus that is homologous to the mammalian nucleus of the optic tract, showed that some neurons encode slow image velocity, typically on the order of 1°/s. Neurons more responsive to fast stimuli were tuned to spatial and/or temporal frequencies. Here we ask if there are species differences in the spatiotemporal tuning of optic flow sensitive neurons among birds with different flight modes. We presented sine wave gratings of varying spatial and temporal frequencies while making extracellular recordings from LM neurons in zebra finches (*Taeniopygia guttata*) and Anna's hummingbirds (*Calypte anna*). Contour plots of spatiotemporal tuning were generated and then fit with a two-dimensional Gaussian. These contour plots allow for the determination of the preferred spatial and temporal frequency combination for each unit (the peak in the plot), and whether the cell is tuned to temporal frequency, spatial frequency, or velocity. Because velocity is the ratio of temporal to spatial frequency, velocity tuned cells have Gaussian peaks oriented at 45°. Comparing new data from zebra finches and hummingbirds with previously published data from pigeons revealed that the three species have LM neurons with distinct spatiotemporal tuning properties. Hummingbird LM neurons were tuned to the fastest stimuli, which were generally of lower spatial frequencies. Of the few hummingbird LM neurons that were tuned to slow stimuli, all preferred optic flow in the temporal-to-nasal direction. The species exhibited substantial differences in the proportion of velocity tuned neurons, with pigeons having the fewest (9%), zebra finches having the most (41%) and hummingbirds with an intermediate value (26%). In pigeons, only 'slow' cells are

velocity tuned, whereas both zebra finches and hummingbirds have 'fast' cells that are velocity tuned. These species-specific differences are suggestive of neural specializations for different optic flow behavior.

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Poster

144. Visual Motion I

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Topic: D.07. Vision

Support: Catalyst award from the Chicago Biomedical Consortium to JNM, SEP, and DJS
Big Ideas Generator Seed Grant from UChicago to JNM and SEP
NSF CAREER Grant 1652617 (to SEP)

Title: Comparison of responses to natural and synthetic visual motion in murine primary visual cortex

Authors: *V. SPURRIER[1], J. N. MACLEAN[2], D. J. SCHWAB[4], S. E. PALMER[3]
[2]Neurobio., [3]Organismal Biol. and Anat., [1]Univ. of Chicago, Chicago, IL; [4]CUNY Inst. for Theoretical Sci., New York, NY

Abstract: The visual world is encoded in multineuronal activity patterns in primary visual cortex (V1). The field has a firm grasp of activity produced by simple synthetic stimuli. However, (withholding the seminal result that natural scenes increase sparsity) the field is far from understanding activity stimulated by natural movies. Here we used planar 2-photon imaging of L2/3 pyramidal neuron somata expressing GCaMP6s in head-fixed, awake, ambulating mice to examine the circuit-level representation of directional motion. The number of neurons imaged ranged from 50 to 250 and imaging speeds were approximately 33 Hz using heuristically optimal path scanning (Sadovsky et al. 2011). Mice were shown two conditions of luminance-matched visual stimuli that were presented in randomized order interleaved with a full-field blank screen: 1) 2 Hz 0.5 cpd gratings drifting in 8 directions separated by 45°; 2) natural flow rotated in the same 8 directions. We simultaneously measured the ambulatory speed of the mouse. We found that neurons are significantly responsive to both the gratings and natural flow (62% to gratings; 76% to natural flow; Dunnett-corrected one-way ANOVA of mean response of each direction versus blank condition, alpha = 0.01). Neurons were also tuned to both the gratings and natural flow (of neurons responsive to gratings, 60% orientation or direction tuned; of neurons responsive to natural flow, 72%; orientation tuning: Hotelling's T^2-test on orientation trial vectors, alpha = 0.01; direction tuning: direction dot product test on direction trial vectors, alpha = 0.01). Surprisingly, neurons that were orientation or direction tuned to both gratings and

natural flow had different angles of maximal response in these two conditions (difference between maximum orientation angle for neurons orientation tuned to both conditions: 52° ± 29°; difference between maximum response direction angle for neurons direction tuned to both conditions: 93° ±50°). These differences were present before and after controlling for the mouse's running speed. We conclude that activity under synthetic and natural optic flow are different, even when movies contain the same global information (in this study, directional flow). These differences are clues as to how to apply theories informed by synthetic visual conditions to naturally stimulated brain computations.

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Poster

144. Visual Motion I

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Topic: D.07. Vision

Support: NIH Grant EY022577

Title: Functional characterization of laminar contributions to speed tuning in mouse primary visual cortex

Authors: *H. WANG[1,2], E. M. CALLAWAY[1,2]
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Abstract: To encode the speed of an object moving across the visual field, a neuron in the central visual system must incorporate information from at least two features that are typically encoded by retinal ganglion cells - spatial frequency (SF, cycles/degrees) and temporal frequency (TF, cycles/ second). In the central visual system, there are neurons that respond best when objects are moving at a particular speed (TF/SF, degrees/second); in other words, they are "speed tuned." While speed tuned neurons are not present in the retina or lateral geniculate nucleus (LGN), a small fraction of neurons is known to be speed tuned in both non-human primate and mouse primary visual cortex (V1) (Preibe 2006, Andermann 2011). Thus, there is a transformation of SF, TF, and speed starting within V1. The circuit mechanisms mediating this transformation are unknown. Given the laminar hierarchy of visual processing in V1 (LGN to layer 4 to layer 2/3 to layers 5/6), speed tuning may arise within particular stages in this layer-specific network. However, whether there are laminar differences in V1 speed tuning is not known.
To characterize the layer-specific speed tuning properties of V1, 64-channel laminar microprobes were used to measure extracellular single-unit neuronal responses to drifting sinusoidal gratings

of varying speed, SF, TF, and direction in awake, head-fixed mice. Current source density (CSD) analysis was performed to determine the laminar depth of each unit. Firing rates from each SF and TF combination were then fitted to a modified 2-D Gaussian along a spatiotemporal frequency plot. The tilt/slope of the Gaussian corresponds to the "speed tuning index" (ξ) of a neuron (Preibe 2006). When ξ equals 1, the cell is perfectly speed tuned as SF and TF tuning vary in proportion to maintain the same preferred speed regardless of SF or TF. When ξ equals 0, the cell is not speed tuned, as SF and TF tuning occur independently, and the preferred speed depends on the SF and TF of the stimulus. Only units that could be well-fit by the 2-D Gaussian were included for subsequent analysis.
Preliminary results suggest that speed tuning arises in a layer specific manner in mouse V1. While the overall fraction of speed tuned units in V1 was small (~10%), consistent with previous reports, these units were primarily found in layer 2/3 or layers 5/6. In layer 4, neurons had a smaller range of speed tuning indices with none greater than 0.72, whereas layer 2/3 and layer 5/6 have a wider range of speed tuning slopes, including those near 1. This suggests that speed tuning only arises in later stages of V1 cortical processing. The inputs to speed tuned neurons within V1 are currently under investigation.

Disclosures: H. Wang: None. **E.M. Callaway:** None.

Poster

144. Visual Motion I

Location: SDCC Halls B-H

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Topic: D.07. Vision

Support: Human Frontier Science Program Long-term Fellowship LT000769/2015
Swiss National Science Foundation Grant 3100330B_163457
ERC Grant 669157 RETMUS
DARPA Grant HR0011-17-C-0038 Cortical Sight

Title: Brain modules for visuomotor integration revealed by whole-brain functional ultrasound imaging

Authors: *E. MACÉ[1,2], G. MONTALDO[3], S. TRENHOLM[4], C. COWAN[1,2], A. BRIGNALL[1,2], A. URBAN[3], B. ROSKA[1,2,5]
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Abstract: Large numbers of brain regions are active during behaviors. A high-resolution, brain-wide activity map could identify brain regions involved in specific behaviors. We have

developed functional ultrasound imaging to record whole-brain activity in behaving mice with a resolution of ~100 µm. We detected 87 active brain regions during visual stimulation that evoked the optokinetic reflex, a visuomotor behavior that stabilizes the gaze both horizontally and vertically. Using a genetic mouse model of congenital nystagmus lacking the horizontal reflex, we identified a subset of regions whose activity was reflex-dependent. By blocking eye motion in control animals, we further separated regions whose activity depended on the reflex's motor output. Remarkably, all reflex-dependent but eye motion-independent regions were located in the thalamus. Our work identifies functional modules of brain regions involved in a sensorimotor integration and provides an experimental approach to monitor whole-brain activity of mice in normal and disease states.

Disclosures: E. Macé: None. **G. Montaldo:** None. **S. Trenholm:** None. **C. Cowan:** None. **A. Brignall:** None. **A. Urban:** None. **B. Roska:** None.

Poster

144. Visual Motion I

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Program #/Poster #: 144.11/GG1

Topic: D.07. Vision

Support: NIH U01NS094330
NIH EY019288
Human Frontier Science Program Grant

Title: Motion discrimination and the motion aftereffect in mouse vision

Authors: *J. M. SAMONDS[1], S. LIEBERMAN[2], N. J. PRIEBE[3]
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Abstract: Prolonged exposure to motion in one direction often leads to the illusion of motion in the opposite direction for stationary objects. This motion aftereffect likely arises across several visual areas from adaptive changes in the balance of activity and competitive interactions. We examined whether or not the mouse was susceptible to this same illusion to determine whether it would be a suitable model for learning about the neural representation of the motion aftereffect. Under a classical conditioning paradigm, mice learned to lick when presented with motion in one direction and not the opposite direction. Mice were given water for one direction of motion and no water for another direction of motion. We initially trained them at directions that were 90 degrees different. Mice initially licked without any timing relationship to the stimulus and at a high rate 400-600 ms after given water. After a few days, mice began to lick up to 2 seconds before water was given after both stimuli were presented. Then after a couple weeks, mice licked

only when presented the rewarded direction of motion. Once they reached criterion performance in the direction discrimination task (d' ≥ 0.8), we rotated the unrewarded direction of motion until reaching criterion again for each rotation until the unrewarded condition was in the opposite direction of motion. Once the mice had reached criterion performance in the opposing direction discrimination task, we assayed the effects of prolonged motion exposure preceding discrimination. When the mice were adapted to low contrast (30%) high coherence (100%) motion preceding this test, their lick behavior for high contrast (100%) zero coherence motion was biased for motion in the opposite direction of the adapting stimulus. Overall, lick count versus motion coherence shifted in the opposite direction of the adapting stimulus. This suggests that although the mouse has a simpler and less organized visual system compared to primates, it still is subject to the motion aftereffect and may elucidate the underlying circuitry.

Disclosures: J.M. Samonds: None. **S. Lieberman:** None. **N.J. Priebe:** None.

Poster

144. Visual Motion I

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Program #/Poster #: 144.12/GG2

Topic: D.07. Vision

Support: NIH Grant R01EY022122

Title: Neurons of the developing lateral geniculate nucleus of the ferret exhibit poor tuning for orientation and direction of moving stimuli

Authors: *A. STACY[1], N. SCHNEIDER[1], S. D. VAN HOOSER[2]
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Abstract: Sensory experience is critical for typical development of particular receptive field properties of neurons in primary sensory cortical areas. Specifically, visual experience is necessary for the development of direction selectivity in the primary visual cortex (V1) of the ferret, while orientation selectivity is already present at the onset of visual experience. However, it remains unknown whether orientation and direction selectivity are present at earlier stages in the visual pathway in the ferret brain, and how sensory experience affects the development of these receptive field properties. We provided visually naïve ferrets with moving sinusoidal grating stimuli known to induce direction selectivity in ferret V1, and investigated its effect on orientation and direction tuning in the lateral geniculate nucleus (LGN), *in vivo*. We developed a novel, custom grid-and-chamber system to facilitate mapping and multichannel recording of ferret LGN. In our preliminary observations, we found that, at the population-level, LGN neurons do not exhibit significant levels of orientation or direction selectivity at the onset of visual experience, or following exposure to moving stimuli. This suggests that direction

selectivity must be computed at a later stage in the visual pathway, providing insight into the neural circuitry underlying the visual processing of motion detection.

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Poster

144. Visual Motion I

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Program #/Poster #: 144.13/GG3

Topic: D.07. Vision

Support: 1R01EY027853-01

Title: Development of motion integration in ferret visual area PSS: Effects of visual stimulation

Authors: *A. A. LEMPEL, K. J. NIELSEN
Neurosci., Johns Hopkins Univ., Baltimore, MD

Abstract: The ferret has become a major animal model for the development of the visual system because of its immature state at birth, and the complexity of its visual system. We have previously demonstrated the existence of a higher order motion area - area PSS - in ferrets, in which motion processing resembles that observed in primate MT. Most importantly, a fraction of PSS neurons shows signatures of local motion integration when probed with coherent plaids (so called pattern responses). Based on our data, pattern responses emerge in PSS during the second week after eye opening around postnatal day (P) 42, and reach mature levels in just a few days (P43-P45). Here, we investigate the role of visual experience in the emergence of pattern responses by studying its impact on the development of multiple motion pathway stages. To test the impact of visual experience, kits between P38 and P40 (just before the natural emergence of pattern responses) were exposed to drifting plaid stimuli for 8h. Throughout the experiment, data were collected simultaneously in V1 and PSS using multi-channel silicone probes. After 8h of controlled visual stimulation, pattern responses - which were absent before the stimulation - could be found in PSS at mature levels. Exposing animals to a gray screen for the same amount of time did not result in pattern response maturation, indicating that certain visual stimulus features are required for development of motion integration. To further investigate this issue, we are now testing the effects of exposing animals to drifting gratings instead of plaids. In addition to changes in PSS, 8h of plaid stimulation caused an increase in the relative response of V1 neurons to plaids versus gratings. Similar changes were also observed during normal development, suggesting that they might be driven by the same mechanisms during controlled stimulation and development. Furthermore, current motion integration models suggest that stronger V1 responses to plaids could be an important factor in generating pattern responses in the subsequent higher motion areas.

Disclosures: A.A. Lempel: None. **K.J. Nielsen:** None.

Poster

144. Visual Motion I

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Program #/Poster #: 144.14/GG4

Topic: D.07. Vision

Support: The study was funded by a grant to ST from the Deutsche Forschungsgemeinschaft (DFG) for the Research Unit 1847 "Physiology of Distributed Computing Underlying Higher Brain Functions in Non-Human Primates"

Title: Determining complex receptive field visual motion preferences in macaque cortical area MSTd

Authors: *B. WILD[1], A. MAAMOUN[2], S. TREUE[3]
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Abstract: The dorsal part of the medial superior temporal area (MSTd) in macaque extrastriate visual cortex is assumed to play a central role in the processing of complex motion patterns. MSTd neurons show tuned responses to linear motion, as well as to 'spiral motion', a continuous circular space of complex motion patterns including expansion, contraction and rotation. In addition, MSTd cells have also been reported to be position-invariant in their responses to spiral motion stimuli.
In order to determine the motion preference profile of MSTd receptive fields, we developed a novel stimulus: a large random dot patterns, formed by the smooth variation of local dot motion between a grid of positions in the stimulus where the local direction and speed were chosen randomly every 100ms from all possible directions and a large range of speeds. We applied a reverse correlation analysis to single unit spike trains, a linear method which has been successfully used to characterize receptive fields in earlier cortical areas (e.g., V1 and MT). With this approach, we could gain a more detailed description of the specific motion preferences of individual MSTd neurons, compared to the simple assumption of linear and/or spiral direction tuning. Separately we also determined the position dependency of the MSTd responses to spiral motion patterns.
We recorded from more than 140 MSTd neurons in three awake rhesus monkeys, foveating a central fixation point during stimulus presentations. For ~30% of the cells the reverse correlation analysis recovered significantly structured spatial motion preference profiles and partial maps of the receptive field. Recovering receptive field maps through reverse correlation worked better in cells that were strongly tuned for linear and spiral motion than in cells with weaker tuning. The recovered maps show a preference to linear motion rather than more complex motion patterns,

but the neural responses to spiral and linear motion patterns was significantly correlated with their motion similarity to the reverse correlation maps in about 50% of those cells. Almost all of the cells showed position invariant responses to spiral motion patterns.

Disclosures: B. Wild: None. **A. Maamoun:** None. **S. Treue:** None.

Poster

144. Visual Motion I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 144.15/GG5

Topic: D.07. Vision

Support: NIH grant EY018613

Title: Tuning dynamics in cortical area MT is predicted by canonical computation

Authors: *A. S. PAWAR[1], S. GEPSHTEIN[2], S. SAVEL'EV[3], T. D. ALBRIGHT[1]
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Abstract: Background: The classical conception of receptive field (CRF) describes selectivity of neurons to various stimulus dimensions: spatial location, orientation, spatiotemporal frequency, direction of motion, etc. Viewed originally as a stable characteristic of neurons, CRF was later challenged by evidence that neuronal selectivity depends on context in the form of stimulation outside CRF. In this revised conception, responses to stimuli inside CRF depend on feedforward connections, whereas stimuli outside CRF modulate responses through lateral and feedback connections. A growing body of literature suggests that lateral and feedback network connections play an even more pervasive role in shaping neuronal selectivity. We used theoretical and physiological methods to investigate how neuronal selectivity arises in the cortex and how it depends on the interaction between stimulus dimensions.**Computational results**: We used a distributed version of the canonical model of cortical computation (Wilson & Cowan, 1972) to study interaction of three stimulus dimensions: luminance contrast, spatial frequency (SF) and temporal frequency (TF). We found that the distributed circuit is intrinsically tuned to stimulus SF. At high contrasts, when circuit behavior is nonlinear, the intrinsically preferred SF varies with contrast, changing up or down depending on whether the circuit is dominated by excitation or inhibition. Intrinsic SF also depends on stimulus TF: at low TF, increasing contrast causes large changes in intrinsically preferred SF. These changes decrease monotonically as TF increases. Intrinsic SF asymptotically approaches an “attractor” value at high TF. **Physiological results**: We tested model predictions by measuring firing rate responses of neurons in cortical visual area MT in two alert macaque monkeys. Stimuli were sinusoidal luminance gratings at different luminance contrasts and spatiotemporal frequencies. In both monkeys, preferred SF of

MT neurons depended on stimulus contrast, similar to model predictions. Increasing contrast caused preferred SF to increase in most cases by an amount that depended on TF. As predicted, the change of preferred SF was large at low TF and small at high TF. Intrinsic SF at low and high contrasts asymptotically approached the same value with increasing TF.**Conclusion**: The results indicate that the so-called "contextual interactions" are more fundamental to cortical selectivity than previously thought. Lateral and feedback connections are as important as feedforward connections in determining neuronal tuning, even to stimuli falling inside CRF.

Disclosures: A.S. Pawar: None. **S. Gepshtein:** None. **S. Savel'ev:** None. **T.D. Albright:** None.

Poster

144. Visual Motion I

Location: SDCC Halls B-H

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Program #/Poster #: 144.16/GG6

Topic: D.07. Vision

Support: NHMRC APP1066588
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ARC CE140100007

Title: Nonlinear temporal integration of visual motion in marmoset MT

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Abstract: It is well established that neuronal tuning curves are malleable. Even though a neuron's average firing rate might reliably represent stimulus properties under a fixed testing protocol, the shape and gain of a tuning curve are affected by small changes in the testing protocol, including factors such as attention, adaptation and eye position. We have previously shown that previous exposure to motion stimuli can compress or laterally shift direction- and speed-tuning curves of neurons in the middle temporal area (MT). The nature of these changes depends on whether the prior motion is near the peak, or flank, of the tuning curve. Here, we examine how neurons in MT of sufentanil-anaesthetised marmosets (*Callithrix jacchus*) encode rapidly changing stimuli, in which the direction changes every 33 ms (4 frames at 120 Hz). In extracellular recordings from 142 neurons across 5 animals, we first characterised the dynamics of direction tuning using a motion reverse correlation technique. Next, we examined how these dynamics depended on pairwise interactions between sequentially presented motion directions. Based on spiking rate predictions from a standard linear-nonlinear cascade model of neural spiking, we argue that additional temporal non-linearities in the way neurons integrate motion are necessary to account for the dynamic changes in direction tuning evident in pairwise interactions between motion periods with different directions. Put simply, motion in one

direction significantly affects the responses to subsequent motion in different directions. Finally we demonstrate that, despite the rapidly changing visual stimulation, we can characterise spike-count correlations between pairs of neurons, allowing us to quantify how populations of neurons collectively represent these rapidly changing stimuli. This work extends previous models of sensory integration in motion-sensitive neurons by explicitly incorporating stimulus history, and the associated rapid, non-linear adaptation.

Disclosures: N.S. Price: None. **B.H. Oakley:** None. **E. Zavitz:** None.

Poster

144. Visual Motion I

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Title: Inactivation of posterior prefrontal cortex compromises processing of visual motion in area MT

Authors: *M. ISLAM[1], D. SAMU[2], K. WIMMER[3], T. PASTERNAK[4]
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Abstract: It is widely accepted that top-down signals can modulate activity of neurons processing sensory information. Posterior regions of the prefrontal cortex (PFC), including the frontal eye fields (FEF), are reciprocally connected with a number of cortical regions processing visual information, and thus, are a likely source of such signals. We examined the role of top-down inputs on neuronal activity in area MT, a region specialized in motion processing, by recording from area MT before and after inactivating the posterior regions within the PFC while monkeys compared directions of two sequentially presented moving random-dot stimuli, S1 and S2. Transient inactivation, produced by injecting 2µl of muscimol (10µg/µl) into three adjacent sites within posterior PFC, resulted in a dramatic increase in firing rates of the majority of recorded units throughout the entire trial. This upward shift in mean activity was accompanied by a proportional increase in variance, suggesting additive increase in firing rates indicative of a shift in the operational regime of neuronal circuits within MT. We asked whether this increase in

the overall activity in MT affected processing of visual motion by focusing on direction selectivity (DS) recorded during S1 and S2. The comparison of activity recorded from the same units before and after inactivation revealed that the inactivation led to a weakening of DS. We found that the effect of inactivation on DS depended on the unit's strength of DS prior to inactivation: the units with the strongest DS showed more pronounced effects than neurons with weak DS. Separate analysis of responses for the preferred and anti-preferred directions revealed that weakening of DS was due largely to the disproportionately greater elevation in firing rates in response to the anti-preferred direction. This effect on firing rates during anti-preferred trials was particularly pronounced for strongly DS units and was nearly absent for units that were less DS. We hypothesize that the excitatory top-down signals provided by the posterior portions of the PFC may enhance motion selectivity in MT by supporting local inhibitory activity contributing to DS. Our results demonstrate that the top-down influences arriving from the PFC contribute to processing of sensory signals.

Disclosures: M. Islam: None. **D. Samu:** None. **K. Wimmer:** None. **T. Pasternak:** None.

Poster

144. Visual Motion I

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Program #/Poster #: 144.18/GG8

Topic: D.07. Vision

Support: NIH Intramural Research Program

Title: Responses to flicker plaids in macaque middle temporal visual area (MT)

Authors: *C. QUAIA, I. KANG, L. M. OPTICAN, B. G. CUMMING
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Abstract: Neurons whose responses are tuned to the direction of motion of patterns containing more than one orientation are first found in area MT: Earlier regions only respond to motion of the individual components. Historically the problem of pattern motion computation has been studied using plaids composed of two moving gratings, or one static and one moving (unikinetic plaid). We have recently introduced a new stimulus, which we called a flicker plaid, in which one grating moves while the other "flickers in place". With noise plaids, this is achieved by simply summing a drifting random line stimulus (RLS) and a RLS with a different orientation that is generated anew for each frame. We showed that ocular following eye movements elicited by such a stimulus are virtually identical to those induced by a unikinetic plaid in which the static component has the same orientation as the flickering component of the flicker plaid. This occurs even though, unlike unikinetic plaids, flicker plaids do not contain a well-defined motion direction.

To study where such selectivity emerges in the visual system, we recorded from 66 MT neurons in one macaque monkey. We presented stimuli in which the static/flickering RLS was rotated either +45 or -45 degrees relative to the drifting RLS. We quantified, for both unikinetic and flicker plaids, the ability of a cell to extract a pattern motion signal by computing the relative rotation of the direction tuning curves for the +45 and -45 plaids. The angle of rotation should be zero for component cells, and +90deg for pattern cells. We found a significant correlation between rotations for unikinetic and flicker plaids (Pearson's r=0.37, p=0.002), but for many cells flicker plaids were associated with negative rotations. This occurs when the response of the cell is dominated by the response to the flickering RLS. Such sensitivity would be removed by an opponent stage, in which cells preferring opposite directions of motion inhibit each other. We simulate this by subtracting from the responses of each neuron the responses of its anti-neuron. This reveals two populations of neurons: one for which unikinetic and flicker plaids produce the same rotation, and one in which only unikinetic plaids produce a tuning curve rotation (complete or partial).
We conclude that ocular following eye movements are either mediated by only those cells that exhibit similar rotation with both plaids, or that a further stage of processing (e.g., MST) is required for the extraction of the pattern motion signal. Since it has been previously shown (Khawaja et al. 2013) that pattern motion signals are more prominent in MST than in MT, the second hypothesis is more likely.

Disclosures: C. Quaia: None. **I. Kang:** None. **L.M. Optican:** None. **B.G. Cumming:** None.

Poster

144. Visual Motion I

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Program #/Poster #: 144.19/GG9

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John and Genevieve Wolfe

Title: Motion sensitivity in the primate lateral geniculate nucleus

Authors: *N. J. KILLIAN[1,3], J. S. PEZARIS[2,3]
[1]Neurosurg., [2]Massachusetts Gen. Hosp., Boston, MA; [3]Neurosurg., Harvard Med. Sch., Boston, MA

Abstract: It is commonly believed that motion sensitivity does not appear in the early visual system of primates until the spatial integration of non-direction-selective lateral geniculate nucleus (LGN) afferents occurs in the primary visual cortex (Chichilnisky and Kalmar, 2003). Challenging this idea, several studies have described weak orientation-sensitive or direction-selective responses in primate LGN neurons, chiefly in New World monkey species (Cheong et al., 2013). Furthermore, motion sensitivity has recently been demonstrated in retinal ganglion cells of the primate retina that project to the magnocellular pathway (Manookin et al., 2018). Here, we show that direction-selectivity is apparent at multiple spatiotemporal scales in receptive fields (RFs) of LGN neurons in awake, behaving Old World monkeys (Macaca mulatta). To study the dynamics of LGN RFs, we developed wide-field chromatic naturalistic (one-over-frequency) noise stimuli and ultra-thin multi-channel electrode arrays for high-throughput extracellular recording. Many neurons had RFs with spatial asymmetry (and thus presumed to exhibit orientation tuning) that dynamically changed shape (and thus presumed to exhibit motion sensitivity). Two temporally-distinct motion response types were observed. First, primarily in magnocellular layers, early narrowly-tuned responses to stimulus movement along a preferred direction occurred from about 100 to 20 msec before spike occurrence. Preferred directions often changed between temporally-adjacent excitatory and inhibitory components, but with matching chromaticity. The second type of motion response occurred later, within 20 msec of the LGN output signal, and represented broadly-tuned outward motion with respect to the RF center. The late response was seen in all cell types and all cell layers (parvocellular, magnocellular, and koniocellular). A variety of mechanisms may give rise to the observed patterns, including retinal circuitry, convergence of retinal inputs within LGN, and cortical feedback. The late response is particularly reminiscent of starburst amacrine cells that form direction-selective retinal ganglion cell circuitry in the rabbit and mouse (Sanes and Masland, 2015), which may have analogs in the primate retina (Dacey et al., 2003).

Disclosures: N.J. Killian: None. **J.S. Pezaris:** None.

Poster

145. Vision: Spatial and Feature-Based Attention

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Program #/Poster #: 145.01/GG10

Topic: D.07. Vision

Support: NIH R01 NS102201

Title: Unexpected sounds globally interrupt active perceptual representations: Evidence for a global inhibitory surprise response?

Authors: *C. SOH[1], J. R. WESSEL[2,3]
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Abstract: Recent research has shown that a fronto-basal ganglia (FBg) brain mechanism for motor inhibition inhibits actions after unexpected events. Moreover, the same mechanism has been shown to exert inhibition on some cognitive representations, suggesting that it could play a more general inhibitory role in cognitive flexibility. To further test this idea, we investigated whether ongoing perceptual representations are subject to suppression via the FBg-network. Subjects undergoing EEG recordings performed two tasks: a cross-modal SSVEP oddball task and a stop signal task (SST). In the SSVEP task, subjects attended to one of two laterally presented visual flickers (12 and 18hz). On a subset of trials, a surprising sound unexpectedly occurred while subjects attended either flicker. We used filter-Hilbert time frequency decomposition to analyze time-resolved attentional tuning at the respective SSVEP frequencies. These analyses showed that surprising sounds induce an abrupt suppression of SSVEP activity in parieto-occipital channels both contralateral (attended) and ipsilateral (unattended) to the cued location, which suggests a surprise-related global interruption of active perceptual representations. Moreover, the onset of this interruption coincided with the typical timing of inhibitory activity originating from the FBg-network. To draw a direct link between this surprise-related SSVEP interruption and inhibitory activity from the FBg-network, we used the SST portion of each subjects' dataset to extract a motor inhibition independent component (MI-IC) using independent component analysis. Previous research has shown that the P3 event-related potential from this MI-IC indexes FBg-activity during successful action-stopping in the SST. In our study, it showed an earlier onset for successful vs. failed stopping within subjects and its onset correlated with the speed of stopping across subjects. Importantly, the activity of this MI-IC was also increased after surprising sounds in the SSVEP portion of the data. Moreover, the time window of this tone-related activity immediately preceded surprise-related SSVEP-suppression. To further illuminate the relation between MI-IC activity and surprise-related SSVEP-suppression, we used single-trial EEG analysis to test whether MI-IC activity after surprising tones in the SSVEP task mediated the inhibitory effect of the tones on ongoing perceptual maintenance. Taken together, our study suggests that both attended and unattended perceptual representations could be subject to interruptions by a generic, universal inhibitory mechanism that also underlies the inhibition of motor activity in the SST.

Disclosures: C. Soh: None. **J.R. Wessel:** None.

Poster

145. Vision: Spatial and Feature-Based Attention

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 145.02/GG11

Topic: D.07. Vision

Support: PNI Innovation Fund

Title: Visual awareness plays a role in the control of visuospatial attention

Authors: *A. I. WILSON, M. S. A. GRAZIANO
Psychology, Princeton Univ., Princeton, NJ

Abstract: We report that visual attention shows rapid adaptation effects, suggesting that an internal model of attention is being updated, just as an internal model of the arm can adapt to an external force field. We also report that the adaptation effects break down when participant's awareness of attention-capturing cues is blocked through the use of masking. Without awareness, only a slow adaptation remains, consistent with model-free learning. These findings support the hypothesis that attention is controlled partly through the use of an internal model, and that visual awareness is closely related to that internal model.
Volunteers were asked to fixate a central point on a screen, and a briefly presented visual cue drew exogenous attention to a peripheral location. 500 ms after the cue, a target stimulus was presented nearby. It could be at exactly the same location or shifted 3 degrees to the left or right of the cue. Subjects were required to discriminate the target. The relative reaction time to different target locations was used as a measure of the spatial distribution of attention on and around the cue. The target appeared to one side of the cue (the "predicted" side) 70% percent of the time, to the opposite side (the "non-predicted" side) 15% of the time, and at the cue location itself 15% of the time. Participants quickly adapted (within the first 50 of 200 trials), shifting attention to the predicted side of the cue. However, only a small minority of participants explicitly noticed the contingency when asked. Thus, visuospatial attention can adapt to a small spatial shift and the adaptation can occur implicitly.
Next, we investigated whether attentional adaptation could occur in the absence of awareness. Using a similar paradigm, we used a mask to block the cue from participant's awareness. No adaptation was observed.
Finally, we tested whether adaptation might still occur in the absence of awareness of the cue, if learning were allowed to continue over a longer sequence of trials. When trained on 600 trials, instead of 200, subjects showed a significant adaptation effect after the first 500 trials. The fundamentally different time course between aware and unaware adaptation supports the division between model-based adaptation (with awareness) and model free adaption (without awareness).

Disclosures: A.I. Wilson: None. **M.S.A. Graziano:** None.

Poster

145. Vision: Spatial and Feature-Based Attention

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Topic: D.07. Vision

Support: NIH 1R01EY02391501A1
DoD - NDSEG
Stanford Center for Mind, Brain and Computation

Title: Selective attention influences visual object category representations across human cortex

Authors: *A. S. KELLER[1,2], J. B. COCJIN[3], A. JAGADEESH[4], L. BUGATUS[4], K. GRILL-SPECTOR[4]
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Abstract: Selective attention allows the brain to focus on relevant information while ignoring distraction. However, the top-down influence of attention on visual category representations is not fully understood. Eleven participants were scanned with fMRI while viewing images from five object categories (faces, houses, bodies, cars, words) and performing one of two tasks: 1) oddball: subjects viewed images of objects from each category and indicated when a phase-scrambled image appeared, and 2) selective attention: subjects viewed superimposed pairs of images from two categories and indicated when an item of the attended category was flipped upside-down. We combined data-driven and hypothesis-driven approaches to investigate how attention modulates category representations across cortex and to determine the sources of this top-down modulation.

Using a data-driven geodesic spotlight search across the cortex in conjunction with a multi-voxel pattern decoding approach, we report: (i) Category information was decoded significantly above-chance from high-level visual areas in lateral occipital and ventral temporal cortex (VTC), posterior parietal cortex, and ventro-lateral prefrontal cortex. (ii) An SVM classifier trained on the oddball task predicts object category for attended objects on average by 26.1% ± 1.2% more than unattended objects, suggesting that attention enhances category representations.

In a complementary hypothesis-driven approach, we developed a method to separate top-down from bottom-up activity in the BOLD signal. We computed correlations between the residual activity (not explained by bottom-up stimuli) in the intra-parietal sulcus (IPS), a key region of the fronto-parietal attention network, and residuals in category-selective regions in VTC. We found significant positive correlations ($p < .05$) between residual activity in the IPS and category-selective regions in VTC. Notably, the correlations were higher between right IPS and most VTC regions than left IPS and VTC ($p < .05$), consistent with prior attention research. We also show that attention and ignoring involve similar top-down modulation, measured as positive correlations in the residuals between IPS and VTC. Finally, we show that fronto-parietal attention network areas (e.g., frontal eye fields) are likely sources of this attentional modulation of visual category representations ($p < .05$), unlike areas outside of this network (e.g., orbitofrontal cortex). Together, our combined approaches provide insights into the neural mechanisms of selective attention on visual category representations and their modulatory role on distributed information.

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Poster

145. Vision: Spatial and Feature-Based Attention

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Title: Utility of peripheral visual field in the SSVEP-based brain-computer interface

Authors: *K. TAKANO[1], N. MORITA-HAYASHI[1], K. KANSAKU[1,2,3]
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Abstract: Introduction
Patients in completely locked-in state (CLIS) are not able to perform voluntary movements, including eye movements. In such a patient, we applied a Steady-State Visual Evoked Potential (SSVEP) based brain-computer interface (BCI), and reported that the patient successfully operated the system (Takano et al. 2015). We asked the patient to use covert attention to a LED, which was placed in the peripheral visual field. To further investigate its physiological and psychological basis, we recruited healthy volunteers and confirmed that these participants were able to modulate SSVEP based on covert attention to the LED in the peripheral visual field (Morita et al. 2018). In this study, we further investigated utilities of the system, by applying psychological testing.
Methods
Eight able-bodied participants (4 female, 32.4 y.o) participated in this study. We prepared a green/blue LED flicker (Sakurada, et al., 2015). The LED was placed in the peripheral visual field at 5, 10, 15, 30 and 45 degrees in the opposite side of the dominant eye. Participants were asked to gaze at a central fixation point, and attend to or ignore the LED (attention/ignore tasks). EEG signals were measured from Oz, PO7, and PO8. We calculated the power of the EEG data. These data were tested with two-way factorial ANOVA for the attention/ignore and the LED positions (5, 10, 15, 30 and 45 degrees). To record the utilities for each participant, we used a visual analogue scale (VAS score, 0-100) for each of 3 questions (ease of attention, ease of ignorance, ease of the whole task). We then computed the correlation between normalized EEG

data (amplitude during attention, amplitude during ignore, and difference of these amplitude) and normalized VAS score.
Results
The main effect was observed for the attention/ignore (ANOVA, $p = 0.0094$). A negative correlation was observed between amplitude during ignorance and ease of ignore ($p = 0.0012$, FDR corrected), and a positive correlation was observed between difference of amplitudes in both attentional states and ease of the whole task ($p = 0.046$, FDR corrected).
Conclusion
This results showed that healthy participants were able to control the amplitude of SSVEP by covert attention to a LED in the peripheral visual field, and that the amplitude was correlated with utilities of the participants. The SSVEP-based BCI system may be useful for patients who lose eye movements.

Disclosures: K. Takano: None. **N. Morita-Hayashi:** None. **K. Kansaku:** None.

Poster

145. Vision: Spatial and Feature-Based Attention

Location: SDCC Halls B-H

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Program #/Poster #: 145.05/GG14

Topic: D.07. Vision

Support: Research to Prevent Blindness Disney Award for Amblyopla Research

Title: Endogenous attention in amblyopic children

Authors: *P. V. RAMESH, L. KIORPES
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Abstract: Amblyopia is a cortical visual disorder caused by unequal visual input to the brain from the two eyes during development. Amblyopes show reduced visual acuity and contrast sensitivity as well as more “global” perceptual losses, such as figure-ground segregation and global form integration. Currently, there is no consensus on the neural basis for these higher-order perceptual losses. One contributing factor could be that amblyopes have deficiencies in attentional processing, such that the attentional processes that control the selection of information favor the better eye. Previous studies in amblyopic adults are conflicting as to whether attentional deficits exist. To test this hypothesis, we studied attentional processing in amblyopic children. We examined covert endogenous attention using a classical spatial cueing paradigm in amblyopic and visually-typical 6-10 year old children – an age range when many visual functions are approaching maturity. In our paradigm, four different shapes were simultaneously presented on a touch-sensitive display screen for a brief interval (400ms) while the child maintained fixation monocularly on a central cross; the non-fixating eye was patched.

Following a short delay, a response cue appeared to indicate which of the 4 locations the child must report on. The task was to select the shape that had appeared at the indicated location. Attention was manipulated by preceding stimulus presentation by a brief informative cue that accurately predicted the location of the upcoming target on half of the trials; other trials were preceded by a neutral, uninformative cue. If endogenous attention was intact, accuracy would be higher and response latency shorter on valid cue trials for all children. Indeed, we found that all children, despite visual condition, benefitted from attentional cueing: they performed significantly better on trials with a valid cue than with the neutral cue. Response latencies were also significantly shorter for the valid cue condition. No difference was found between performance of the amblyopic and the visually-typical children, or between dominant and non-dominant eyes of all children. The results showed that covert spatial attention is intact in amblyopic and visually-typical children, and is therefore not likely to account for higher-order perceptual losses in amblyopia.

Disclosures: P.V. Ramesh: None. **L. Kiorpes:** None.

Poster

145. Vision: Spatial and Feature-Based Attention

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Program #/Poster #: 145.06/GG15

Topic: D.07. Vision

Support: DFG TH 425/12-2

Title: How the brain integrates top-down information to optimize geometrical gaze following

Authors: *M. GÖRNER[1], P. KRAEMER[1,2], H. RAMEZANPOUR[1], P. W. DICKE[1], P. THIER[1]
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Abstract: We use the other´s gaze to identify her/his object of interest and to shift our attention to the same object, i.e. to establish joint attention. To identify the target object, information on the other´s gaze vector has to be transferred into the ego-centric frame of reference of the observer. The vector direction can be determined by integrating information on eye, head and body orientation. However, the direction may not be sufficient as gaze may hit more than one object. In this case, information on the target distance relative to the other one, i.e. information on vector length, is additionally needed. Only in case this distance is small enough, eye convergence can be exploited to determine the vector length. We hypothesize that in all other cases, the observer must resort to “top-down” estimates of the relative values of the objects for the other person or to other information useful to disambiguate the scene and to identify the target object. In an event-related fMRI experiment we tried to localize the brain areas responsible

for the integration of information on vector direction and length. Our subjects (n=18, 10 females) saw an image of an actor looking at an array of objects from two classes, houses and hands. The actor´s gaze always hit three objects and subjects had to make a saccade to the gazed at object from the class, identified as target class by a spoken instruction coming with 2/3 of the trials. This instruction could either unambiguously identify the target object (only 1 object from the relevant class hit by the gaze vector=condition 1), or fail to do so (2 or more objects from that class hit=condition 2; no instruction=condition 3). In accordance with previous studies we identified a 'gaze following patch' (GFP) in the pSTS, activated by gaze following. The fact that BOLD activity here turned out to be independent of condition, may suggest that the GFP is confined to the extraction of gaze direction. Running an exploratory whole-brain-analysis, in which we searched for regions influenced by condition, we identified two, one in the inferior frontal junction (IFJ) and a second one corresponding to the posterior parietal cortex (PPC). A time course analysis of BOLD activity showed that the condition-dependent modulation in the IFJ started clearly earlier than in the PPC, suggesting its relevance for the disambiguation of the target. The later condition-dependent modulation in the PPC is consistent with the PPC´s role in the allocation of spatial attention.
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Poster

145. Vision: Spatial and Feature-Based Attention

Location: SDCC Halls B-H

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Topic: D.07. Vision

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Title: Using pharmaceuticals to study how cognition affects perception

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Abstract: We showed recently that cognitive processes that improve perceptual performance such as arousal, spatial attention and learning affect neuronal populations in visual cortex in similar ways, even while acting on very different time scales. This work led to a strong hypothesis, that perceptual performance can be predicted by simple signatures of neuronal population activity, regardless of how performance is at a particular level. Here, we develop a framework to test this hypothesis using commonly used stimulants, such as caffeine, as well as pharmaceutical drugs used to treat Attention Deficit Hyperactivity Disorder (ADHD), such as methylphenidate and amphetamine. Our goal is to compare their effects on performance on perceptual tasks and on neuronal population activity with those caused by cognitive processes such as arousal, attention, and learning. We measured the effects of pharmaceuticals on the performance of rhesus monkeys during a visual change-detection task that manipulates spatial attention. Using an approach inspired by signal detection theory, we measured effects of these stimulants on several measures of visual perceptual performance, including sensitivity (*d*'), criterion (*c*), spatial bias, and the effects of a randomly administered bonus reward which affects general cognitive processes like arousal and motivation. Our preliminary results suggest that stimulants affect these measures in ways that are similar to but dissociable from the effects of cognitive processes. Understanding the effects of stimulant drugs on behavioral performance, as well as on the activity on neuronal populations in future studies, may answer fundamental questions about the neuronal mechanisms underlying perceptual performance as well as clinical questions about the different behavioral and neuronal effects of these commonly prescribed drugs.

Disclosures: B.S. Bowes: None. **A.M. Ni:** None. **M.R. Cohen:** None.

Poster

145. Vision: Spatial and Feature-Based Attention

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Title: Reliance on central vs. peripheral vision for visual search in younger and older adults

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Abstract: It has been suggested that older adults tend to rely more on their central vision compared to peripheral vision when processing a visual scene. To test this, we examined how older and younger participants performed visual search tasks when their central vision was occluded to different degrees. Visual search can measure two types of visual information processing with two different tasks: the pop-out version relies on bottom-up processing of the entire visual scene (i.e. global processing) whereas the serial version requires top-down processing of each feature serially (i.e. local processing). 13 healthy younger (*M* = 21.8, *SD* = 1.5) and 15 older adults (*M* = 69.1 years, *SD* = 7.3) performed a pop-out and a serial version of a visual search tasks in the presence of different sized gaze-contingent artificial central scotomata (no scotoma, 3° diameter, 5° and 7°). Participants were asked to indicate as quickly as possible whether a target was present or not among distractors whose number varied (16, 32 or 64 objects). We found evidence for a greater decline in peripheral processing in older adults compared to younger in pop-out but not in serial search. For the pop-out control condition with no scotoma, we found that the further the target in the periphery, the longer the search time for both groups. Importantly, the increase in search time was much greater in older than in younger participants. Further, in the scotoma conditions, we observed that both groups took longer to find the target with increasing sized central scotoma, but that this increase in search time was much greater for older adults than for younger participants. For the serial condition, we observed similar overall effects of target distance from center and scotoma size however there was no difference across groups. We surmise that this may be due to the difficulty of the task, such that central vision is necessary for serial search and therefore, occluding central vision is equally disruptive for both older and younger groups. In conclusion, we suggest that global bottom-up peripheral visual scene processing is decreased in older compared to younger adults.

Disclosures: J. Ouerfelli-Éthier: None. **A. Laurin:** None. **T. Tran:** None. **G. Blohm:** None. **L. Pisella:** None. **A.Z. Khan:** None.

Poster

145. Vision: Spatial and Feature-Based Attention

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Topic: D.07. Vision

Support: National Eye Institute Intramural Research Program at the National Institutes of Health

Title: Correlated variability in populations of midbrain neurons constrains perceptual choice

Authors: *J. P. HERMAN, L. N. KATZ, R. J. KRAUZLIS
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Abstract: Recent work has demonstrated the importance of considering “noise correlations” in determining what can be read out from neuronal populations during perceptual choices. The midbrain superior colliculus (SC) participates in covert perceptual choices but the role of correlations in SC neurons has not been examined. Here, we considered the theoretical consequences of correlations within and between pools of neurons in the SC, as well as measuring those correlations in 1 monkey.
We considered how varying degrees of correlation would affect read-out in a simple difference model comparing pooled activity between the right and left SC. We found that in this model positive correlations between right and left SC would facilitate read-out, whereas both negative between-SC correlations and positive within-SC correlations would hinder read-out. We identified specific combinations of between- and within-SC correlation values that best predicted the monkey’s behavior, and others that would prevent accurate prediction.
We measured the extracellular activity of left and right SC neurons simultaneously with multi-contact probes while the monkey made covert perceptual choices. The monkey’s task was to decide whether a relevant (cued) peripheral stimulus had changed color saturation - requiring a joystick release, or if the irrelevant (foil) stimulus had changed, requiring him to maintain joystick hold. In 3 sessions, we recorded the activity of 80 visual-movement neurons, slightly more than 1000 between- and within-pool pairs. On average, SC neurons displayed weak but significantly positive pairwise correlations, which were stronger within (0.09) than between pools (0.047). Our measurements of within- and between-SC correlations nicely matched a subset of the values that allowed our pooling model to best predict monkey performance.
Our findings support the conclusion that covert perceptual choices can be decoded from the pooled activity of midbrain neurons. The match between our theoretical calculations and empirical measurements suggests that the read-out of covert perceptual choices from the SC depends on the relative levels of activity in the right and left SC. These results also raise questions about the particular inputs to the midbrain that give rise to the correlation structure we observed, and how the correlation structure might change with task instructions or during learning.

Disclosures: J.P. Herman: None. **L.N. Katz:** None. **R.J. Krauzlis:** None.

Poster

145. Vision: Spatial and Feature-Based Attention

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Support: CIN (German Excellence Initiative of the German Research Foundation (DFG) greant EXC307)
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Title: Feature-based attention selectively modulates connectivity between sensory regions and attention and default networks

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Abstract: While numerous previous studies investigated how feature-based attention modulates mean neural activity, little is known about effects of feature-based attention on connectivity between feature-selective regions and nodes of the dorsal attention network or of the default mode network. Here we used a block design with ultra-long trials lasting 3 minutes that alternated between color attention, motion attention and passive viewing, involving the same stimuli. This paradigm allowed for high-quality functional connectivity measurements free of confounds related to on- and offset effects of stimulus blocks. Functional connectivity was measured between visual regions V4 and V5/MT+ (responsive to color and motion, respectively) with nodes of the dorsal attention network (DAN) and of the default mode network (DMN). Attention to color versus motion enhanced the connectivity between DAN and V4 more than between DAN and V5/MT, revealing selective enhancement of connectivity depending on the attended feature. Conversely, color versus motion attention reduced the connectivity between DMN and V4 more than between DMN and V5/MT+, showing feature-selective reduction in connectivity. The results hence show that not only nodes of the DAN selectively modulate connectivity with specific visual regions depending on the attended feature, but that the same holds true for nodes of the DMN, yet with inversed sign. The current study raises a role of DMN, previously known to be involved in rest, mind wandering or inversely in attention to external features, to interact with visual regions in a selective manner during feature selective attention.

Disclosures: S. Kwon: None. **A. Bartels:** None.

Poster

145. Vision: Spatial and Feature-Based Attention

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Title: Signal transmission between monkey areas V2 and V4 causally depends on gamma-band phase synchronization

Authors: *E. DREBITZ, L.-P. RAUSCH, H. STEMMANN, A. K. KREITER
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Abstract: Successful behavior in our complex environment relies on the brain's ability to adapt neuronal information processing fast and flexible to changing demands. Attention-dependent selective processing of a relevant stimulus despite of the presence of other, irrelevant stimuli is a characteristic example. Numerous studies have shown that neurons, which receive signals of multiple different stimuli, respond selectively to the currently attended stimulus, almost "as if" only the attended stimulus would be presented within their RF. The routing-by-synchronization hypothesis proposes that such selective processing of the attended stimulus results from enhanced synaptic transmission caused by selective γ-band synchronization between these neurons and the subset of afferent inputs representing the attended stimulus. Furthermore, this hypothesis states that afferent inputs representing non-attended and therefore irrelevant stimuli do not synchronize their activity with the downstream neurons, which decreases their impact onto these neurons. Indeed, we showed previously that V4 neurons synchronize their γ-band activity up to eight times stronger with afferent V1 neurons representing the attended stimulus, than with V1 neurons providing signals of a non-attended stimulus. Here we investigate, whether such changes in synchronization are causally responsible for a change in effective signal transmission. To this end, monkeys performed a demanding shape tracking task that required attending one of four stimuli, which continuously changed their shape. Two of the stimuli were located in the same V4 RF and each stimulus could become target or distractor with equal probability. During trial periods which required monkeys to recognize the reappearance of the initial shape we stimulated a small set of neurons in supra-granular layers of area V2 that encoded one of the stimuli located in the same V4 RF with a single biphasic electrical pulse. LFP and spikes were recorded in V4 with up to four microelectrodes. We found that the impact of electrically evoked V2 spikes strongly depended on the phase of the current γ-cycle in the V4 population. The animals' behavioral response was significantly slower when the spikes arrived at a specific phase of the V4 γ-oscillation, whereas there was no such effect for other γ-phases. Furthermore, the potency of the electrically evoked V2 spikes to trigger spikes in area V4 neurons strongly depended on the phase of the γ-oscillation at their arrival. In summary, these results indicate that adjustment of γ-band phase synchronization is causally responsible for selective signal routing and information processing.

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Poster

145. Vision: Spatial and Feature-Based Attention

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Topic: D.07. Vision

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Title: Coordination of cortical state within and between areas V1 and V4 of the Macaque

Authors: *J. VAN KEMPEN[1], M. BOYD[1], T. A. ENGEL[2], M. A. GIESELMANN[1], A. THIELE[1]
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Abstract: Cortical activity reveals spontaneous activity fluctuations that are not solely determined by external inputs, but reflect changes to the underlying excitability of neurons, referred to as cortical state. Cortical states fluctuate strongly between sleep-wake states, but even during wakefulness state fluctuations do occur, which influence sensory processing as well as behavioural performance. During less active/inactive states, cortical activity displays highly synchronous activity, characterized by increased low-frequency oscillations and high spike-spike correlations. During active states, the cortex is more desynchronized and displays suppressed low-frequency and increased high-frequency activity, as well as lower correlations in population spiking activity. Recent evidence (Engel et al., 2016. Science) revealed that global fluctuations in cortical state are modulated locally by spatially selective, top down attention in V4, and influence behavioural performance. Global cortical states are thus coordinated by cognitive demands and can operate on a local scale. Whether similar local attention induced state changes occur in primary sensory cortex, and whether (and how) cortical state changes are coordinated between cortical areas is unknown. To investigate this, we recorded simultaneously from V1 and V4 using 16-contact laminar electrodes in 3 awake, behaving Macaque monkeys performing a selective attention task. We used a Hidden Markov Model (HMM) to characterize the On-Off dynamics in multi-unit activity independently for V1 and V4, and investigated the effects of these dynamics on activity within and across both areas. We find that cortical states in V1 and V4 are correlated during both (1) passive fixation when no stimuli are presented, as well as (2) during stimulus presentation. State changes in either area induce polarity reversals in LFPs both within and across brain areas. On to Off switches result in a positive LFP deflection (indicative of less cellular excitability), whereas Off to On switches result in a negative deflection. During Off episodes in V1 or V4, low frequency oscillations (<10 Hz) are more prominent within and across areas. During On phases, power at low frequencies is suppressed whereas gamma-range frequencies (40-80 Hz) are enhanced. Thus, cortical state changes are coordinated between corresponding retinotopic locations in V1 and V4, state changes in V1 or V4 can trigger state changes in the corresponding areas, and they are linked to changes in local excitability within these areas.

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Poster

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University of Bremen, Center for Cognitive Neuroscience

Title: Attentional allocation increases mean firing rates of target representations in V1 over nearby distractor representations via two distinct mechanisms

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Abstract: Selective attention serves selective processing of behaviourally relevant stimuli at the expense of irrelevant stimuli. It has often been observed to result in an increase of neural activity associated with the attended stimulus (target), a decrease of activity associated with the non-attended stimulus (distracter), or a combination of both mechanisms. Which of these possibilities is actually used in primary visual cortex and how the magnitude of these modulatory effects depend on the stimulus strength of closely spaced target and distracter stimuli is still an open question. In this context, neural models [1] employing phase shifts between oscillating populations to realize selective stimulus routing between V1 and V4 [2] actually predict an imbalance between the strength of the population responses in V1 for target and distractor in favour of the target representation for successfully gating these afferent signals at V4.
Here we investigate how attention acts on V1 populations representing nearby target and distractor stimuli under different contrast combinations implying different strength of population responses. Microelectrode recordings were performed in area V1 of awake behaving macaque monkeys engaged in a demanding shape tracking task which required the animals to attend a target in close proximity to a distracter. When target and distracter stimuli were of equal contrast, the firing rate of the V1 population representing the target was increased, and the firing rate of the distracter population was decreased. Interestingly, we found that the rate of the target population is increased more strongly if the target stimulus has a lower contrast than the distracter stimulus, as if compensating for the contrast difference. Thus, in both conditions attentional intervention induces a rate imbalance in favour of the target population in V1 over the nearby distracter population. Analysis of errors in trials where a low contrast target was combined with a high contrast distracter revealed that if attentional allocation fails, the firing rate

of the target population was not increased over the rate of the distractor population. Furthermore, analysis of effect sizes and time courses of attentional rate effects suggests that target facilitation and distracter suppression stem from two distinct mechanism and are not two sides of the same coin.

[1] Harnack D., Ernst U.A., and Pawelzik K.R. (2015), J. Neurophysiol. 114, 1593-1605, https://doi.org/10.1152/jn.01038.2014
[2] Grothe I., Rotermund D., Neitzel S.D., Mandon S., Ernst U.A., Kreiter A.K. and Pawelzik K.R. (2018), J Neurosci 38 (14) 3441-3452; DOI: https://doi.org/10.1523/JNEUROSCI.2221-17.2018

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Poster

145. Vision: Spatial and Feature-Based Attention

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Title: Assessing attentional resources allocated to irrelevant stimuli in primates

Authors: *J. T. ARSENAULT[1], S. R. MURRIS[2], R. VOGELS[3], W. VANDUFFEL[4]
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Abstract: Both reinforcement and attention play important roles in gating perceptual learning. Nonetheless, a full understanding of these mechanisms requires isolation of their respective contributions. However, attention and reinforcement are difficult to disentangle as attention is often oriented towards reinforced stimuli. Task-irrelevant learning offers a way to investigate the role of reinforcement in learning irrelevant stimuli while potentially avoiding stimulus-oriented attention. Importantly though, irrelevant stimuli can still capture attention under many conditions. To examine conditions whereby irrelevant stimuli captured attention, we examined task-relevant performance while varying the saliency of task-irrelevant stimuli. Monkeys were trained on a relevant color discrimination task. In this task, monkeys fixated centrally for a randomized period (1500 - 3000 ms), after which a color target (size 0.3° x 0.3°, 7.5° above) was shown for 33 - 500 ms. A hand response was then used to indicate the color target (e.g. blue-left,

red-right). Monkeys showed a clear relationship between target duration and task performance with ceiling level performance after 200-500 ms. Grayscale face and body images on a static background (oriented rightward or leftward, shown at the diagonal at 3° below the fixation point) were utilized as irrelevant stimuli. To determine appropriate SNR levels of these irrelevant stimuli, monkeys first performed an orientation discrimination task (face/body). During subsequent testing with the color task, irrelevant stimuli were shown at SNR levels corresponding to 60% performance at the face/body orientation task, and color task performance was compared with and without irrelevant stimuli. Presence of a low SNR irrelevant stimulus on a static background reduced color task performance, suggesting that the irrelevant stimuli captured attention. To further reduce the saliency of irrelevant stimuli, a background of consistently refreshed (60 Hz) white noise was used. Because this noise further degraded the saliency of the irrelevant stimuli, they were shown at slightly higher SNR levels (corresponding to 70% correct during the orientation task). Reduced performance on the relevant color task persisted. Further tests at a reduced SNR level (corresponding to 60% correct during the orientation task) yielded no difference in performance between trials with and without irrelevant stimuli, suggesting that they became unattended stimuli. Overall these results demonstrate the capacity of even weak stimuli to divert attentional resources and underline the importance of empirical tests for assessing the allocation of attention.

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Poster

145. Vision: Spatial and Feature-Based Attention

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Title: Enhanced neural processing by covert attention only during microsaccades directed towards the attended stimulus

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Abstract: Attention can be 'covertly' directed without eye movements, yet, even during visual fixation there are continuous microsaccades (MS) occurring at about every 300ms. In areas V4 and IT of macaques performing a sustained attention task, we found that firing rates and stimulus representations were enhanced by attention, but only following a MS towards the attended stimulus. For MS away from the cued stimulus the attentional modulations were absent. The onset of neural attentional modulations after a cue was tightly coupled to the MS onset. Because MS direction switched continuously at single trial level, attention effects emerged and vanished locked to MS several times within the same trial. These effects could neither be explained by differences in eye positions nor did artificial microsaccadic-like movements of stimulus reproduce the observations suggesting that the linkage between MS and attention is extra-retinal. The results reveal a major link between the effects of covert attention on visual processing and the overt movement of the eyes during visual fixation.

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Poster

145. Vision: Spatial and Feature-Based Attention

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Title: Task dependence of spatial attentional modulations in the cortex: The C1 and beyond

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Abstract: Recent interest has piqued regarding whether or not the first component of the visual evoked potential, the C1, is modulated by spatial attention (Slotnick, 2018). We have recently suggested three factors that may determine whether this modulation is seen in the context of a target detection task: the similarity of stimulus features between targets and non-targets, motivation/performance feedback, and the net luminance levels of the background display/stimulus compound (Kelly and Mohr, 2018). Here, we report experiments with two

groups of participants designed to test the first two of these hypotheses, which did not show any attentional modulation of the C1 (investigation into the luminance factor is ongoing). In all cases, participants maintained spatial attention at one of two locations where they monitored upcoming stimuli to detect targets. All stimuli consisted of a standard stimulus (a +/-45deg tilted Gabor) with targets superimposed on top. The first group performed the task with similar and dissimilar targets (in two blocked conditions) under motivated conditions in both cases. The second group always detected a similar target but performed both with and without a motivation manipulation. The similar target was an orthogonal Gabor with the dissimilar target being a disc. The motivation manipulation (which produced significant improvement in task performance, $p<.01$) was provided by a "gamified" version of the task, which included online feedback of performance and difficulty level, as well as a detailed end-of-block, graphical display of "level progression". In the unmotivated condition participants were unaware of this gamified task structure and were given limited end-of-block feedback only. We note that this is not a pure manipulation of motivation as the differential performance feedback may facilitate other processes, such as learning.
Although attention did not modulate the C1, we observed significant attentional enhancements of the P1, N1, P3b, and suppression of the P3a ($p<.001$ in all cases). We further observed greater attentional enhancement in the motivated condition. Our design included the motivation manipulation both between and within groups (although in the latter case the motivated condition was always completed second, allowing for practice effects). Within groups the interaction was present in all four components (P1, $p<.01$; N1, $p<.05$; P3a, $p<.001$; P3b, $p<.0001$) whereas between groups it was present only for the P3a ($p<.01$) and P3b ($p<.05$). We interpret these results as an enhancement of top-down attentional processes by our motivation manipulation, with a practice effect emerging additionally via early sensory components.

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Poster

145. Vision: Spatial and Feature-Based Attention

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Topic: D.07. Vision

Title: Feature-based attention spreads within and between objects

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Abstract: Feature-based attention has been characterized by two core mechanisms: 1) when attending to a feature in one part of the visual field, perceptual processing is enhanced for items sharing that feature across the visual field (location-spreading), and 2) when attending to a single

feature, attention spreads to other features belonging to the same object (within-object spreading). Here, we test whether and how these two mechanisms interact. Participants attended to two independent fields of moving dots positioned to the left and right of fixation; each dot field contained two sets of spatially intermingled dots, one group of dots moving upwards, the other group of dots moving downwards. In one visual half-field (left or right), each group of dots had a distinct color (green or red), while in the other visual half-field all dots shared the same color (white). On each trial, participants were cued to attend to one set of colored dots and were instructed to detect a luminance decrease of the attended colored dots. At the same time, they were asked to attend to the white dots on the other side to detect a brief increase in motion speed, regardless of motion direction. We hypothesized that feature-based attention would spread from the attended color to the direction of motion of these dots (within-object spreading), and subsequently spread to other regions across the visual field (location-spreading). If this were the case, we would expect an increase in detection rate of the speed changes in the matching direction relative to the non-matching direction. Consistent with this spreading account, in the first experiment (N=20) we found that participants had higher accuracy in detecting speed changes that matched the motion direction of the attended colored dots relative to the non-matching motion direction (8.7% difference in hit rate, $p < .001$). To investigate whether this spreading can be flexibly controlled, in a second experiment we manipulated the proportion of trials in which the speed increase matched the motion direction of the attended colored dots. Participants (N=48) were randomly assigned to two groups with 80% or 20% match trials, so that spreading across feature dimensions would be more or less beneficial to performance, respectively. There was no reliable difference between the groups in detecting speed changes as a function of the direction of the attended color dots (8.2% vs 6.1% difference in hit rate, $p = .512$), suggesting that participants were unable to control the spreading of feature-based attention. Broadly, these data suggest that attention to a single feature results in spreading within objects and across locations, even when detrimental for task performance.

Disclosures: A.F. Chapman: None. **V.S. Störmer:** None.

Poster

145. Vision: Spatial and Feature-Based Attention

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 145.18/HH10

Topic: D.07. Vision

Support: NSERC
University of Toronto Scarborough
University of Toronto

Title: Decoding electrophysiological correlates of task-driven attention to shape and surface features

Authors: *N. LEE[1], L. GUO[1], A. NESTOR[1], M. NIEMEIER[1,2]
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Abstract: Attention to visual features such as colour, motion, or contours has been shown to aid vision throughout the visual field. This feature-based attention appears to often operate at early stages of processing, and it seems to work along parallel, additive channels if more than one feature is attended. We have recently found that multiple features continue to have additive effects in a difficult object perception task that includes perceptual integration and decision processes, consistent with the idea that attention might be applied to entire objects, and thus, to later stages of perception. To clarify the time frames of attention to object features here we recorded electrophysiological signals from 64 scalp electrodes in human participants while they viewed real objects. Objects had one of two shapes and colours, respectively, where the colours indicated the weight of the objects. Further, to manipulate attention participants either grasped and lifted the objects or touched them with their knuckle so that shape and colour were more or less task-relevant and to different degrees. Pattern classification of shape and colour based on spatiotemporal EEG data found that accuracy peaked at the N1, around 100-200 ms after stimulus presentation. Shape and colour classification were higher during grasping compared to knuckling and this difference was especially robust in the case of shape. Taken together, this suggests that task-related attention modulates representation of features based on their relevance for executing an action at intermediate latencies.

Disclosures: N. Lee: None. **L. Guo:** None. **A. Nestor:** None. **M. Niemeier:** None.

Poster

145. Vision: Spatial and Feature-Based Attention

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Program #/Poster #: 145.19/HH11

Topic: D.07. Vision

Support: NSF IRFP 0965110
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GT Neural Engineering Center 1241384

Title: Spatial anticipation modulates the gain of visual responses in mouse primary visual cortex

Authors: *A. SPEED, N. MIKAIL, D. COBB, B. HAIDER
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Abstract: Fundamental studies in primates show that cognitive factors such as anticipation and attention modulate the amplitude (or gain) of neural responses to sensory stimuli. It remains debated if these cognitive factors and their neural effects exist in mice. Do mice show behavioral effects of stimulus anticipation? What are the neural correlates of these behavioral effects?
We developed a behavioral paradigm in head-fixed mice where they learn to detect visual stimuli localized in discrete portions of visual space. By presenting stimuli for many repeated trials in the same location, we measured if stimulus anticipation affected detection speed and accuracy. For a block of trials at a given spatial location (typically 15-25 trials), reaction times were significantly slower on early versus late trials (-12.6% or -85.9 ms, $p < 0.01$, $n = 15$ mice,). Moreover, when the stimulus unexpectedly appeared in a spatially distant location (>90°away), detection probability was markedly reduced on early versus late trials at the new location (36% failures early, 23% late). These observations indicate that mice learn to anticipate the spatial location of visual stimuli, and use this to improve their behavior, consistent with effects of spatial anticipation and attention in higher mammals.
To assess the effects of stimulus anticipation on neural responses, we performed multi-site silicon probe recordings in primary visual cortex (V1) from neurons whose receptive fields (RF) overlapped the spatial location of stimuli detected for rewards. In between detection trials, we measured neural responses to unrewarded “probe” stimuli. We compared the amplitude of local field potential (LFP) responses to the same probe stimuli across two conditions: trials when detected stimuli appeared at the location of maximal neural sensitivity (central RF), and trials when the detected stimuli appeared far outside this region. On trials when detected stimuli appeared in the central RF, probes evoked LFP responses that were larger than trials when detected stimuli appeared elsewhere (193 ± 107 μV vs 123 ± 48 μV). Since stimuli appeared repeatedly in the same location, the gain of LFP responses to probes increased across trials: responses were 24% percent larger later versus earlier in the block. These results suggest that anticipation improves behavioral performance and modulates the gain of cortical sensory responses in a spatially specific manner, as observed in higher mammals. We are currently investigating the laminar and cellular basis of these effects.

Disclosures: A. Speed: None. **N. Mikail:** None. **D. Cobb:** None. **B. Haider:** None.

Poster

145. Vision: Spatial and Feature-Based Attention

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Program #/Poster #: 145.20/HH12

Topic: D.07. Vision

Support: NIH Grant EY017921

Title: Effects of large-scale bilateral optogenetic inactivation of the lateral prefrontal cortex on feature attention and working memory

Authors: *D. MENDOZA-HALLIDAY[1], H. XU[1], F. A. C. AZEVEDO[1,2], R. DESIMONE[1]
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Abstract: Numerous studies have suggested that the lateral prefrontal cortex (LPFC) plays a role in visual feature attention and working memory. However, in most studies, the relationship between LPFC activity and these cognitive functions has been merely correlational; furthermore, studies that have attempted to show a causal link have mostly done so by inactivating LPFC in a temporally non-specific manner. To overcome this limitation, here we devised a method for large-scale bilateral optogenetic LPFC inactivation in the macaque monkey during specific periods of a working memory-guided feature attention task. In each trial, a sample stimulus - a full-screen random-dot surface with motion in one direction - was presented for 0.8 s. After a 3.2 s delay period, two overlapping full-screen random dot surfaces were presented - one with motion in the sample direction (i.e., the target), and one with motion in the opposite direction (i.e., the distractor). A brief speed change occurred in a randomly-located and randomly-timed small patch of dots, and the animal was rewarded for reporting this change if it happened in the target but not the distractor. Three levels of task difficulty were achieved by varying the percentage of speed-changing dots within the patch. The task required maintaining the sample motion direction in working memory and then selectively allocating attention to this direction in a spatially-global manner. To achieve large-scale bilateral LPFC optogenetic inactivation, we replaced the native dura with transparent artificial duras over both lateral prefrontal cortices and made over 170 injections of the inhibitory opsin Jaws covering a surface area of ~50 mm^2 per hemisphere. Each LPFC was optogenetically stimulated with a high-power 635 nm laser 18 mm above the cortical surface. We recorded neuronal activity in visual areas MST and MT. Stimulation was delivered in half of the trials, specifically during the target/distractor presentation. We found that bilateral LPFC optogenetic inactivation caused a remarkable deficit in task performance compared to control trials. Interestingly, this deficit scaled with task difficulty, with a 30% decrease in performance in the most difficult trials. A decrease in task performance also occurred when optogenetic stimulation was delivered during the delay period. Moreover, LPFC inactivation also caused a decrease in the responses of motion direction-selective neurons in MT and MST, far from the optogenetically-stimulated regions. Our results suggest that LPFC plays a key role in visual feature attention and working memory and that such role may involve the top-down modulation of activity in visual cortical neurons.

Disclosures: D. Mendoza-Halliday: None. **H. Xu:** None. **F.A.C. Azevedo:** None. **R. Desimone:** None.

Poster

145. Vision: Spatial and Feature-Based Attention

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Topic: D.07. Vision

Support: F31 EY028018-02
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Title: Separable codes for behavior in mouse primary visual cortex across attentional states

Authors: *A. M. WILSON[1], J. M. BECK[1], L. L. GLICKFELD[2]
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Abstract: Fluctuations in neuronal activity in sensory cortices are tightly linked to perception and can be used to identify the contribution of specific neuronal populations to behavior. However, since flexible behavior likely requires engaging distinct circuits, this relationship between neuronal activity and behavior may depend on context. Thus, we developed a multi-modal attention task for head-fixed mice in order to better understand the context-dependent relationship between neuronal activity and behavior. In this task, mice press a lever to initiate the presentation of a series of oriented gratings ("distractor" stimuli). On visual trials, the mouse learns to release the lever in response to a "target" stimulus of a different orientation. On auditory trials, the distractor visual stimulus is present, but mice are instead cued to detect an auditory target. Mice performed worse when tested with rare, invalidly cued targets, demonstrating a modality-specific shift of attention. We used two-photon imaging to record from neurons in primary visual cortex (V1) while mice performed the task and found that neurons are more strongly driven by the visual stimulus when vision is attended. To investigate the neuronal specificity of this attentional modulation, we performed a logistic regression analysis on a population of tuned, task-responsive neurons. Neurons were weighted based on how well their activity predicted either the presence of a visual target or, in a separate analysis, how well they predicted the mouse's behavior from trial-to-trial. We find a sub-linear relationship between target-weight and behavior-weight in which neurons with positive target weights have similar weights for behavior, but neurons with negative target weights have near-zero weights for behavior. Thus, the activity of these cells is predictive of the distractor stimulus, but not correlated with behavioral response. This suggests that subgroups of V1 neurons make unique contributions to the decision-making process. Surprisingly, when this analysis was performed on auditory trials, we found auditory target-weights and behavior-weights similar in magnitude to visual trial weights. However, the weights found to predict visual and auditory behavior were highly uncorrelated. Taken together, this suggests that when the mouse is using visual

information, a unique population of neurons in V1 informs behavior. Furthermore, this demonstrates that modulation of V1 neurons by attention acts in a task-specific manner. We are currently investigating how the activity of neurons that are predictive of behavior on visual or auditory trials is modulated by attentional state.

Disclosures: A.M. Wilson: None. **J.M. Beck:** None. **L.L. Glickfeld:** None.

Poster

145. Vision: Spatial and Feature-Based Attention

Location: SDCC Halls B-H

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Program #/Poster #: 145.22/HH14

Topic: D.07. Vision

Title: Manipulating the attentional field of primate V1 neurons

Authors: *M. A. GIESELMANN, A. THIELE
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Abstract: The Normalization Model of Attention (Reynolds and Heeger , 2009) predicts that attentional modulation in V1 depends on the stimulus size and on the size of the attentional field. The effects of stimulus size on V1 responses have been widely described. However, manipulating the size of the attentional field in the context of a classic visual attention task is largely unstudied at the cellular level. Here, we manipulated the size of the attentional field by changing the spatial certainty of an attended detection target. We trained two macaque monkeys to detect the occurrence of a small circular patch, occurring on (or offset from) a small or large stationary sinewave grating, presented centered on the neuron's receptive field. Cuing of attention was towards the receptive field or into the opposite hemifield. Two different foci of attention were investigated. In the "narrow focus" condition (spatial certainty) the target appeared in the center of a V1 receptive field. In the "wide focus" condition (spatial uncertainty) the target could appear in one of 12 possible positions distributed across an area of 4 by 4 degrees centered on the RF. Conditions varying the site and the focus of attention were presented in blocks with a 100% and 93% cuing validity.
We analyzed 91 V1 thresholded, spiking multi-units recorded in V1. We computed the average firing rate during the sustained part of the visual response (300-500 ms from stimulus-onset) just before the detection target appeared. In 31 units (34%) the size of the attentional focus affected attentional modulation. A widened focus of attention could increase or decrease attentional modulation (n=15, n=16, respectively). Thus, while the size of attentional focus affects attentional modulation, the effects vary across the neuronal population.
References:
Reynolds, J.H., and Heeger, D.J. (2009). The normalization model of attention. Neuron 61, 168-185.

Disclosures: M.A. Gieselmann: None. **A. Thiele:** None.

Poster

145. Vision: Spatial and Feature-Based Attention

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Support: The study was funded by a grant to ST from the Deutsche Forschungsgemeinschaft (DFG): Collaborative Research Center 889 "Cellular Mechanisms of Sensory Processing" (Project C04).

Title: Optogenetic inhibition reveals a causal role of the direct anatomical connection from the FEF to extrastriate visual area MT in mediating attentional modulation in non-human primates

Authors: *J. HÜER[1], M. G. FORTUNA[1], H. GUO[1], L.-T. SCHILLER[2], A. GAIL[1], J. GRUBER[2], H. SCHERBERGER[3], J. STAIGER[4], S. TREUE[1]
[1]Cognitive Neurosci. Lab., [2]Med. RNA Biol., [3]Neurobio. Lab., German Primate Ctr., Göttingen, Germany; [4]Institue for Neuroanatomy, Univ. Med. Ctr., Göttingen, Germany

Abstract: Firing rates in primate extrastriate visual areas are modulated by spatial attention. Previous studies suggest that the frontal eye field (FEF) plays a major role in controlling the attentional modulation of sensory responses in visual areas. These studies, however, used electrical microstimulation and pharmacological inactivation, which cannot differentiate effects arising via direct or indirect pathways originating in FEF. We used optogenetics to probe the role of the direct anatomical connection from the FEF to visual area MT in mediating attentional effects by inhibiting this connection. We injected a viral vector (AAV5-αCaMKII-eNpHR3.0-mCherry) into the FEF of four rhesus macaques. Two of the animals were used for histological confirmation that the inhibitory opsins are incorporated in presynaptic terminals of FEF projections to area MT. The other two animals were trained in a spatial attention task. Two moving random dot patterns were presented at 2 to 10 degrees eccentricity relative to a fixation point. We manipulated the attentional focus of the animals by cueing the target stimulus at the beginning of each trial. They were rewarded for responding to a direction change in the target RDP while ignoring direction changes in the distractor RDP. Several months after virus injection, we recorded single cell activity in area MT and delivered a continuous laser pulse to the vicinity of the recording electrode tip to inhibit FEF input during the sustained response of MT neurons. Trials with and without optical stimulation were randomly interleaved. Optogenetic inhibition reduced attentional modulation of MT neurons significantly during the stimulation period. More specifically, firing rates were decreased when the RDP placed in the receptive field of the recorded neuron was the target, whereas firing rates were increased when the same stimulus was the distractor. Our results reveal that the direct anatomical projection from the FEF

to visual area MT plays a causal role in both, enhancing target responses and reducing distractor responses during a spatial attention task.

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Poster

145. Vision: Spatial and Feature-Based Attention

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Program #/Poster #: 145.24/HH16

Topic: D.07. Vision

Support: DFG WE 5469/2-1

Title: Phase shifts of monkey V1-MT cross-frequency coupling predict reaction time

Authors: *D. WEGENER, B. SCHLEDDE
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Abstract: In an ever-changing sensory environment with plentiful information, attention-dependent cortical processing prioritizes relevant information for conscious perception. On a behavioral level, attention accelerates the reaction time (RT) for detecting a change in visual input. In area MT, fast RTs are associated with shorter latencies of transient firing rate changes in response to a speed-change of a moving stimulus (Galashan et al., Neuron 2013), occurring independent of differences in firing rate. Here, we investigate the physiological mechanism underlying this reduced latency of MT neurons. We conducted simultaneous electrophysiological recordings in areas MT and V1 in two macaque monkeys. Animals were required to covertly attend a drifting grating and to detect an abrupt acceleration of the motion signal. We measured cross frequency correlations (CFC) by means of phase-amplitude coupling (PAC) in the 400 ms preceding the target speed change occurring within the receptive fields of pairs of MT-V1 neurons. We found that low γ-band amplitude (around 35 Hz) of the local field potential (LFP) in one area was temporally modulated along the phase of a low-frequent LFP oscillation in the other area. The specific low frequency band for phase-providing signals differed between animals but was confined to frequencies in the α- and β- bands. Crucially, the preferred phase of coupling, i.e. the mean angular direction of the PAC, was shifted between trials with fast, medium, or slow RT. Preferred phases were different between animals but for both we found significant shifts along the cosine of the low frequent oscillation between slow and fast RT trails. Using the spiking activity of paired recordings from area MT, we approved our original finding (Galashan et al., Neuron 2013) that trials with fast RT, although having no difference in pre-change firing rate, possess shorter latencies and higher transient peaks than trials with slow RT. The new data now suggest that these differences in the transient response to the stimulus change

are caused by differences in CFC and moreover, that the specific phase of PAC has a functional relevance for perceptual performance. Our results promote current models of selective attention incorporating the temporal modulation of local γ synchrony as a mean of long range coordination of feedforward signal Transmission.

Disclosures: D. Wegener: None. **B. Schledde:** None.

Poster

145. Vision: Spatial and Feature-Based Attention

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Topic: D.07. Vision

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Title: The dorsal pulvinar periodically synchronizes cortical hubs of the attention network, regulating engagement at an attended location

Authors: *I. C. FIEBELKORN, M. A. PINSK, S. KASTNER
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Abstract: The pulvinar is the largest thalamic nucleus in the primate brain. It is organized into subdivisions that are characterized by their anatomical connectivity with both cortical and subcortical structures. The dorsal pulvinar, for example, projects to and receives projections from cortical hubs of the fronto-parietal network that directs spatial attention. Lesions in this region of the pulvinar result in symptoms that are similar to hemineglect following lesions of parietal cortex. Despite such clear evidence of a critical role in attentional function, there have been few studies characterizing neuronal responses in the dorsal pulvinar, let alone investigating its functional role in the broader attention network. We therefore simultaneously recorded from the dorsal pulvinar and two well-characterized, cortical hubs of the attention network in macaques: the frontal eye fields (FEF) and the lateral intraparietal area (LIP). We first compared local neural activity (both local field potentials and single unit activity) across these cortical and subcortical regions, showing that neural activity in the dorsal pulvinar and LIP are relatively similar during spatial attention. We then examined between-region interactions. Our results indicate that the dorsal pulvinar synchronizes neural activity in FEF and LIP, with this synchronization occurring in the low-beta range (~10–20 Hz). We previously demonstrated that spatial attention samples the visual environment in theta-rhythmic cycles, with theta phase organizing neural activity in the attention network into alternating states associated with either

better or worse behavioral performance. Pulvinar-mediated synchronization is specific to periods of rhythmic engagement at the presently attended location (i.e., the attentional state associated with relatively better behavioral performance at the cued location). In comparison, LIP-mediated synchronization seems to characterize periods of relative disengagement from the presently attended location (i.e., the attentional state associated with relatively worse behavioral performance at the cued location), perhaps in anticipation of a potential attentional shift. Our findings thus elucidate the role of the dorsal pulvinar during the dynamic network interactions that shape environmental sampling.

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Poster

145. Vision: Spatial and Feature-Based Attention

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Program #/Poster #: 145.26/II1

Topic: D.07. Vision

Title: Microsaccades predict spatial attention: Feature learning of microsaccade properties for oculo-feedback

Authors: J. EMOTO[1], *Y. HIRATA[2]
[1]Chubu Univ. Grad. Sch. of Engin., Aichi, Japan; [2]Chubu Univ. Col. of Engin., Aichi, Japan

Abstract: Microsaccade (MSC) has been considered to be a potential objective indicator of spatial attention. Previously, we developed the oculo-feedback (FB) system that detects each MSC highly accurately (>98%) in realtime and feeds-back the event to the subject thru sensory stimulation (Emoto & Hirata, 2016). It was demonstrated in humans that MSC properties can be modified by repetitive application of the oculo-FB, suggesting that the subject's mental states reflected in MSCs can be self-controlled by this scheme. Presently, we aimed at developing a novel method to evaluate relationship between MSC properties (e.g. rate, direction, timing) and attentional states. We designed a new network architecture of variational autoencoder (VAE) in order to learn the feature space of MSC properties, and to predict subject's task performance (reaction time: RT) based on the learned features. The proposed method is intended to estimate the best MSC spatiotemporal pattern that achieves the optimal task performance. The designed VAE is composed of encoder, decoder, and translator. The encoder converts MSC properties to a feature vector, while the decoder converts them inversely. The translator converts a feature vector to a corresponding task performance. We optimized the parameters of the VAE by using eye and task performance data collected in the following experiment. A subject wearing an eye tracker (EyeSeeCam) sat in a dark room at 31 cm from a 35-inch PC monitor with one's head fixed by a chin rest, and was instructed to look at a small dot (0.1 deg visual angle) displayed on the monitor during each trial. A trial started when the

subject pushed a button, and after a random interval between 1.25 s and 1.75 s, a visual target (bar extending 0.73 deg) appeared somewhere at 8 deg from the dot. The bar was tilted 15 deg either in clockwise (CW) or counter-clockwise (CCW) direction. After around 30 ms from the target appearance, a white circle occluded the target. The subject had to answer the tilting direction of the target by pressing a button assigned to CW or CCW. Eleven subjects participated and underwent 120 trials each. From each trial, data in the 1-sec interval from the trial start was used because it is assumed that MSCs in this interval are affected only by top-down attention. Data except for those from 3 subjects highly contaminated by noise were randomly divided into a training and validation data set. As a result of optimizing the VAE, the mean squared error of RT prediction was 0.12 s with respect to the mean RT of 1.14 s, suggesting that specific spatiotemporal patterns of MSCs reflect reliably top-down attentional states, and the VAE currently configured successfully mapped their relationship.

Disclosures: J. Emoto: None. **Y. Hirata:** None.

Poster

145. Vision: Spatial and Feature-Based Attention

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Topic: D.07. Vision

Support: EU project #6819978:BRAIN 3.0
ANR-14-ASTR-0011-01 BRAINAPP

Title: Decoding multiplexed attention, temporal expectation and response preparation information from the prefrontal cortex

Authors: *S. BEN HAMED, F. DI BELLO, S. BEN HADJ HASSEN, C. P. GAILLARD
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Abstract: The frontal eye fields (FEF) plays a key role in top-down attentional control (Ibos, et al. 2013). In a recent study, we estimate in real-time the (x,y) position of the covert spatial attentional spotlight, using classification methods applied to the ongoing monkey FEF multi-unit activity (Astrand et al., 2016). Like in other prefrontal cortical areas, information in the FEF is highly multiplexed both at the single cell and at the population level. Here, we demonstrate that taking into account temporal expectation and response preparation related-information, drastically improves the real-time access to the attentional spotlight. In our previous work, the attentional spotlight was decoded, during a cued spatial target detection task, achieving an average 4-class classification performance of 64% (chance = 25%). Here, we show that decoding is drastically improved when performed on trials from the same CTOA length categories (av. 75% performance). Importantly, interpolating the classifier weight changes as a function of

CTOA length to infer a continuous weight = f(time from cue) function further improved the decoding performance. We likewise show that retroactively taking into account reaction time duration also improves decoding performance (av. 70% performance). These results demonstrate that although spatial attention, temporal expectation and response preparation information are multiplexed they can be independently accessed throughout the trial, thus providing important cues on how information is encoded in the prefrontal cortex. This also provides very specific methods to improve the performance of attention-driven cognitive brain machine interfaces.

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Poster

145. Vision: Spatial and Feature-Based Attention

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Topic: D.07. Vision

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Title: Real-time decoding of covert attentional spotlight from monkey prefrontal local field potentials

Authors: *C. DE SOUSA FERREIRA, C. P. GAILLARD, S. BEN HADJ HASSEN, F. DI BELLO, S. BEN HAMED
Inst. Des Sci. Cognitives Marc Jeannerod, Bron, France

Abstract: The ability to access brain information in real-time is important both for a better understanding of cognitive functions and for the development of therapeutic applications based on brain-machine interfaces. Great success has been achieved in the field of neural prosthesis. Progress is still needed in decoding higher-order cognitive processes such as covert attention. Recently, we showed that we can access the position of the covert attentional spotlight in real-time using classification methods on frontal eye fields multi-unit activity (MUA) in the non-human primate (Astrand et al., 2016). Importantly, we demonstrated that the (x,y) decoded covert attentional spotlight parametrically correlates with the behavioural perceptual responses of the monkeys thus validating our decoding of covert attention. To extend our findings and get closer to non-invasive techniques, we here replicate our previous work using local field potentials (LFP) signals collected during a cued spatial target detection task. Specifically, we evaluate the performance of major machine learning methods at extracting the covert attentional spotlight both from the overall LFP frequency content, and from specific functional frequency bands. We further quantify how much this extracted information (whether a discrete attentional

locus or a continuous (x,y) attentional locus) correlates with overt behaviour. These results are compared to our previous MUA decoding results. Overall, this study confirms that the covert attention spotlight can be accessed from LFP frequency content, in real-time, and can be used to drive high-information content cognitive brain machine interfaces for the development of new therapeutic strategies.

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Poster

145. Vision: Spatial and Feature-Based Attention

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Topic: D.07. Vision

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Title: The prefrontal cortex attention spotlight explores space rhytmically

Authors: *C. P. GAILLARD, F. DI BELLO, S. BEN HADJ HASSEN, Y. BIHAN-POUDEC, S. BEN HAMED
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Abstract: Recent behavioral studies suggest that attention samples space rhythmically (Landau and Fries, 2012, Kastner et al., 2013; VanRullen et al., 2013, Dugué et al., 2016). Oscillations in brain activity have been described as a possible mechanism supporting attentional processes. However, the precise mechanism through which this rhythmic exploration of space is subserved remains unknown. In a previous study (Astrand et al., 2016), we applied machine learning methods to ongoing monkey prefrontal multi-unit population activity, to decode, in real-time, the (x,y) location of the attentional spotlight. Here, we further demonstrate that the overall decoded spatial attention information that can be extracted from population multi-unit activity oscillates at a 7-12Hz rates. These oscillations in attentional information account for stimulus encoding. On trials in which the target is correctly detected, how much information about the target is available in the neuronal population oscillates at the same frequency as attentional information. The same is true for the encoding of the distractor on false alarm trials, in which the distractor is mistaken for a target. Oscillations in the decoded attentional spotlight also account for variations in overt behavior, whether hit rates in response to a target or false alarm rates in response to a distractor. Importantly, these oscillations characterize displacements of the decoded attentional spotlight. While these oscillations are task-independent, we demonstrate that how space is explored by the decoded attentional spotlight is task specific. In other words, while 7-12Hz oscillations mediate

attentional displacement, top-down control flexibly adjusts these displacements to the ongoing behavioral demands.

Disclosures: C.P. Gaillard: None. **F. Di Bello:** None. **S. Ben Hadj Hassen:** None. **Y. Bihan-Poudec:** None. **S. Ben Hamed:** None.

Poster

146. Cellular Physiology in the Basal Ganglia: Novel Cell Types and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 146.01/II5

Topic: E.03. Basal Ganglia

Support: NINDS K08

Title: A novel tool for studying striosome connectivity and function

Authors: *M. M. MCGREGOR[1], G. MCKINSEY[3], C. J. BAIR-MARSHALL[4], A. E. GIRASOLE[2], M. RYAN[5], J. L. RUBENSTEIN[6], A. B. NELSON[2]
[2]Neurol., [1]UCSF, San Francisco, CA; [3]Psychiatry, Stanford, Menlo Park, CA; [4]Neurol., [5]UC San Francisco, San Francisco, CA; [6]Nina Ireland Lab. Dev Neurobiol, Univ. of California San Francisco, San Francisco, CA

Abstract: The striatum is the major input nucleus of the basal ganglia. While lacking gross anatomical divisions, neurochemical stains reveal that the striatum is composed of distinct compartments termed striosomes and matrix. Output neurons within striosome compartments, identified by markers such as enriched expression of μ-opioid receptors and diminished expression of calbindin, are thought to have distinct connectivity with upstream and downstream targets, as compared to those in the matrix. Anatomical tracing studies suggest that striosome neurons receive preferential input from regions involved in limbic functions (basolateral amygdala, prelimbic cortex, bed nucleus of the stria terminalis), while those in the matrix receive greater input from sensorimotor regions (primary motor and sensory cortex). Additionally, striosome neurons are thought to uniquely project monosynaptically to dopamine neurons in the substantia nigra pars compacta (SNc), which exert powerful control over striatal activity. These anatomical differences have led to the hypothesis that striosome neurons play a distinct behavioral role from other striatal output neurons. However, until recently, difficulty identifying striosome neurons outside of post mortem tissue has limited understanding of their physiology and behavioral function. Furthermore, more recent tools for targeting striatal output neurons, termed medium spiny neurons (MSNs), in striosomes have produced variable results in regards to earlier studies. Thus, the advent of novel tools for identifying and manipulating striosome neurons is critical to advance our understanding of their function. Here, we establish the Meis2^{CreER} mouse line as a novel tool for identifying and manipulating striosome MSNs *in vivo*.

Using a combination of optogenetics, histology, and in vivo and in vitro electrophysiological recordings, we show that 1) Meis2^{CreER} mice enable targeting of striatal neurons within striosome compartments. 2) Targeting of striatal neurons is restricted to MSNs. 3) Labelled MSNs within striosome compartments receive differential input from limbic and motor regions, as compared to those in the matrix. 4) Activation of labelled neurons is sufficient to inhibit firing of SNc dopamine neurons. Preliminary results also examine differences in electrophysiological properties of striosome and matrix neurons within either the direct or indirect pathway.

Disclosures: M.M. McGregor: None. **G. McKinsey:** None. **C.J. Bair-Marshall:** None. **A.E. Girasole:** None. **M. Ryan:** None. **J.L. Rubenstein:** None. **A.B. Nelson:** None.

Poster

146. Cellular Physiology in the Basal Ganglia: Novel Cell Types and Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 146.02/II6

Topic: E.03. Basal Ganglia

Support: NIH Grant NS097185

Title: Firing patterns and resonance in striatal fast-spiking interneurons

Authors: *M. H. HIGGS[1], C. J. WILSON[2]
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Abstract: Striatal fast-spiking interneurons (FSIs) fire runs of spikes separated by pauses, a pattern known as stuttering, and entrain their spikes to a preferred frequency component of their input, a phenomenon called spiking resonance. In principle, spiking resonance could result from subthreshold voltage oscillations during the pauses, and/or the characteristic frequency and rhythmicity of the spike runs. To investigate these possibilities, we obtained perforated-patch recordings from FSIs in striatal slices from male and female parvalbumin-Cre-tdTomato mice. Cells were stimulated with current noise at several mean current levels, producing firing patterns varying from largely irregular spiking at the lowest current to dense stuttering at the highest current. Spiking resonance was measured from the spectrum of the spike-triggered average current (STA). The resonant frequency (f_R) was indicated by the spectral peak, and the resonance strength (s_R) was taken as the relative increase in spectral magnitude from $f_R/2$ to f_R. If spiking resonance arose from subthreshold voltage oscillations, we would predict high s_R even in the low-current condition with irregular spiking. However, the data showed low s_R in this condition but much higher s_R with increased mean current, and s_R was highly correlated with the content of regular inter-spike intervals in the spike trains. These results suggest that the subthreshold oscillations produce only weak spiking resonance, but spike runs give stronger resonance. As a

further test of this idea, we analyzed the average current before spikes preceded by pauses (isolated-spike-triggered average, iSTA), correcting for the prior condition of spike absence for the specified period. The iSTA spectra showed lower s_{res} compared to the regular STA spectra, confirming that rhythmic firing enhanced spiking resonance. The iSTA spectra had greater magnitude below f_R and similar magnitude at higher frequencies, indicating that FSIs in the paused, depolarized state have high sensitivity to a broad range of input frequencies. Our results suggest that signal transmission by FSIs depends on their firing pattern; sparse spikes can encode a wide range of input frequencies, whereas rhythmic bursts or runs transmit gamma-frequency signals more selectively.

Disclosures: M.H. Higgs: None. **C.J. Wilson:** None.

Poster

146. Cellular Physiology in the Basal Ganglia: Novel Cell Types and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 146.03/II7

Topic: E.03. Basal Ganglia

Support: NIH Grant NS097185

Title: Signal transmission from globus pallidus to substantia nigra pars reticulata

Authors: *D. V. SIMMONS, M. H. HIGGS, C. J. WILSON
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Abstract: The indirect pathway is the projection from external globus pallidus (GPe) to the subthalamic nucleus, and from there onto basal ganglia output nuclei. However, the GPe also projects directly to substantia nigra pars reticulata (SNr), making strong inhibitory connections onto SNr neurons. To investigate the impact of this pathway, we recorded GPe firing activity in response to patterned current stimuli, characterized GPe-mediated synaptic currents in SNr neurons, and simulated the effects of GPe output on the spiking activity of an SNr neuron model. Using perforated-patch recording, broadband current noise and beta-frequency (20 Hz) currents were injected into GPe neurons, and the spiking output was recorded. To characterize GPe to SNr synaptic inhibition, a virus encoding a Cre-inducible channelrhodopsin was injected into GPe of Parvalbumin-Cre mice, targeting the type of GPe neurons that project to SNr. Brain slices were prepared, and unitary IPSCs from GPe axons were evoked by minimal photo-stimulation and recorded in whole-cell voltage-clamp mode. Based on these synaptic data, the output spike trains from GPe were converted to synaptic conductance waveforms, incorporating the measured synaptic conductance amplitudes, rise time, decay time constant, and number of unitary connections detected, as well as previously described synaptic depression. Conductance waveforms representing 1-3 unitary GPe inputs were applied to an SNr neuron model

constructed based on our phase resetting data, and the model spike times were analyzed. The results suggest that in addition to regulating the firing rates of SNr neurons, the GPe-to-SNr pathway can effectively transmit signals in the beta and gamma frequency bands.

Disclosures: D.V. Simmons: None. **M.H. Higgs:** None. **C.J. Wilson:** None.

Poster

146. Cellular Physiology in the Basal Ganglia: Novel Cell Types and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 146.04/II8

Topic: E.03. Basal Ganglia

Support: NARSAD Young Investigator Grant from the Brain & Behavior Research Foundation

Title: Differential modulation of striosome and matrix spiny projection neurons by dopamine in the dorsal striatum

Authors: *E. M. PRAGER, J. L. PLOTKIN
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Abstract: As the primary input nucleus of the basal ganglia, the striatum integrates highly convergent afferents from cortical and subcortical brain regions to promote or suppress action initiation and guide motor-decision making behaviors. This requires the engagement of two distinct populations of striatal spiny projection neurons (SPNs): D_1 dopamine receptor-expressing direct pathway SPNs (dSPNs) and D_2 dopamine receptor-expressing indirect pathway SPNs (iSPNs). Adding a further level of complexity, both dSPNs and iSPNs reside in one of two chemically- and anatomically- defined compartments, known as striosomes (or patches) and matrix. We have recently shown that dSPNs in striosome and matrix compartments of the dorsal striatum respond similarly to convergent excitatory synaptic stimulation: coordinated stimulation of distal dendritic synapses induces long-lasting depolarized somatic “up-states” that are similar in both dSPN types. Here we explore how this form of synaptic integration is modulated by dopamine, using Nr4a1-eGFP transgenic mice (to identify striosomes) crossed with pathway-specific reporter lines (to identify SPN type). D_1-receptor-mediated modulation of up-states differed in a compartment-specific manner: D_1-receptor activation significantly attenuated up-state duration in striosome dSPNs and prolonged it in matrix dSPNs. The opposing effects of D_1-receptor activation were Ca^{2+} dependent, and due to reduced density of L-type voltage gated Ca^{2+} channels in striosome dSPN dendrites. Blocking L-type Ca^{2+} channels in matrix dSPNs caused D_1-receptor activation to attenuate up-state duration; enhancing L-type Ca^{2+} channels in striosome dSPNs caused D_1-receptor activation to prolong up-state duration. Current experiments are underway to determine if dopaminergic modulation of iSPNs is compartment-

specific as well. These data suggest that the balance of striosome-matrix output of the dorsal striatum is shaped by fluctuations in dopamine.

Disclosures: E.M. Prager: None. **J.L. Plotkin:** None.

Poster

146. Cellular Physiology in the Basal Ganglia: Novel Cell Types and Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 146.05/II9

Topic: E.03. Basal Ganglia

Support: The goverment of Japan

Title: Absence of direct synaptic connectivity between striatal compartments in identified striosome neurons

Authors: *T. HERNANDEZ FLORES, Y. NAKANO, M. GARCIA MUNOZ, G. W. ARBUTHNOTT
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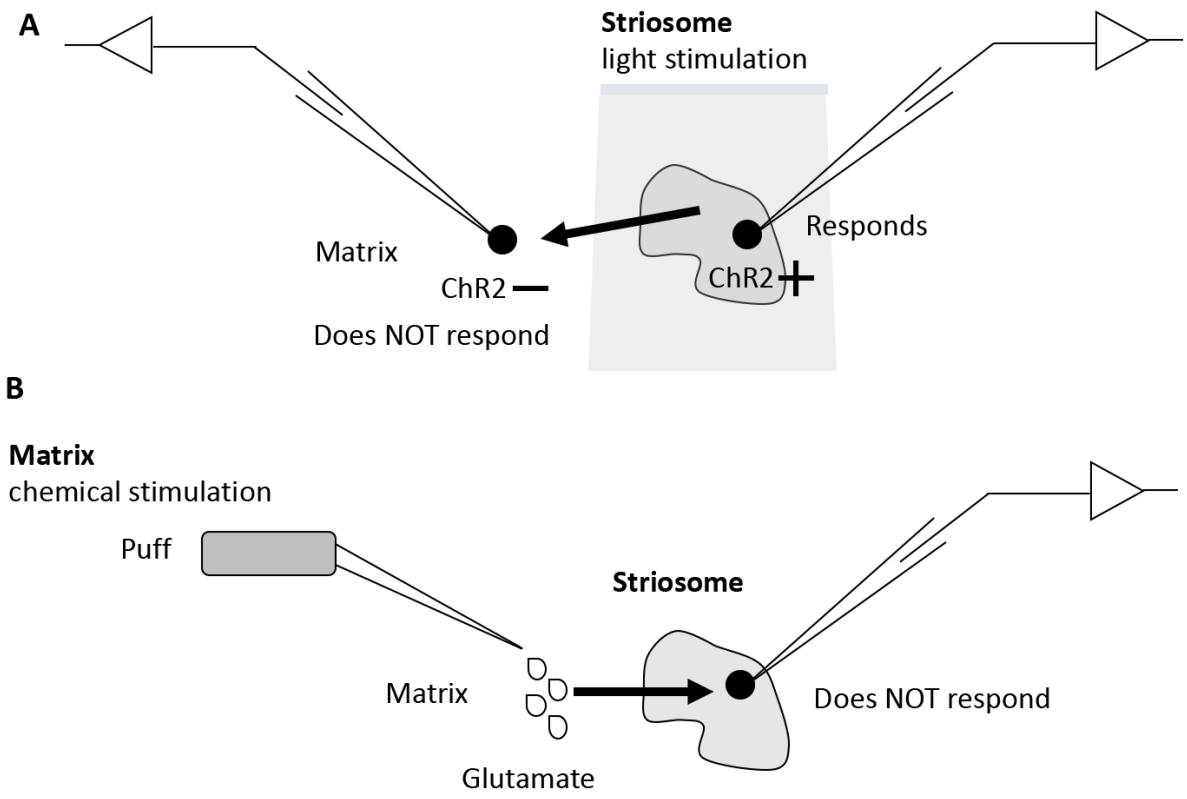
Abstract: Performance of motor and motivation-related behaviors is a function of the largest nucleus of the basal ganglia- striatum. Its neurons are distributed in two compartments: matrix and striosomes (review: *Brimblecombe & Cragg, ACS chemical neurosci. 8, 235,* 2017). The matrix is enriched with cholinergic markers and receives afferents from sensory and motor cortex. Striosomes, interdigitated in the matrix, form a three-dimensional labyrinth-like structure reactive to mu-opiod receptors that receives input from prelimbic, sensory and motor cortex. Medium spiny neurons of direct and indirect pathways are present in matrix and striosomes. Importantly, dendritic and axonal processes remain confined to their own compartments. In a previous work, channel rhodopsin 2 (ChR2) was expressed with an adeno-associated viral vector type 10 (AAV10) in the matrix compartment only. In this case, matrix photoactivation selectively and exclusively induces inhibitory postsynaptic currents (IPSCs) within the matrix (Lopez-Huerta, V.G., et al. *Brain structure & function,* 221, 1737, 2015). Here we asked if the reversed procedure renders similar results. We used Cre Sepw1-NP67 mice 25-30 days old, that selectively targets striosomal neurons (Gerfen, C.R. et al. *Neuron,* 80, 1368, 2013). We injected a Cre-dependent ChR2 (AAV1-ChR2-dflox-mCherry) to be able to activate striosomal neurons. After a two-week survival period, animals were perfused and whole-cell patch-clamp recordings of both striosome ChR2-positive and ChR2-negative (matrix) were performed. Photoactivation evoked action potentials and IPSCs within the striosome (A) but not the matrix. Additionally, when the matrix was activated by a glutamate puff delivered close to a recorded area (B), synaptic responses were not observed in striosomes. Our results confirm absence of synaptic connectivity in the direction matrix to striosome and viceversa.



Disclosures: T. Hernandez Flores: None. **Y. Nakano:** None. **M. Garcia Munoz:** None. **G.W. Arbuthnott:** None.

Poster

146. Cellular Physiology in the Basal Ganglia: Novel Cell Types and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 146.06/II10

Topic: E.03. Basal Ganglia

Support: JSPS KAKENHI Grant Number JP16J02300

Title: Distinct subdomains of the mouse striatum based on the diversity of the striosomes and matrix compartments

Authors: *Y. MIYAMOTO[1], S. KATAYAMA[1], N. SHIGEMATSU[1], A. NISHI[2], T. FUKUDA[1]

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Abstract: The striatum receives extensive input from the cerebral cortex and acts as the major input nucleus of the basal ganglia. The internal structure of the striatum is divided into two distinct compartments called striosomes (patches) and matrix, both of which contain two types of projection neurons expressing the dopamine D1 or D2 receptors. Although both compartments have been distinguished by differential chemical markers, their immunoreactivities are extremely diverse, and the distribution pattern of these striosomes remains to be clarified. In this study spatial distribution of the striosomes/matrix compartments in the mouse striatum was analyzed by immunohistochemistry combining antibodies against μ opioid receptor (MOR), Substance P (SP), Enkephalin (Enk), and Calbindin (CB). We found that the striatum could be divided into 3 major parts, 1) the compartment-rich area, 2) striosome-free space, 3) the most caudal tri-laminar part. The compartment-rich area had several domains containing 5 types of striosomes; those detectable only by MOR or SP, those detectable by both MOR and SP, those detectable by both MOR and Enk, and those detectable by all of MOR, SP, and Enk. The striosome-free space occupied a large volume in the lateral part of the striatum and showed low immunoreactivity for CB, contrasted with high CB-immunoreactivity in the matrix. The most caudal tri-laminar part located just lateral to the globus pallidus, where the most medial band was characterized by highly intense labeling for SP and absence of Enk labeling, the most lateral band with homogeneous Enk labeling and absence of SP, and the intermediate band was a caudal extension of striosome-free space. Furthermore, anterograde tracers were injected in various area of the cerebral cortex and the distribution patterns of labeled axon terminals were investigated in the striatum. Axon terminals originating from primary motor and sensory cortical areas were located in the striosome-free space, whereas axons from the associational/limbic cortical areas were distributed mainly in the compartment-rich area. Finally, the relationship between the striatal subdomains and the distribution of D1 and D2 receptor-expressing neurons was quantitatively analyzed. The proportion of D1-expressing neurons was approximately 70% in all SP-containing striosomes, 40% in SP-negative striosomes, as low as nearly 30% in striosome-free space, and about 50% in the matrix. We concluded that the new subdomains based on striatal internal structure are closely related to both the topography of cortical afferents and the structural organization of the striatum in terms of the direct and indirect parallel streams.

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Poster

146. Cellular Physiology in the Basal Ganglia: Novel Cell Types and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 146.07/II11

Topic: E.03. Basal Ganglia

Support: NS003134

Title: Dendrite specific inhibition of SNc dopamine neurons

Authors: *R. C. EVANS, E. TWEDELL, M. ZHU, J. ASCENCIO, Z. M. KHALIQ
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Abstract: Dopamine neurons of the substantia nigra pars compacta (SNc) have two specific dendrite types, lateral dendrites which run through the SNc and ventral dendrites which project into the substantia nigra pars reticulata (SNr). Anatomical evidence suggests that these ventrally-projecting 'SNr dendrites' receive strong inhibitory input (Henny et al., 2012) and that striatal axons wrap around this dendrite to form 'dendron bouquets' (Crittenden et al., 2016). However, functional differences between these two dendrite types remain undefined. Here we use morphological reconstructions and one-photon laser activation of channel rhodopsin (CoChR) to directly compare the synaptic and structural properties of the 'SNc dendrites' to the 'SNr dendrites' of dopamine neurons. We find that these dendrite types have distinct morphologies with SNc dendrites branching proximal to the soma, and SNr dendrites branching distal to the soma, and that these two structures receive differential inhibitory input from the striatum. Putting these morphological characteristics into our computational model of an SNc dopamine neuron shows that the morphology of the SNr dendrite facilitates the transmission of slow inhibitory signals to the soma. Using spatially-specific optogenetic stimulation of striatal fibers in the substantia nigra, we show that laser activation along SNr dendrites more effectively stops tonic firing in dopamine neurons than laser activation along SNc dendrites. In voltage clamp, we run a similar experiment measuring inhibitory currents in the presence of TTX and 4-AP to prevent axonal propagation. We find that stimulation of striatal patch (striosome) axons on the SNr dendrite of dopamine neurons results in larger currents than similar stimulation on the SNc dendrite. These inhibitory currents involve both GABA-A and GABA-B receptors. We contrast this finding to inhibitory inputs onto dopamine neurons from the Globus Pallidus (GPe), which show equally strong currents on both SNr and SNc dendrites, and do not show evidence of GABA-B receptor activation. Our experimental results demonstrate a functional, dendrite-specific connection between the striatal patch (striosome) compartment and SNc dopamine neurons.

Disclosures: R.C. Evans: None. **E. Twedell:** None. **M. Zhu:** None. **J. Ascencio:** None. **Z.M. Khaliq:** None.

Poster

146. Cellular Physiology in the Basal Ganglia: Novel Cell Types and Mechanisms

Location: SDCC Halls B-H

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William N. and Bernice E. Bumpus Foundation – Innovation Award to YK

Title: Convergent modulatory systems regulate striatal spinogenesis

Authors: *N. M. BANNON, C. M. GRAGE, Y. KOZOROVITSKIY
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Abstract: In the striatum, multiple neuromodulatory systems converge onto spiny projection neurons (SPNs), regulating key aspects of motivated behavior, locomotion, and goal-directed learning. Critical among these modulators are dopamine and adenosine, which activate distinct G protein coupled receptors (GPCRs) and bidirectionally regulate the activity of a key cellular enzyme, protein kinase A (PKA).

During postnatal development, SPNs produce new dendritic spines and excitatory synapses in response to focal glutamatergic stimulation. This process, although mechanistically dependent on glutamate, is powerfully modulated by GPCR activation and PKA activity. Increases in $G\alpha_s$ GPCR signaling and PKA activity enhance the probability of synaptogenesis evoked by 2-photon glutamate uncaging *ex vivo*, and enhance corticostriatal synaptic connectivity *in vivo*. To determine how different classes of GPCRs regulate *de novo* spinogenesis, we combined simultaneous 2-photon imaging of GFP-expressing indirect pathway SPNs with 2-photon glutamate uncaging. Short uncaging pulses delivered at 2 Hz next to a dendrite can induce the growth of new dendritic spines within seconds. First, we replicate data demonstrating the application of the adenosine A2AR agonist CGS21680 increases the probability of spinogenesis in response to 2-photon glutamate uncaging in the acute brain slice. Applying the $G\alpha_{i/o}$-coupled Drd2 agonist quinpirole suppressed evoked spinogenesis, demonstrating that two GPCRs with opposing effects on PKA activity have reciprocal effects on spinogenesis. When drug application was restricted to the local dendrite immediately prior to induction, these modulatory effects were still present, demonstrating that GPCR modulation of spinogenesis can act on fast temporal and small spatial scales.

These data show that dynamic modulatory signaling can control plasticity with rapidly defined subcellular domains. These advances lay a framework to help us understand how striatal plasticity can forge specific and meaningful connections in a recurrent circuit known for activity-dependent plasticity. Furthermore, multiple modulatory systems converge to control this phenomenon highlighting the role of SPNs as integrators of multiple signaling systems. This point is particularly relevant for neurodegenerative disorders such as Parkinson's, where the loss of dopaminergic signaling interacts with endogenous convergent modulatory systems.

Disclosures: N.M. Bannon: None. **C.M. Grage:** None. **Y. Kozorovitskiy:** None.

Poster

146. Cellular Physiology in the Basal Ganglia: Novel Cell Types and Mechanisms

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Program #/Poster #: 146.09/II13

Topic: E.03. Basal Ganglia

Support: NIH grant NS034645

Title: Cholinergic regulation of striatal GABAergic interneurons

Authors: *M. ASSOUS[1], J. M. TEPPER[2]
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Abstract: Striatal cholinergic interneurons (CINs) have long been known to play a crucial role in striatum acting directly on SPNs via neuromodulatory muscarinic receptors that have been demonstrated to regulate many aspects of striatal functioning. However, recent data have shown that CINs are also part of a fast synaptic circuitry involving multiple postsynaptic nicotinic receptors on striatal GABAergic interneurons. This notion was first suggested by indirect evidence demonstrating nicotinic activation of recurrent inhibition in CINs. Subsequently, we demonstrated that optogenetic activation of CINs elicits very large, disynaptic compound GABAergic IPSP/Cs in SPNs that are secondary to nicotinic receptor activation. These IPSCs involve NPY-NGF interneurons as well as other GABAergic interneurons targeted by viral transduction in Htr3a-Cre mice. Here we show that in addition to NGF interneurons at least 3 other striatal GABAergic interneuron populations receive direct innervation from CINs. Using double transgenic strategies, where ChAT-ChR2 mice were crossed with different Cre lines targeting striatal GABAergic interneurons, we found that tyrosine hydroxylase interneurons (THINs), fast-adapting interneurons (FAIs) spontaneously active bursty interneurons (SABIs) receive suprathreshold cholinergic input. This input originates primarily or exclusively from striatal CINs as lesioning of cholinergic brainstem neurons projecting to striatum do not significantly alter the excitatory responses. Interestingly, the nicotinic EPSP/Cs in different interneurons are heterogeneous with respect to receptor subtypes. While the β_2-subunit selective agent DHβE blocks the EPSP in NGFs, the disynaptic innervation of SPNs, the recurrent inhibition of CINS and the EPSs in a subset of FAIs, it only partially blocks the nicotinic input to THINs, SABIs and the EPSP in a subset of FAIs. We also provide evidence showing that THINs project back to CINs and through this pathway contribute to some extent to the recurrent inhibition measured in CINs after optogenetic cholinergic stimulation. Altogether, these data suggest that in addition to providing a modulatory muscarinic effect on SPNs, striatal CINs provide strong excitatory nicotinic input to a majority of striatal GABAergic interneurons and receive feedback projection from some of them. Hence, striatal CINs and GABAergic

interneurons form a specialized intricate bidirectional network which may be involved in many functions originally attributed solely to CINs.

Disclosures: M. Assous: None. **J.M. Tepper:** None.

Poster

146. Cellular Physiology in the Basal Ganglia: Novel Cell Types and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 146.10/II14

Topic: E.03. Basal Ganglia

Title: A new type of striatal neurons characterized by their large size, immunoreactivity with the antibody SMI-32, and selective distribution in the most caudal striatum

Authors: *S. OGATA, Y. MIYAMOTO, N. SHIGEMATSU, T. FUKUDA
Dept. of Anat. and Neurobio., Kumamoto Univ., Kumamoto, Japan

Abstract: The striatum is the main input nucleus of the basal ganglia. It contains one class of principal neurons, called medium spiny neurons, and at least four kinds of interneurons. We found a distinctive type of neurons immunostained by SMI-32, an antibody specific to non-phosphorylated form of high molecular weight neurofilament proteins. SMI-32 positive neurons did not belong to any type of striatal neurons so far identified. They were distributed in a region adjacent to the globus pallidus, thus in the most caudal part of the striatum. Their somata were as large as those of choline acetyltransferase (ChAT) positive neurons, which are known to be the largest type in the striatum, but SMI-32 neurons were immunonegative for ChAT. All SMI-32 positive neurons showed immunoreactivity for parvalbumin (PV), whereas the majority of PV neurons were negative for SMI-32. Because SMI-32 neurons showed immunoreactivity for glutamic acid decarboxylase (GAD), the GABA synthetic enzyme, they were found to be a subpopulation of striatal GABAergic neurons. SMI-32 neurons showed unique dendritic morphology in that their dendrites extended only to the lateral direction, avoiding medially located globus pallidus, and that the dendrites received numerous GAD and enkephalin (Enk) positive boutons so densely that almost all surfaces of the dendrites were covered by these boutons. With regard to the excitatory inputs, vesicular glutamate transporter1-immunoreactive boutons did not make contact with SMI-32 neurons, whereas vesicular glutamate transporter 2-immunoreactive boutons were in contact with these neurons. This suggests that excitatory inputs to SMI-32 positive neurons were mainly of the subcortical origin. Another novel finding was that the region where distal part of the dendrites reached was a specific domain characterized by the weak immunoreactivity for tyrosine hydroxylase as compared to the surrounding tissue of the striatum. Moreover, this domain received axon terminals labeled by anterograde tracers that were injected into the auditory cortex. From the above, it was concluded that SMI-32 positive neurons are GABAergic neurons that receive dense innervation from Enk-positive, most probably

indirect-pathway striatal neurons, and that their dendrites ramify in a specific domain that receive projection from the auditory cortex but is less innervated by dopaminergic neurons.

Disclosures: S. Ogata: None. **Y. Miyamoto:** None. **N. Shigematsu:** None. **T. Fukuda:** None.

Poster

146. Cellular Physiology in the Basal Ganglia: Novel Cell Types and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 146.11/II15

Topic: E.03. Basal Ganglia

Support: SNF Grant 166130

Title: Dopamine depletion induces circuit specific alterations of GABAergic transmission in the striatum

Authors: *L. RUBI, L. CRISTIÁ LARA, I. L. BOCCALARO, C. SCHWERDEL, J.-M. FRITSCHY
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Abstract: Parkinson's disease (PD) is caused mainly by the loss of dopaminergic innervation to the striatum, leading to changes in striatal circuit activity affecting the thalamo-cortical pathway, and hence to movement impairments. As the main input nucleus of the basal ganglia, the striatum regulates major brain functions and a dysfunction of striatal circuits causes not only motor, but also cognitive, and emotional deficiencies.
The striatum is composed mainly of GABAergic medium spiny neurons (MSN), expressing either D_1 dopamine receptors (D_1-MSN) and forming the direct, movement-promoting pathway, or D_2 dopamine receptors (D_2-MSN), forming the indirect movement-suppressing pathway. Besides D_1- and D_2-MSN, the striatum contains several subtypes of GABAergic and cholinergic interneurons, which modulate the activity and synchronization of MSN. So far, the local inhibitory synaptic circuits of the striatum remain poorly characterized, and little is known about the distribution of $GABA_A$ receptor ($GABA_AR$) subtypes distinguished by the α subunit variant on MSN and striatal interneurons. Here, we wanted to further investigate the specificity of certain $GABA_AR$ subtypes in D_1- and D_2-MSN, as well as in one group of low-threshold spiking (NPY^+) interneurons and their alterations upon dopaminergic denervation. Dopamine depletion was achieved by unilateral injections of the neurotoxin 6-Hydroxydopamine (6-OHDA) into the dorsal striatum of D1-EGFP, D2-EGFP, and NPY-EGFP reporter mice. Control animals underwent the same procedure and were injected with an equal amount of vehicle (ctrl). By using immunofluorescence and electrophysiology, we investigated the cell type-specific distribution of major $GABA_AR$ subtypes (α_1, α_2, α_3,) in a ctrl and 6-OHDA-injected group. In ctrl, GABAergic synapses on the soma of D_1- and D_2-MSN contain α_1 and α_2, but not α_3 $GABA_ARs$, with D_1-

MSN preferentially bearing α_2 $GABA_A$Rs. In contrast, we found specific expression of α_1 and α_3 $GABA_A$Rs in NPY^+ interneurons. D_2-MSN, which contain preferably α_1 $GABA_A$Rs, received more synapses from striatal interneurons than D_1-MSN. Dopamine depletion caused overall increased amplitude and frequency of sIPSCs in the three cell types, indicative of enhanced activity and possible disinhibition of interneuron circuits. Our data suggest that the striatum contains specialized intrastriatal GABAergic circuits, characterized by cell-specific expression of $GABA_A$R subtypes, with altered connectivity and function in the dopamine-denervated striatum. These GABAergic dysfunctions might act to compensate, in part, the imbalance between the direct and indirect pathway underlying motor deficits in PD.

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Poster

146. Cellular Physiology in the Basal Ganglia: Novel Cell Types and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 146.12/II16

Topic: E.03. Basal Ganglia

Support: NIH Grant R01 NS088528
NIH Grant T32 NS007480-17

Title: Striatal ERK activation in a knock-in mouse model of DOPA-responsive dystonia

Authors: *M. BRISCIONE, C. DONSANTE, X. FAN, A. P. SHANNON, S. BONNO, S. CAMPBELL, D. BERNHARD, A. DOWNS, D. GUTMAN, T. SARDAR, D. J. SUTCLIFFE, H. A. JINNAH, E. J. HESS
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Abstract: Abnormal dopamine (DA) neurotransmission is associated with many dystonic disorders; however, the precise nature of the pre- and postsynaptic defects in DA transmission that result in dystonia are not known. To address this, we used a knock-in mouse model of L-DOPA-responsive dystonia (DRD). DRD mice display the core features of the human disorder, including reduced striatal DA levels and abnormal movement that improves in response to L-DOPA, the precursor to DA. In the striatum, DA promotes movement by activating D1 receptors (D1Rs) expressed on direct pathway medium spiny neurons (MSNs) and inhibits movement by activating D2 receptors (D2Rs) expressed on indirect pathway MSNs. D1Rs stimulate adenylate cyclase (AC) activity and D2Rs inhibit AC; however, in DRD mice, D1Rs are supersensitive and D2Rs exhibit a change in valence whereby D2R agonists increase AC. Our objective was to determine signaling alterations in both MSN subtypes downstream of AC, specifically phosphorylated ERK (p-ERK), that contribute to the alleviation of dystonia by L-DOPA to

identify downstream targets that may serve as novel therapeutic targets. DRD mice and normal control mice were subcutaneously injected with L-DOPA (10 mg/kg), the D1R agonist SKF 81297 (0.2 mg/kg), the D2R agonist quinpirole (0.1 mg/kg), or saline. Mice were perfused 45 min after drug treatment and striatal sections were immunostained for p-ERK. DRD mice showed a significant and robust increase in p-ERK levels in the striatum in response to L-DOPA treatment compared to normal mice and saline controls. SKF 81297 treatment paralleled this response. This effect was specific to D1R-containing-MSNs. In contrast, quinpirole treatment did not evoke increases in p-ERK immunoreactivity. Future studies are aimed to elucidate additional cell-type specific mechanisms underlying increased p-ERK in DRD mice to refine downstream signaling mechanisms that are instrumental to the therapeutic effects. We will explore the role of D1R and D2R co-activation in regulating p-ERK and whether p-ERK may serve as a coincidence detector for convergent DA and glutamatergic signaling that occurs when endogenous DA signaling is restored in the striatum. We will also utilize the DRD mouse model to explore the downstream signaling pathways involved in the antidystonic effects of novel compounds that prove to alleviate abnormal movements.

Disclosures: M. Briscione: None. **C. Donsante:** None. **X. Fan:** None. **A.P. Shannon:** None. **S. Bonno:** None. **S. Campbell:** None. **D. Bernhard:** None. **A. Downs:** None. **D. Gutman:** None. **T. Sardar:** None. **D.J. Sutcliffe:** None. **H.A. Jinnah:** None. **E.J. Hess:** None.

Poster

146. Cellular Physiology in the Basal Ganglia: Novel Cell Types and Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 146.13/II17

Topic: E.03. Basal Ganglia

Support: Neurological Foundation of New Zealand (14-12 and 09-35)

Title: Substantia nigra pars reticulata-ventroanterior motor thalamus synapses are altered in parkinsonian rats

Authors: *L. C. PARR-BROWNLIE[1], R. A. SIZEMORE[2], R. A. SMITHER[2], S. M. HUGHES[3], C. BOSCH-BOUJU[4]
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Abstract: Profound alterations in basal ganglia neuronal activity underlie movement deficits in Parkinson’s disease (PD). We have previously shown that motor thalamus neuronal activity is also impaired, with reduced inhibitory modulations in movement-related and low threshold calcium spike burst activities. While impaired activity in basal ganglia afferents may underlie motor thalamus neuronal changes in the parkinsonian state, it is possible that structural changes

also contribute. To investigate structural changes at synapses formed by basal ganglia afferents in the motor thalamus, we injected a lentiviral vector that contained the GAD67 promoter to restrict expression of GFP to GABAergic neurons in the substantia nigra pars reticulata (SNr) of control (sham) and 6-hydroxydopamine (6-OHDA) lesioned rats. Using confocal microscopy, we found GFP-filled somata of neurons in the SNr and their axon terminals in the ventroanterior (VA) motor thalamus. We used immunohistochemistry to convert GFP to an electron dense label (3'3'5 diaminobenzidinetetrahydrochloride, DAB) to visualise SNr-VA boutons using cryo tomography electron microscopy (Zeiss 2200fs, 10,000x). Tomograms from a tilt series (+60 to -60 degrees) showed that DAB labelled SNr axon terminals contained multiple mitochondria, pleomorphic vesicles, and formed symmetric synapses typical of inhibitory connections. Consistent with previous studies of ventromedial thalamus in control animals, transduced SNr axon terminals formed large calyx-like boutons in the VA thalamus in sham rats (n = 5), with the majority (4/5, 80%) forming multiple synapses (3.3 ± 0.7 synapses). In contrast, there were significantly fewer synapses per SNr bouton (1.5 ± 0.5 synapses, p = 0.049, t-test) in 6-OHDA lesioned rats (n = 6), with multiple synapses present on 36% (5/14) of terminals. Associated with SNr-VA synapse changes, SNr boutons were 50% smaller in 6-OHDA lesioned than sham rats (274.9 ± 71.9 μm^2 versus 560.0 ± 125.4 μm^2, p = 0.029). Data indicate that SNr boutons in VA thalamus are altered following degeneration of midbrain dopamine neurons. These morphology changes are likely to alter synaptic transmission at SNr-VA synapses, and could contribute to the dysfunctional motor thalamus activity reported in the parkinsonian state.

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Poster

146. Cellular Physiology in the Basal Ganglia: Novel Cell Types and Mechanisms

Location: SDCC Halls B-H

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Topic: E.03. Basal Ganglia

Support: KAKENHI JP (25282247 and 15K12770)
"Adaptive Circuit Shift" (26112001)

Title: The unique distribution of D1 and D2 dopamine receptors in the lateral caudal striatum of rodents

Authors: *K. OGATA, F. KARUBE, Y. HIRAI, F. FUJIYAMA
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Abstract: The striatum is the main target of the dopamine release from the substantia nigra pars compacta, and is known to mainly have two subtypes of dopamine receptors, D1 receptor (D1R)

and D2 receptor (D2R). D1R is expressed in direct pathway medium spiny neurons (dMSNs) directly projecting to the output nuclei, the substantia nigra pars reticulata and the entopeduncular nucleus. D2R is expressed in indirect pathway MSNs (iMSNs) projecting to the globus pallidus. These two types of neurons were homogeneously and randomly distributed in most of the dorsal striatum. However, in the specific sub-region of the lateral caudal striatum adjacent to the globus pallidus, D2R and pre-pro Enkephalin (PPE), the markers for iMSNs, were almost absent. This area is called D2R-expressing MSNs-poor zone (D2R-poor zone). In the present study, we firstly demonstrated the area where the expression of D1R was significantly poor than the other striatal area, so called D1R-poor zone. This region was located lateral to the D2R-poor zone, and the immunoreactivity for tyrosine hydroxylase and dopamine transporter were poor in both D1R-and D2R-poor zones. To confirm whether the proportion of dMSNs was certainly small in the D1R-poor zone, a retrograde tracer was injected in the entire output nuclei. As the result, dMSNs were significantly less distributed in the D1R-poor zone and more distributed in the D2R-poor zone than in the dorsal striatum. Since the cell density was stable across these three area, the proportion of iMSNs should differ among them. Indeed, we confirmed the ratio of PPE+ neurons to NeuN+ neurons was higher in the D1R-poor zone than in the dorsal striatum. Our findings indicate that dMSNs and iMSNs are not homogeneously distributed in the specific sub-regions of the lateral caudal striatum. The D1R-poor zone comprises less dMSNs and more iMSNs. The distribution of D1R and D2R was biased, whereas the D1R- and D2R-poor zone receives less input of dopamine. This discrepancy suggests that these specific regions should play a different role from the dorsal striatum.

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Poster

146. Cellular Physiology in the Basal Ganglia: Novel Cell Types and Mechanisms

Location: SDCC Halls B-H

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Topic: E.03. Basal Ganglia

Support: SMRC 2014-3804, 2013-4657
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Title: Targeting VGLUT2 in mature dopamine neurons decreases mesoaccumnal glutamatergic transmission and identifies a role for glutamate co-release in synaptic plasticity by increasing baseline AMPA/NMDA ratio

Authors: *M. PAPATHANOU[1], M. CREED[2,3], M. C. DORST[4], Z. BIMPISIDIS[1], S. DUMAS[5], H. PETTERSSON[1], C. BELLONE[3], G. SILBERBERG[4], C. LUSCHER[3], Å. WALLEN-MACKENZIE[1]
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Abstract: The ventral midbrain is a heterogeneous structure of the basal ganglia consisting of the ventral tegmental area and the *substantia nigra* that project to the ventral and dorsal striatum, respectively. The nigrostriatal pathway is involved in movement, whilst the mesoaccumbal pathway is involved in motivated and goal-directed behavior. As such changes in neuronal transmission of these pathways are associated with psychiatric disorders and Parkinson's disease (PD). Expression of the *Vglut2* gene encoding the Vesicular glutamate transporter 2 (VGLUT2) in midbrain dopamine neurons has been proposed to contribute to behavioral reinforcement in adult mice upon their consumption of sugar or addictive drugs. The mechanism has been difficult to pin-point due to developmental onset of *Vglut2* gene expression, although the presence of VGLUT2 molecules in pre-synaptic vesicles has been proposed to increase the packaging efficiency of dopamine. In addition to supporting dopaminergic function, VGLUT2-mediated glutamate co-release by dopaminergic terminals in the nucleus accumbens (NAc) may actively participate in maintaining synaptic plasticity of excitatory transmission. Repeated exposure to cocaine is known to cause lasting adaptations of excitatory synaptic transmission onto medium spiny neurons (MSNs) in the NAc but a putative contribution from VGLUT2-mediated glutamate co-release has never been investigated.
In this study, we implemented a tamoxifen-inducible strategy to selectively probe VGLUT2 in mature dopamine neurons. We observed a mildly heightened consumption of sugar, while locomotor sensitization to cocaine remained unaffected. In contrast, glutamatergic transmission within the NAc was significantly reduced. Specifically, ablation of VGLUT2 in mature dopamine neurons enhanced baseline AMPA/NMDA ratio in dopamine receptor subtype 1 (DRD1)-expressing accumbal MSNs and occluded the effect of cocaine on synaptic transmission. We conclude that VGLUT2 in mature dopamine neurons actively contributes to glutamatergic neurotransmission in the NAc, a finding which for the first time highlights VGLUT2-mediated glutamate co-release in the complex mechanisms of synaptic plasticity in behavioral reinforcement, non-motor aspects of PD and drug addiction.

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Poster

146. Cellular Physiology in the Basal Ganglia: Novel Cell Types and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 146.16/JJ2

Topic: E.03. Basal Ganglia

Support: NIH-NINDS R01 NS095809

Title: Dopamine cells balance regional differences in striatal cholinergic transmission via dopamine and glutamate co-release

Authors: *Y. CAI, C. FORD
Pharmacol., Univ. of Colorado Denver, Aurora, CO

Abstract: Dopamine cells balance regional differences in striatal cholinergic transmission via dopamine and glutamate co-release
In the dorsal striatum, dopaminergic inputs from the substantia nigra compacta (SNc) drive pauses in cholinergic interneuron (ChI) firing by activating D2-receptors. Dopamine neurons also co-release other transmitters, which may vary across striatal regions. To examine whether dopamine inputs differentially regulate cholinergic activity across in the dorsomedial and dorsolateral striatum, we overexpressed channelrhodopsin in dopamine neurons in DAT-Cre mice to evoke the release of dopamine while recording from ChIs in striatal slices. Optogenetic stimulation of dopamine terminals evoked a pause (~1 second) of firing in dorsomedial ChIs, but drove a burst (~1.5 seconds) of action potentials in dorsolateral ChIs. Sulpiride (500nM) blocked the pause in dorsomedial ChIs, confirming the pause resulted from activation of D2 dopamine receptor. The burst firing of dorsolateral ChIs was eliminated by antagonists of group I metabotropic glutamate receptors (mGluRs), revealing the co-transmission of glutamate was responsible for the increase in ChI firing. To study how dopaminergic input regulates acetylcholine transmission via M4 acetylcholine receptors (M4-AChRs) in direct pathway medium spiny neurons (dMSNs), we virally overexpressed G protein-coupled inwardly rectifying potassium (GIRK) channels in dMSNs. Spontaneous ChI firing drove M4-mediated inhibitory postsynaptic potassium current (IPSC) in GIRK positive dMSNs. We found the frequency of spontaneous M4-IPSCs was higher in dorsomedial dMSNs than in dorsolateral dMSNs, indicating the level of acetylcholine transmission was higher in the dorsomedial striatum. Optogenetic stimulation of dopamine terminals caused a pause (~1 second) in spontaneous IPSCs in dorsomedial dMSNs, but potentiated (~2 seconds) IPSCs in dorsolateral dMSNs. These results suggest that dopaminergic inputs use co-release in the dorsal striatum to balance regional differences in cholinergic transmission.

Disclosures: Y. Cai: None. **C. Ford:** None.

Poster

146. Cellular Physiology in the Basal Ganglia: Novel Cell Types and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 146.17/JJ3

Topic: G.02. Motivation

Support: NIH Grant T32 DA07278

Title: Genetic dissection of the role potassium channels of midbrain dopamine neurons play in physiology and behavior

Authors: *B. JUAREZ[1], Y. S. JO[2], A. C. HUNKER[1], A. MENDEZ[1], L. S. ZWEIFEL[1]
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Abstract: Dopamine neurons encode a number of behaviors due to precise transitions between silence and two different activity states: single-spike tonic firing and multi-spike phasic firing. Emerging evidence suggests that imbalances in these well-controlled transitions can lead to disorders of information processing, including neurodevelopmental and neuropsychiatric disorders. Ion channels expressed on midbrain dopamine neurons contribute to the pattern of these physiological transitions. Furthermore, it is increasingly appreciated that rare, spontaneous mutations in ion channels can contribute to the etiology of these disorders. Here, in order to understand how ion channels contribute to pathological behaviors, we cross-referenced a database of single-nucleotide polymorphisms associated with neurodevelopmental disorders to RiboTag RNAseq data of midbrain dopamine neurons to identify which potassium channels may contribute to pathological dopaminergic firing. Two of these ion channels were KCND3 (Kv4.3, A-type potassium current) and KCNMA1 (KCa1.1, calcium-activated, big potassium current). Using cutting-edge, viral-mediated cre-inducible CRISPR-Cas9 technology *in vivo*, we selectively knocked out KCND3 or KCNMA1 in dopamine neurons of the ventral tegmental area in mice. We next characterized the altered intrinsic properties of these dopamine neurons to confirm functional knockout using patch-clamp electrophysiology. In order to determine if KCND3 and KCNMA1 contribute to different aspects of dopamine-mediated behaviors, we performed a behavioral profile of these knockout mice. Finally, we determined how knocking out these ion channels of interest differentially contributed to tonic and phasic firing of dopamine neurons *in vivo* using optrodes for multi-unit electrophysiology. This study aims to provide novel information of the physiological contribution ion channels have in mediating healthy and pathological information processing of the dopaminergic system.

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Poster

146. Cellular Physiology in the Basal Ganglia: Novel Cell Types and Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 146.18/JJ4

Topic: E.03. Basal Ganglia

Support: NIH grant R01NS097671
Safa Bouabid was a recipient of the University of Tennessee Neuroscience Institute FY2017 postdoctoral fellowship

Title: Chemogenetic manipulation of basal ganglia indirect pathway *in vitro* and *in vivo*

Authors: *S. BOUABID, Q. WANG, F.-M. ZHOU
Pharmacol., Univ. of Tennessee Col. of Med., Memphis, TN

Abstract: The striatal medium spiny neurons (MSNs) are critically involved in motor and non-motor brain functions. The MSNs forming the direct pathway (dMSNs) express the D_1 dopamine receptors, while those forming the indirect pathway (iMSNs) express the D_2 dopamine receptors (D_2Rs) and cAMP-increasing adenosine A2a receptors (A2aRs). Thus, monitoring the neural activity in identified MSNs is essential to further understanding the cellular mechanisms of these neurons in brain functions. Here, we report the activation of iMSNs both *in vitro* and *in vivo*, using transgenic chemogenetic mice in which the cAMP-producing G protein Gs-coupled designer receptor exclusively activated by designer drug (Gs-DREADD) is expressed in the D_2R expressing iMSNs. We first show that the whole cell patch clamp recordings in current clamp and voltage clamp in striatal brain slices of D2 Gs-DREADD indicate that the DREADD agonist clozapine-N-oxide (CNO) activation of cAMP-producing Gs-DREADDs inhibits the inwardly rectifying potassium current in iMSNs and thus increases the intrinsic excitability and evoked spike firing of these neurons. Second, *in vivo* recording in freely moving mice shows that intraperitoneal injection of CNO consistently increases the spike firing in 50% of the recorded MSNs in D2 Gs-DREADD positive mice, without affecting the MSNs firing in Gs-DREADD-negative mice confirming that CNO's effect on MSNs is selectively mediated by Gs-DREADD. To further confirm the reliability of the spike recording and cell identification, we also monitored the spike firing of globus pallidus external segment (GPe) neurons, the main target of the inhibitory output from iMSNs, and neurons of the subthalamic nucleus (STN), a key target of the inhibitory GPe output. We show that CNO substantially inhibits the spiking firing of GPe neurons and excites a subgroup of STN neurons, which is coherent with iMSNs excitation. Moreover, these changes in the spiking firing in the indirect pathway after CNO injection are correlated with the locomotor inhibition in D2 Gs-DREADD positive mice. Altogether, our results provide insights into the cellular functions of the cAMP-producing A2aRs in iMSNs and

suggest that cAMP production in iMSNs can increase iMSN spiking activity and cause motor inhibition.

Disclosures: S. Bouabid: None. **Q. Wang:** None. **F. Zhou:** None.

Poster

146. Cellular Physiology in the Basal Ganglia: Novel Cell Types and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 146.19/JJ5

Topic: E.03. Basal Ganglia

Title: Dorsal striatum medial spiny neurons encode motor skill learning

Authors: *L. ZHANG, B. LIANG, G. BARBERA, Y. LI, D.-T. LIN
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Abstract: Dorsal striatum is important for motor skill learning. Two types of medium spiny neurons (MSNs) exist in the dorsal striatum: the direct pathway MSNs expressing dopamine D1 receptor (D1-MSN) and the indirect pathway MSNs expressing dopamine D2 receptor (D2-MSN). However, the mechanisms by which the D1- and D2- MSNs encode motor skill learning remain largely unknown. To address this question, we employed miniScope to concurrently record calcium activities from hundreds of MSNs longitudinally while mice trained on an accelerating rotarod. We found that both D1- and D2-MSNs were highly dynamic throughout motor skill learning. New active neurons were recruited throughout training, and the number of new D1-MSNs was significantly higher than that of new D2-MSNs, especially at early learning stage. Moreover, we identified two types of neurons within both D1- and D2- MSNs: the speed-depressing neurons (SDN) and speed-potentiating neurons (SPN). We found that the numbers of both D1-SDNs and D2-SDNs increased rapidly on training day 1, and gradually reached a plateau over the subsequent days, similar to the behavior learning curve, while the number of D1- or D2- SPNs didn't change over learning. Together, our results suggest that D1-MSNs are more relevant to motor skill learning, and the emergence of SDN may encode motor skill learning. This work was supported by NIDA/NIH.

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Poster

146. Cellular Physiology in the Basal Ganglia: Novel Cell Types and Mechanisms

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Program #/Poster #: 146.20/JJ6

Topic: E.03. Basal Ganglia

Support: NIH R01 EB016407
NIH R01 MN085074

Title: Local axon collaterals shape spike timings of basal ganglia output neurons

Authors: *K. M. LAMBERT[1,2], F. R. FERNANDEZ[1], J. A. WHITE[1]
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Abstract: As the primary output nucleus of the rodent basal ganglia, the substantia nigra pars reticulata (SNr) is responsible for integrating excitatory and inhibitory inputs from the three major upstream pathways. Unlike many other brain integration centers, it does this in a network built nearly entirely from GABAergic projection neurons which have been shown to be interconnected only through sparse inhibitory axon collaterals. These local axon collaterals have been proposed to either provide an output gain modulation or to desynchronize SNr activity. Here, we attempt to resolve these disparate roles using a combination of pharmacology, optogenetics, and dynamic clamp in sagittal brain slices containing SNr projection neurons. Blockade of spontaneous, basal inhibitory activity using the $GABA_A$ antagonist gabazine resulted in minimal change to SNr spike rates but significantly impacted the timing of these events by reducing the width of the interspike interval (ISI) histogram. An increase in the levels of extracellular potassium, used to increase the spiking activity of the network, did not change the impact of gabazine blockade on spike rate or timings. This suggested that the steady state role of the inhibitory network was to shape the timings of spike outputs, rather than determine the spike rate. We used mice expressing channelrhodopsin-2 (ChR2) under the Thy-1 antigen promoter to transiently activate the SNr neurons. Recording the inhibitory currents during optogenetic activation revealed a circuit which quickly reaches a maximally-activated state that causes a stereotyped pause in postsynaptic firing in response to increasing pulse durations. Repetitive stimulation also indicated the presence of synaptic depression, as the fast-firing neurons were able to track stimulation frequencies >60 Hz for at least 30 seconds, yet postsynaptic currents quickly failed to track or greatly decayed in amplitude. Finally, dynamic clamp experiments revealed that the sparse nature of these inhibitory inputs is the likely reason for their inability to cause large changes in firing rate. Overall, our results suggest an inhibitory network responsible for shaping output timings, but not net output rates.

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Poster

146. Cellular Physiology in the Basal Ganglia: Novel Cell Types and Mechanisms

Location: SDCC Halls B-H

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Topic: E.03. Basal Ganglia

Support: Tourette Association of America
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Howard Hughes Medical Institute

Title: Organization of parallel basal ganglia output pathways

Authors: *L. E. MCELVAIN[1,2], Y. CHEN[3], B. LIM[2], R. M. COSTA[4], D. KLEINFELD[1]
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Abstract: The basal ganglia (BG) are an interconnected set of subcortical nuclei that regulate diverse aspects of behavior. While much experimental and conceptual emphasis has been placed on the role of ascending BG projections to thalamus and BG-thalamo-cortico loops, the majority of structures targeted by BG outputs are in the brainstem. Here, we focus on the largest BG output nucleus, the substantia nigra pars reticulata (SNr) and delineate the organization of its projections in mice. Combining viral strategies to label SNr axons and boutons with high-resolution (0.23um/pixel) whole-brain scanning, we demonstrate SNr projections are more extensive than previously thought; SNr targets include 12 diencephalic, 27 brainstem, and 3 BG nuclei. Rather than projecting in a one-to-one or one-to-all organization, SNr projections arise from 7 distinct subpopulations that project via specific axon collaterals. Each subpopulation targets functionally specific domains of the brainstem and emits ascending collateral projections to the diencephalon, such that 6 of 7 brainstem-projecting subpopulations collateralize to topographically distinct subdomains of motor and intralaminar thalamic nuclei. Analyses of SNr bouton density in downstream targets demonstrate that they cover an approximately exponentially distributed continuum of densities, rather than a trinary scale of “strong”, “weak”, and “absent”. This axonal architecture positions SNr to operate as a hub that can channel BG signals to specific, functionally related brainstem premotor and thalamic regions. We further combine mapping of BG outputs with our ongoing efforts to generate a 3D atlas of the brainstem—including fine-scale mapping of identified premotor neurons in reticular formation subdivisions—to link SNr activity to behavioral responses via identified intermediate neurons.

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Poster

146. Cellular Physiology in the Basal Ganglia: Novel Cell Types and Mechanisms

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Program #/Poster #: 146.22/JJ8

Topic: E.03. Basal Ganglia

Title: Neurons in layer 1 and 6b of mouse cerebral cortex

Authors: *E. LAI, E. S. ALBERT, M. GARCIA-MUNOZ, G. ARBUTHNOTT
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Abstract: A major output from the basal ganglia arrives in layer 1 of frontal cortex from the ventromedial thalamus in rodents. This thalamocortical input is excitatory, since thalamic cells make glutamate and the fibers are positive for the vesicular glutamate transporter 2 (vGluT2). Recent electrophysiology has identified responses of pyramidal neurons in layer 2/3 to activation of thalamic fibers, as inhibitory, carried by GABA inhibitory interneurons in layer 1. While exploring the sources of input to layer 1 of cortex, we discovered some retrogradely identified neurons with fluorogold just above the corpus callosum, in an area either called layer 6b. Now we are studying layers 1 and 6b with several questions in mind: Do all GAD67 neurons in layer 1 respond to inputs from motor thalamus? Are the cells that we have seen in layer 6b part of the same circuit? Does either layer have privileged access to layer 5 cortical output (pyramidal tract, corticofugal or corticostriatal) or layer 6 corticothalamic output? Is there a functional connection between the top and bottom of the cortex that would help us better understand cortical function? In order to start answering these questions we performed *in vitro* whole-cell patch-clamp recordings of motor cortex in C57BL/6 mice.
Layer 6b neurons showed (mean ± SD) -68 ± 5.55 mV resting membrane potential, and an input resistance of 206.4 ± 13.9 MΩ at a holding potential -60 mV. Similarly, layer 1 GFP interneurons of GAD67-GFP mice, had resting membrane potentials of -60 ± 8.1mV and input resistances at rest of 236.1 ± 55.9 MΩ.
Most cortical electrophysiology and anatomy has concentrated, for good reasons, on the middle layers where most neurons are located and from where the major cortical outputs are derived. We hope that a study of the less populated areas of cortex will illuminate the detailed interactions of the motor thalamus and cortical mechanisms involved in motor control.

Disclosures: E. Lai: None. **E.S. Albert:** None. **M. Garcia-Munoz:** None. **G. Arbuthnott:** None.

Poster

146. Cellular Physiology in the Basal Ganglia: Novel Cell Types and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 146.23/KK1

Topic: E.03. Basal Ganglia

Support: ERC starting grant to G. S.
Wallenberg Academy Fellowship to G. S.

Title: Synaptic properties of cortical and thalamic projections onto different types of striatal neurons

Authors: *Y. M. JOHANSSON, G. SILBERBERG
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Abstract: The generation of movements depends on the integration of a vast amount of information in the striatum. The striatal microcircuit receives inputs from the primary motor cortex (M1) and the primary somatosensory cortex (S1) as well as from thalamus. These afferents target medium spiny neurons (MSN) and different types of interneurons. While the synaptic properties of inputs to MSN are well established, only little is known about the properties of afferents targeting different striatal interneurons. Here we mapped the synaptic properties of cortical and thalamic projections targeting distinct types of striatal neurons. Five different cell types were distinguished by crossing a tdTomato-reporter line with either D1-, D2-, PV-, ChAT- or SOM-cre mice. Unilateral virus injections (AAV2-CamKIIa-YFP-ChR2) in S1, M1 or thalamus allowed the selective expression of channelrhodopsin in different striatal input structures. We obtained simultaneous whole-cell recordings of MSNs and neighboring interneurons in acute striatal slices while stimulating the afferent axons with optogenetics. In the presence of gabazine, monosynaptic excitatory postsynaptic potentials (EPSP) were recorded following brief stimulation of the presynaptic terminals. We found that fast-spiking (FS) interneurons receive stronger inputs from S1 and M1 than simultaneously recorded D1- and D2-MSNs. Low-threshold spiking (LTS) and especially cholinergic (ChIN) interneurons responded only partially and weakly to cortical input. In contrast, thalamic stimulation reliably evoked responses in ChINs, which were characterized by a large NMDA component. Afferents from M1 provided input to both ipsi- and contralateral striatum, while afferents originating in S1 and thalamus evoked only ipsilaterally responses. Notably, LTS interneurons received the largest EPSPs for contralateral M1 input and did not respond to thalamic stimulation. Taken together, our findings indicate that excitatory inputs elicit highly specific responses in the striatum, which are determined by the identity of the postsynaptic neuron type as well as the afferent cortical or thalamic region.

Disclosures: Y.M. Johansson: None. **G. Silberberg:** None.

Poster

146. Cellular Physiology in the Basal Ganglia: Novel Cell Types and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 146.24/KK2

Topic: G.02. Motivation

Title: Characterization of transgenic mouse lines for targeting major neuromodulatory systems reveals organizational principles of the dorsal raphe nucleus

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Abstract: Transgenic Cre driver mouse lines enabling transgene expression under control of cell-type specific promoters have proven to be critical tools for dissecting the enormous diversity of cell types in the mammalian brain. Following the report of "off-target" effects in the TH-Cre line used to target dopamine (DA) neurons (Lammel et al., 2015; Neuron), we carried out a systematic evaluation of the cell-type specificity of five transgenic lines used to target major neuromodulatory systems: (1) ChAT-Cre mice for targeting acetylcholine neurons, (2) Pitx3-Cre and (3) DAT-Cre mice for targeting DA neurons, and (4) ePET-Cre and (5) SERT-Cre mice for targeting serotonin (5HT) neurons. We find dramatic differences in cell-type specificity between the DAT-Cre and PITX3-Cre lines, and a two-fold difference in penetrance between the SERT-Cre and ePET-Cre mouse lines. Next, we iteratively leveraged these Cre lines to show that genetically defined 5HT, DA, GABA, and glutamate neuron populations in the DR are arranged in a previously unappreciated topographical pattern that includes two anatomically distinct glutamatergic subpopulations: one with minimal, and one with significant co-expression of 5HT cell markers. Analyzing axon terminals from these DR subpopulations revealed both convergent projections (e.g. to the lateral hypothalamus, ventral tegmental area) and divergent projections that distribute predominantly DA input to the lateral division of the central amygdala (CeA), GABA input to the medial division of the CeA, and glutamatergic input to the capsular part of the CeA. Lastly, we used monosynaptic rabies tracing to map out the presynaptic partners of DR 5HT and DA neurons and find that while these populations receive input from qualitatively similar brain regions, DR DA neurons receive quantitatively more input from the nucleus accumbens (NAc), medial habenula, and septum compared to DR 5HT neurons. Importantly, DR DA neurons targeted using DAT-Cre mice received a greater proportion of their input from rostral brain regions such as the NAc compared to DR DA neurons targeted using the Pitx3-Cre mice, which receive a greater proportion of input from caudal brain areas like the laterodorsal tegmentum and parabrachial nucleus. Collectively, our study presents a comparative analysis of

Cre expression patterns in five transgenic mouse lines across the major neuromodulatory systems of the brain to contextualize literature predicated on these mouse lines, inform the design of future optogenetic experiments, and demonstrate how a systematic understanding of these tools can be applied to elucidate novel organizational principles of heterogeneous brain nuclei.

Disclosures: D.F. Cardozo Pinto: None. **V.J. Han:** None. **I. Pollak Dorocic:** None. **K.T. Beier:** None. **S. Lammel:** None.

Poster

146. Cellular Physiology in the Basal Ganglia: Novel Cell Types and Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 146.25/KK3

Topic: G.02. Motivation

Title: Architecture of habenula circuitry underlies a distinct stress-induced depression phenotype

Authors: *I. CERNIAUSKAS[1], J. WINTERER[2], J. W. DE JONG[1], D. LUKACSOVICH[2], H. YANG[1], F. KHAN[1], J. R. PECK[1], V. LILASCHAROEN[3], B. LIM[3], C. FÖLDY[2], S. LAMMEL[1]
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Abstract: Chronic stress can lead to long-term changes in the brain and to major depressive disorder. Our understanding of depression is limited due to the variability of symptoms and poorly-defined animal models of this disease. To overcome these challenges, we developed a novel approach that helps to investigate whether stress-induced changes in neural circuits correlate with specific depression-like behaviors. We exposed mice to chronic mild stress (CMS) and subsequently screened for depression-related behaviors using three different behavioral paradigms (tail suspension test, sucrose preference test and elevated plus maze, which screen for behavioral despair, anhedonia and anxiety, respectively). We found that mice display a high degree of individual variability in their responses to CMS. Therefore, we employed unbiased statistical procedures to separate mice into different subgroups based on individual behavioral phenotypes. Next, we sought to uncover whether CMS-induced neural adaptations correlate with specific single- or multi-dimensional depression phenotypes. To do this, we focused on the lateral habenula (LHb), which provides ‘negative value’ to ventral tegmental area (VTA) dopamine neurons and has emerged as a key brain region in the pathophysiology of depression. By combing retrograde tracing with patch-clamp recordings and optogenetics with in vivo electrophysiology, we demonstrate that hyperactivity of VTA-projecting but not dorsal raphe (DR)-projecting LHb neurons represents a common neural substrate for multi-dimensional depression-like phenotypes. Rabies virus tracing shows that VTA- and DR-projecting LHb neurons receive similar monosynaptic inputs from diverse brain regions. However, synaptic physiology reveals a specific excitatory synaptic connection between the entopeduncular nucleus

(EP) and VTA-projecting LHb neurons that undergoes CMS-induced synaptic adaptations. Lastly, by performing bidirectional chemogenetic manipulations of EP inputs to the LHb we demonstrate a selective role of this pathway in behavioral despair. We are currently investigating if exposure to CMS also leads to projection-specific changes in gene expression using single-cell RNA-seq. Collectively, these results suggest that individual symptoms of depression may be caused by pathology localized to specific neural circuits as we demonstrate for the EP-LHb-VTA pathway. Identifying and characterizing these pathways will be critical for defining novel drug targets and understanding depression not as a single entity but as a diverse set of symptoms, each of which can be treated independently using targeted pharmaceuticals.

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Poster

146. Cellular Physiology in the Basal Ganglia: Novel Cell Types and Mechanisms

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Program #/Poster #: 146.26/KK4

Topic: G.02. Motivation

Title: A neural circuit mechanism for coding negative motivational stimuli in the mesolimbic dopamine system

Authors: *J. W. DE JONG[1], S. A. AFJEI[1], I. POLLAK DOROCIC[1], J. PECK[1], C. K. KIM[2], K. DEISSEROTH[3], S. LAMMEL[1]
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Abstract: Ventral tegmental area (VTA) dopamine (DA) neurons play a central role in reward processing and reinforcement learning and have been implicated in addiction, depression and other conditions of dysfunctional motivated behavior. Although some VTA DA neurons have been implicated in encoding both appetitive and negative motivational stimuli, the neural circuitry through which these cells signal such stimuli remains incompletely understood. Using *in vivo* fiber photometry, we simultaneously record DA terminal activity in distinct subnuclei of the nucleus accumbens (NAc) during an aversive and reward conditioning task. We find that DA terminals in the ventral NAc medial shell (vNAcMed), which originate from VTA DA neurons, respond strongly to unexpected aversive outcomes and cues that predict them, whereas DA terminals in the NAc lateral shell (NAcLat) and dorsal NAc medial shell are persistently depressed. Surprisingly, although vNAcMed DA terminals respond to both appetitive and aversive motivational stimuli, phasic responses to reward-predictive cues dominate in the NAcLat and are largely absent in the vNAcMed. Moreover, by combining viral, optical and

physiological tools, we characterize the functional contribution of two major inputs to vNAcMed and NAcLat-projecting DA neurons. We demonstrate that a genetically defined subset of glutamatergic (i.e., VGLUT2-expressing) neurons in the lateral hypothalamus represents a key afferent integrator of aversive activation in vNAcMed-projecting DA neurons. Conversely, glutamatergic neurons in the dorsal raphe (i.e., VGLUT3-expressing) predominantly target NAcLat-projecting DA neurons and activation of these inputs to the VTA promotes reward. Collectively, our results provide a novel framework that delineates the distinct contribution of functionally and anatomically separate mesolimbic DA subsystems and their afferent pathways in the regulation of positive and negative motivational states.

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Poster

147. Basal Ganglia Systems in Motivated Behaviors

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Topic: E.03. Basal Ganglia

Support: NIAAA Division of Intramural Clinical and Biological Research

Title: Real-time *in vivo* monitoring of striatal GPCR signaling using FRET-based biosensors

Authors: *S. M. AUGUSTIN, J. O. LEE, Y. KIM, H. L. PUHL, III, S. S. VOGEL, D. M. LOVINGER
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Abstract: Dynamic modulation by G-protein-coupled receptor (GPCR)-mediated intracellular signaling cascades underlies synaptic plasticity and neuronal activity to ultimately affect basal ganglia function. The dorsal striatum is a critical basal ganglia region that integrates incoming signals from cortex, thalamus, and midbrain to control action learning and performance. These afferents form synapses onto indirect and direct projecting medium spiny neurons (MSNs). Intracellular signaling cascades within both subtypes of MSNs are important for synaptic modulation and plasticity. The cAMP-PKA signaling pathway has been shown to be a key synaptic modulator not only in striatum, but throughout the brain. However, little is known about real-time intracellular cAMP-PKA signaling following GPCR activation *in vivo*. Combining genetically-encoded Forster Resonance Energy Transfer (FRET)-based sensors and optical measurement techniques we can assess intracellular cAMP accumulation and PKA phosphorylation using EPAC and AKAR biosensors, respectively. In vitro, the EPAC biosensor can measure activity-induced changes in cAMP accumulation in cells measured by 2-photon fluorescence lifetime imaging microscopy (2p-FLIM). Both biosensors, EPAC and AKAR, can

be virally expressed in striatal MSNs. Using fiber photometry to measure fluorescence lifetime as a quantification of FRET activity, we assessed real-time intracellular signaling in distinct striatal MSNs in freely moving animals. PKA activity is altered by a general anesthesia, as measured by changes in the AKAR biosensor donor lifetime. Various pharmacological manipulations targeting GPCRs in striatal neurons show receptor and circuit level changes in freely moving animals. Further analyses in on-going work will validate the biosensors and assess cell-type-specific signaling changes *in vivo*.

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Poster

147. Basal Ganglia Systems in Motivated Behaviors

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Program #/Poster #: 147.02/KK6

Topic: E.03. Basal Ganglia

Title: Modulation of the activity of striatal fast-spiking interneurons by action and outcome in a choice context

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Abstract: The fast-spiking interneurons (FSIs) of the striatum, thought to correspond to the parvalbumin-containing GABAergic interneurons, have generated widespread interest due to their powerful influence on striatal circuit function and their specific contributions to hyperkinetic movement disorders, including dystonia, tics, and stereotypies. Although an influential view held that FSIs may be involved in action selection, there is little evidence from neuronal recording studies in awake animals to support this claim. To learn more about the role of these local circuit elements, we investigated their functional properties in macaque monkeys performing reaching movements under two task conditions: one in which they chose among targets of movement based on the expected reward outcome, and another in which they were simply assigned one option for movement. Monkeys usually took longer to initiate movements when there is a choice between options. In this condition, we found that 24 of 58 FSIs (41%) recorded in different regions of the striatum changed their firing rate around the time of movement initiation, with approximately equal proportions of activations and inhibitions. We identified two groups of activated FSIs that reached their peak just before (*early*) or after (*late*) movement onset. When comparing their activity between the choice and no-choice conditions, only the *early* group showed a slight tendency to display the highest level of activity in the presence of a choice requirement, suggesting that this subset of FSIs might provide signals to facilitate one chosen action over the others. Almost all of the neurons (88%) modulated by

movement were also modulated around reward receipt with increases or decreases in activity, indicating that action and outcome signals are both present in individual FSIs. These neuronal modulations occurring during distinct epochs of task performance suggest that changes in the activity of FSIs may be required to facilitate a transition from movement initiation and execution to outcome evaluation which may be crucial for the dynamic processing of information in the striatum. This influence could be altered in certain pathological conditions involving deficient striatal FSI function, leading to uncontrolled behaviors.

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Poster

147. Basal Ganglia Systems in Motivated Behaviors

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Topic: E.03. Basal Ganglia

Support: NINDS Grant K08-NS072183

Title: Experience-dependent contributions of striatal dopamine to dexterous limb movements

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Abstract: Striatal dopamine (DA) is suggested to influence motor function via dual roles regulating moment-by-moment performance and reinforcement learning. However, most evidence for these aspects of DA function comes from tasks that require no movement (e.g., classically conditioned tasks), simple movements (e.g., lever presses), or innate movements (e.g., locomotion). To determine if and how DA mediates these functions during cortically-dependent, dexterous skills, we employed a rat skilled reaching task with real-time high-speed video analysis to trigger optogenetic DA neuron stimulation during reach-to-grasp movements. Stimulation during, but not between, reaches gradually impaired performance. Impaired reaches exhibited movement patterns similar to successful reaches, but were characterized by early transitions between reach sub-movements. That is, rats attempted to grasp before their paw was fully extended over the pellet. Furthermore, once poor reaching patterns were established, rats rapidly transitioned between truncated reaches with optogenetic stimulation and normal reaches without stimulation, typically taking one reach to switch between movement patterns. These results suggest that nigrostriatal DA regulates skilled motor performance in an experience-dependent manner, and influences both motor learning and performance. Ongoing studies are directed towards determining how the precise timing of DA manipulations dissociates the role of DA as a “learning” or “performance” signal.

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Poster

147. Basal Ganglia Systems in Motivated Behaviors

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Support: CNPq
CAPES

Title: Putative place-to-go cells in the rat nucleus accumbens

Authors: *C. DA CUNHA[1], D. LEVCIK[2], A. SUGI[1], L. PULIDO[1], V. CYRUS[1], M. AGUILAR-RIVERA[3], R. A. FUENTES[4], C. D. BLAHA[5], K. H. LEE[6]
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Abstract: It is broadly accepted that representation of space is encoded within hippocampal formation and action selection is driven by the basal ganglia. However, no study so far has shown a particular mechanism for triggering the action of approaching a specific place in the environment. We propose that the action of returning to a place where the animal was previously rewarded is triggered by neurons in the nucleus accumbens. The nucleus accumbens is part of basal ganglia, receives direct inputs from the hippocampal formation, and is involved in reward-driven actions. To test our hypothesis, we recorded activity of individual neurons in the nucleus accumbens while rats previously trained to collect chocolate drops consistently located in the end of three particular arms of an eight-arm radial maze were performing this task. Our results showed that some of the recorded neurons had higher activity before entering a specific rewarded arm, but not before entering the other rewarded arms. We propose that this activity might reflect the decision to go to a particular place. Neurons that exhibit such activity might be putative place-to-go cells, i.e. neurons responsible for initiating the action of approaching a particular place.

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Poster

147. Basal Ganglia Systems in Motivated Behaviors

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Topic: E.03. Basal Ganglia

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Title: Striatal dopamine D2 receptors regulate cost sensitivity and behavioral thrift

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Abstract: The role of the dopamine D2 receptor (D2R) in regulating feeding behavior and obesity remains controversial. Earlier literature suggests that reduced D2R signaling decreases appetitive motivation, yet more recent theories suggest reduced D2R drives compulsive behavior leading to overconsumption in obesity. How D2R signaling modulates behavioral choice remains poorly understood. Using a semi-naturalistic homecage foraging paradigm where the only source of food was through lever pressing on a progressive ratio schedule, we revisit classic pharmacological studies that led to the 'extinction mimicry' hypothesis. We found administration of haloperidol, a dopamine D2 receptor antagonist, shifts foraging strategy toward energy conserving strategy in pursuit of food (behavioral thrift) without altering consumption or bodyweight, inconsistent with the idea of extinction mimicry. To isolate the contributions of striatal postsynaptic D2R signaling, we selectively deleted D2R from indirect pathway medium spiny neurons (iMSNs) by crossing mice expressing cre-recombinase regulated by the adenosine 2A receptor promoter (Adora-cre) with mice homozygote for a conditional Drd2 null alleles. We assess food demand and behavioral energy expenditure in a homecage paradigm and examine the response of this line to conditions of escalating costs. The D2R selective knockout reduces the animals' willingness to expend energy in pursuit of food, even under conditions of increasing, chronic hunger. Unlike the mice administered haloperidol, the D2R selective knockouts do not exhibit a compensatory shift in foraging to more frequent but smaller, more inexpensive meals. Instead, they work and eat less. Our finding supports a model in which D2R, particularly on iMSNs gates behavioral energy expenditure by regulating cost-sensitivity.
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Poster

147. Basal Ganglia Systems in Motivated Behaviors

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Topic: E.03. Basal Ganglia

Title: Amphetamine inhibits locomotor activity via dopamine D4 receptor stimulation in rats with enhanced D1 receptor activation

Authors: D. ERLIJ[1], B. CAMPOS[2], M. RODRÍGUEZ[3], F. PAZ-BERMÚDEZ[4], *G. B. FLORAN[5]
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Abstract: Although amphetamine and other inhibitors of dopamine reuptake typically increase motor activity in normal subjects, their administration is the therapy of choice for the treatment of hyperactivity disorders. We previously showed that activation of D4Rs in SNr and nRT inhibits motor activity. In the current study we examined whether amphetamine can inhibit locomotor activity via activation of D4Rs. In our experiments administration of amphetamine to rats (1 mgr/kg i.p.) consistently enhanced locomotor activity. We hypothesized that this stimulatory effect is mediated mainly by activation of D1Rs. This stimulation then may mask the inhibitory effect of simultaneous D4R activation. We administered high intraperitoneal doses (10 mgrs/kg i.p.) of SKF 38393 to maximally activate D1Rs. These injections produced a marked increased in locomotor activity that was inhibited by i.p. injections of amphetamine. These inhibitory effects of amphetamine were prevented by the selective D4R antagonist L 745,870 (0.1 mgr/kg i.p.) but not by the selective D3R antagonist U-99149A (20 mgrs/kg i.p). These results show that when D1Rs are maximally activated, amphetamine inhibits locomotor activity via activation of D4Rs. This response suggest that the therapeutic inhibitory effects of amphetamine are produced because in the hyperactive patients the D1R system is nearly fully activated. They also suggest that selective D4R agonists may be an effective tool to treat hyperactive disorders.

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Poster

147. Basal Ganglia Systems in Motivated Behaviors

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Topic: E.03. Basal Ganglia

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Title: Rearrangement of oscillatory activity within the cortico-thalamic-basal ganglia circuit evoked by epidural spinal cord stimulation

Authors: *C. I. ASTUDILLO[1], P. PETERSSON[2], R. A. FUENTES[3]
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Abstract: Introduction: Epidural spinal cord stimulation (SCS) is a neuromodulatory strategy showing promising results for the treatment of the motor symptoms of Parkinson's disease (PD), especially for patients with predominance of postural and gait disturbances. SCS produces benefits over the motor symptoms of PD and also modifies the oscillatory component of the electrical activity within the cortico - thalamic - basal ganglia (Cx-Th-BG) circuit. Inded, the increased oscillatory activity around 8-30 Hz (beta band), normally found in motor cortices and striata of patients and PD animal models, is disrupted throughSCS, although the mechanisms underlying this effect are not known. To understand how SCS alters the oscillatory activity within the Cx-Th-BG circuit, using an animal model of PD, we investigated the effect of the most elemental unit of SCS, the unitary pulse. **Methods:** 4 Sprague Dawley rats were used in this study and parkinsonism was induced injecting 6-OHDA bilaterally in the striatum. Custom made tungsten electrodes were chronically implanted to record the local field potential (LFP) in motor cortices, striatum and thalamus. To deliver SCS, custom made platinum electrodes were chronically implanted at the T5 vertebral level. Recording sessions were carried on while the animals were awake and freely moving. **Results:** By averaging the LFP signals around 600-1000 pulses, the evoked response to the pulse was constructed. In a broadband spectral analysis, we found that evoked oscillatory beta activity is triggered after the pulse, but without any significant change of the spectral power in the LFP signal. These results suggest that single SCS pulses cause a re-arrangement of the beta oscillations in the motor circuit. Noteworthy, in the evoked activity two different frequency peaks within the commonly denominate beta band are observed; one peak around 7-12 Hz and a second peak around 23-28 Hz. **Discussion:** The rearrangement of the oscillatory activity observed after a single pulse could be a phenomena constantly occurring during the continuous delivery of SCS and it could explain the disruption of the beta band in

therapeutic paradigms of stimulation. We are currently expanding our electrophysiological analysis to explore if the two observed peaks of oscillatory activity within the beta band respond differently to the SCS, and also if the rearrangement of the oscillatory activity is a widespread phenomenon ocurring in other areas of the Cx-Th-BG circuit.

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Poster

147. Basal Ganglia Systems in Motivated Behaviors

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Title: A role for striatal tyrosine hydroxylase interneurons in behavior

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Abstract: The striatum is one of the main input nuclei of the basal ganglia and has been implicated in goal-directed and habitual behavior. Within the striatum is a rich diversity of interneurons with distinct molecular and physiological subtypes. The activity of these neurons *in-vivo* and their involvement in learning and behavior are poorly understood. To explore this relationship, we focus on the tyrosine hydroxylase-containing interneuron (THIN). We used a TH-cre mouse line to target THINs in the striatum. To visualize the activity of THINs in awake, behaving mice, we injected the calcium indicator gCAMP6f with a cre-dependent viral vector into the striatum, then imaged the cells in a variety of behavioral contexts. We found that, while performing a simple operant task, there is a complete inhibition of the calcium signal. This inhibition is observed both during a successful trial and unsuccessful attempts, indicating that this response is related to the action, not reward collection. We also only observed this inhibition in a proportion of recorded cells (about 70%). This is consistent with in-vitro data indicating four distinct electrophysiological types of THINs. Preliminary analysis indicates some indirect relationship between the calcium signal and certain movements. To further understand the relationship between physiology and behavior, we selectively lesioned THINs using a cre-dependent diphtheria toxin A (dTA) virus in the same mouse line. Animals were trained in an operant conditioning chamber, where pressing one lever gave a chocolate reward, and the other lever gave a grain reward. Both control and lesioned animals were able to learn to press the

levers at equal rates. In a devaluation test, control animals pressed the valued lever significantly more than the devalued lever, but lesioned animals pressed on both levers equally, indicating a lack of understanding of the action-outcome contingencies. Conversely, reinstated lever pressing is stronger when presented with flavor congruent to the presented lever than an incongruent flavor in both groups, suggesting otherwise. Our results indicate an important role for THINs in the coordination of goal directed behavior. Specifically, THIN activity outside of operant behavior is likely important for normal striatal function.

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Poster

147. Basal Ganglia Systems in Motivated Behaviors

Location: SDCC Halls B-H

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Topic: G.02. Motivation

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Title: Transcriptional profiles of adult neurons involved in vision and reward

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Abstract: A thorough understanding of cell types is a foundation for studying neural circuits and behaviors. Single-cell RNAseq (scRNAseq) provides transcriptional profiles for thousands of cells in parallel. This massively parallel technique has been applied to mouse brains to reveal neuronal diversity. However, few attempts have been made to characterize nonhuman primate (NHP) neurons. NHP studies are a cornerstone of systems neuroscience and a stepping stone to translational application of gene therapies. Massively parallel scRNAseq is an ideal method to characterize NHP brains and identify rare transcripts that permit cell type-specific access to this nervous system. Therefore, we set out to profile brain regions involved in reward and vision, including midbrain, striatum, frontal cortex, and retina.
We developed protocols to isolate mature neurons from retina, cortex, striatum, and midbrain using 8-10 m.o. rats. These brains pose similar challenges as NHPs – namely abundant myelin and mature neurons. We compared different protocols using qPCR to identify important transcripts. Suspensions with no doublets that contained TH, DRD1, or DRD2 were reliably obtained. About 10k cells each from rat cortex, striatum, and midbrain were profiled and sequenced to more than 500 million reads. We found robust neuron and glial markers in all brain regions. In the midbrain, we found dopamine neuron markers including TH, SLc18a2, and Ddc,

but these were only found in a small minority of cells suggesting that dopamine neurons were highly sensitive to sample preparation. Likewise, the cortical sample revealed VGLUT1 and VGLUT2 but again in a minority of neurons. Striatal preparations were rich with GAD1 positive inhibitory neurons. Glial markers were robust in all samples. These results validate the use of scRNAseq in adult tissue but highlight the difficulty in robust analysis of neuron populations in mature brains. Moving forward, we will explore alternative methods for isolating neurons and characterize the transcriptional profile of brain regions for vision and reward.

Disclosures: J. He: None. **L.C. Byrne:** None. **W.R. Stauffer:** None.

Poster

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Topic: E.03. Basal Ganglia

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Title: Motor thalamic preparatory activity and its modulation by basal ganglia input using optogenetics in a mouse licking task

Authors: *J. CATANESE, D. JAEGER
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Abstract: Movement preparation requires information flow through specific brain circuits involving sensory and motor areas according to the behavioral context. Within these brain circuits, the thalamus plays a central role as it processes and relays both sensory and motor signals. Even basic knowledge about ongoing thalamic processing during motor preparation and movement is still lacking. In addition, thalamic contributions to important neurological disorders like Parkinson's disease remain unknown.
In order to start addressing this gap in our understanding, we monitored the neuronal activity in the motor thalamic nuclei receiving direct input from the basal ganglia. Head-fixed mice were trained to lick left or right according to air-puffs to the left or right whiskers. The mice were required to withhold their lick during a short delay period while movement preparation takes place and preparatory activity is maintained in short term memory for movement initiation. During behavior we recorded from motor thalamus using silicon electrode arrays (Neuronexus, Inc) and stimulated Substantia Nigra pars reticulata (SNr) terminals that express ChR2 or ArchT in vGAT-cre mice. Recent results from our lab have shown that SNr terminal stimulation will influence the lick choice behavior of mice. Our new study allows us to correlate neural activity changes during behavior with responsivity to nigral inputs. Our results indicate that at the end of

the delay period just before the lick, the firing rate of thalamic neurons either ramp up, decrease or remains steady. Interestingly, we also observe a variety of responses after optogenetic stimulation of the SNr terminals in thalamus, including activation or inhibition with distinct latencies.
These results suggest different pathways -monosynaptic or multisynaptic - from the SNr to the motor thalamus. We will next test the hypothesis that neurons with a certain type of optogenetically evoked responses are associated with a certain type of responses in the task. This data will help us deconstruct the how basal ganglia input impacts the neural components of motor planning and execution that occur in the motor thalamus.

Disclosures: J. Catanese: None. **D. Jaeger:** None.

Poster

147. Basal Ganglia Systems in Motivated Behaviors

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Title: Projection pathways in ventrolateral striatum modulate operant control of licking

Authors: *S. MUTLU, E. LOTTEM, A. MACHADO, M. CAREY, Z. F. MAINEN, R. M. COSTA
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Abstract: Ventrolateral striatum (VLS) neurons receive input mainly from orofacial sensory-motor regions, and respond to orofacial movements, suggesting that VLS could be involved in the control of orofacial movements. However, the role of this region in goal-directed licking behavior, and particularly the interplay between striatal direct- and indirect- pathways during licking remain largely unknown. In a first set of experiments we found that optogenetic stimulation of direct pathway neurons induced licking while stimulation of indirect pathway did not affect ongoing movements of freely moving mice.To further investigate the role of VLS direct and indirect pathways in operant licking under different motivational conditions, we developed a head-fixed olfactory-guided operant task in which mice were trained to modulate licking differently in response to four different odors (go/nogo/wait/neutral).Calcium imaging during task performance showed that both direct and indirect pathways contain subpopulations of

neurons that respond to different task parameters, such as go-cue, licking initiation, termination and reward. However, optogenetic inhibition of these neurons did not affect correct licking responses during task performance. In contrast, we found that during odor-outcome associative learning, inhibition of direct pathway neurons delayed learning, while silencing indirect pathway facilitated learning.Our results suggest that direct and indirect pathway activation is sufficient for antagonistic modulation of licking, while the activity of these pathways is only necessary in early stages of learning and might be disengaging from execution in late stages of learning, after action-outcome associations are well established.

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Poster

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Topic: E.03. Basal Ganglia

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Title: The striatum organizes 3D behavior via moment-to-moment action selection

Authors: *J. E. MARKOWITZ[1], W. F. GILLIS[1], C. C. BERON[1], S. Q. NEUFELD[1], K. ROBERTSON[1], N. D. BHAGAT[1], R. E. PETERSON[1], E. PETERSON[1], M. HYUN[1], S. W. LINDERMAN[2], B. L. SABATINI[1], S. R. DATTA[1]
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Abstract: Many naturalistic behaviors are built from stereotyped, modular components that are flexibly arranged to form sequences. Although striatal circuits have been implicated in action selection and implementation, the neural mechanisms that compose behavior in unrestrained animals are not well understood. Here we simultaneously record neural activity in the direct and indirect pathways of dorsolateral striatum (DLS) and monitor 3D pose dynamics as mice spontaneously express action sequences. These experiments demonstrate that DLS neurons systematically encode information about the identity and sequential ordering of stereotyped sub-second 3D behavioral motifs; this encoding is facilitated by fast-timescale decorrelations between the direct and indirect pathways. Furthermore, perturbing the DLS prevents appropriate

sequence assembly during both exploratory or odor-evoked behaviors. By characterizing naturalistic behavior at neural timescales, these experiments identify a code for 3D pose dynamics built from complementary pathway dynamics, support a role for DLS in constructing meaningful behavioral sequences, and suggest models for how actions are sculpted over time.

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Poster

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Topic: E.03. Basal Ganglia

Support: The Human Brain Project (HBP), EU/Horizon 2020 no 720270 (HBP SGA1), 785907 (Human Brain Project, SGA2)

Title: Investigating action selection in the basal ganglia - computational approaches at different levels of biological description

Authors: *J. J. JOHANNES HJORTH[1], S. M. SURYANARAYANA[2], A. KOZLOV[1,2], J. FROST NYLÉN[2], G. SILBERBERG[3], K. N. GURNEY[4], J. HELLGREN KOTALESKI[1,2], S. GRILLNER[2]
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Abstract: The basal ganglia of the forebrain play a major role in deciding when to initiate an action. Detailed, although incomplete, information exist on the major building blocks in terms of striatum which receives input from cortex and thalamus, intrinsic nuclei such as GPe and the subthalamic nucleus, and the output nuclei, targeting different brainstem centres and thalamus. . We report here 1) a data-driven simulation of the striatal micro-circuitry based on compartmental modelling, and 2) a hypothesis-driven model of the different parts of the basal ganglia based on point neurons.
In the detailed striatal model, reconstructed morphologies of GABA-ergic projection neurons (D1 and D2), fast spiking and cholinergic interneurons are connected based on neurite overlap. Realistic cell densities are used, and the synaptic connectivity is pruned to match experimental data. Electrophysiological properties are optimised using BluePyOpt. Reconstructed axonal

fibres from cortex and thalamus provide input to the micro-circuitry. Here 70,0000 striatal neurons are simulated using parallel neuron on a Cray Supercomputer. The effect of dopamine modulation on the circuit, and the impact of clusters of cortical and thalamic axons are investigated.
The second part is based on a network model by Gurney et al. (2001) expanded to include the newly revealed subdivision in GPe - the arkypallidal cells, which have an extensive projection to the striatum and the prototypical subtypes. The extended connectivity enhanced the selection of actions, providing additional support for the action selection hypothesis. The prototypical cells were the principal subpopulation influencing action selection and via the arkypallidal cells, they could 'switch-on' or 'switch-off' the striatum. The model also captured tonic dopaminergic modulation of SPN activity from the SNc, with excitatory effects on D1 SPNs and inhibitory effects on D2 SPNs, and investigated their role in action selection at various levels of dopamine activity. This revealed a novel basic circuitry consisting of arkypallidal projections to the striatum, underlying theta oscillations in dopamine-depleted conditions, which could be relevant for Parkinson's disease. In all, the results highlight the GPe as a major control hub of the basal ganglia and provide a mechanistic account for its control function.

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Poster

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Topic: E.03. Basal Ganglia

Title: Time-delay based reservoir computing modeling of the basal ganglia

Authors: *Y. G. TIRAT-GEFEN
GMU / Maxwave Res. LLC, Rockville, MD

Abstract: This presentation discusses the use time-delay based reservoir computing models of the behavior of the basal ganglia.The basal ganglia plays a key role in many movement disorders such as Parkinson"s disease. Current therapies for basal ganglia related disorders include pharmaceutical intervention affecting neurotransmitter levels (e.g. L-Dopa in Parkinson's disease) and medical devices, e.g. deploying deep brain stimulation (DBS).
The basal ganglia role in movement learning indicates that machine learning is a fruitful approach for modeling. The basal ganglia biological neural circuits seem to even mimic traditional methods in machine learning such as reinforcement learning and dimensional reduction. The chaotic nature of some the basal ganglia oscillatory behavior is a good target for

time-delay nonlinear modeling methods such as multidimensional time-delay differential equations.
Our work describes the modeling of basal ganglia circuits using reservoir computing, a machine learning approach, to capture the chaotic nature of basal ganglia oscillations. On top of this model we evaluate mathematical formulations and algorithms to fine tune the use of medications (e.g. dosage and timing of dose) and medical device based interventions. Our models indicate that these formulations and algorithms may offer a new avenue of personalized treatment of patients suffering from movement disorders.

Disclosures: Y.G. Tirat-Gefen: None.

Poster

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Title: Inhibitory basal ganglia inputs induce excitatory motor signals in the thalamus

Authors: *J. KIM[1], Y. KIM[2], R. NAKAJIMA[1], A. SHIN[2], G. AUGUSTINE[3], D. KIM[2]
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Abstract: Basal ganglia (BG) circuits orchestrate complex motor behaviors including action selection, patterning and coordination by modulating downstream motor areas such as thalamus and brainstem. However, the circuit specific mechanisms in BG output structures for multiple motor coding remain poorly understood. Here, we used optogenetics to selectively perturb specific basal ganglia outputs and concurrently examined cortical, muscular, and behavioral motor responses. We found that photostimulation of basal ganglia inhibitory inputs from the globus pallidus to the thalamus induces multiple motor dysfunctions including suppressed locomotion, tremor and enhanced rigidity through rebound excitation of target neurons. Reducing the neuronal population with this rebound excitability by direct photoinhibition of VL neurons abolished multiple motor dysfunctions. In a low dopamine state, the number of VL neurons showing post-inhibitory firing increases, while reducing the number of active VL

neurons via photoinhibition of BG input, effectively prevents Parkinson disease (PD)-like motor symptoms. Contrary to the modulation of thalamic inputs, movement was facilitated when we applied photostimulation of basal ganglia inputs from the globus pallidus to the brainstem. These results suggest that basal ganglia inhibitory inputs generate multiple motor commands depending on downstream output regions and their non-canonical responsiveness. Thus, the conventional view of the role of BG circuits should be reconsidered with these aspects. Targeting BG output structures could yield alternative approaches for treating debilitating motor disorders.

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Poster

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Topic: E.03. Basal Ganglia

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Title: Disruption and rescuing cued-turning in rats by silencing and activating, respectively, striatal cholinergic interneurons

Authors: *C. AVILA, A. KUCINSKI, M. SARTER
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Abstract: Impairments in balance and complex movement control in patients with Parkinson's disease (PD) are associated with loss of basal forebrain cholinergic neurons. We previously demonstrated that loss of cholinergic signaling in the cortex results in deficits in top-down control and associated failures to detect behaviorally significant task cues. PD patients with combined cortical cholinergic and striatal dopaminergic losses exhibit a high risk for falls. We have hypothesized that such falls reflect the failure to process exteroceptive and interoceptive movement cues and to detect movement errors, and that this information therefore is no longer transferred to the striatum. In the striatum, cholinergic interneurons (ChIs) are positioned to integrate cortico-striatal information with the dopaminergic mediation of action selection. Thus, we predicted that inhibition of ChIs is sufficient to produce complex movement deficits and, in rats modeling the dual cholinergic-dopaminergic losses of PF fallers (DL rats), activation of ChIs will rescue their ability to employ cues to guide complex movements. As falls in PD patients are readily provoked by demands on cued turns, here, we tested this hypothesis in rats by employing a cued turning treadmill task (C3T). Rats were trained to walk on a treadmill until the onset of a "stop" or a "turn" cue, following which the treadmill restarted in the same or the reverse

direction, respectively. DL rats, which exhibit frequent falls when traversing rotating rods (Kucinski et al., 2013), were impaired in responding accurately to the turn cue in the C3T, supporting the validity of this task in revealing the disruption of the cognitive-motor interface. DREADD-mediated inhibition of ChIs in the dorsomedial striatum, in otherwise intact ChAT::Cre rats, fully reproduced this selective deficit of DL rats. Moreover, DREADD-mediated activation of these neurons partially attenuated the cued turning deficit of DL rats. These results support the hypothesis that striatal cholinergic interneurons mediate the integration of cues into complex movement sequences, such as turning. Activation of ChIs may be a therapeutic strategy for reducing falls in PD patients.

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Poster

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Topic: E.03. Basal Ganglia

Support: NARSAD Young Investigator Award 23840
BSF 2015255

Title: Repeated activation of amygdala inputs to the dorsolateral striatum induces compulsive motor behavior

Authors: C. CUNHA, D. ASHUROV, B. JONES, K. MAYIL, *J. L. PLOTKIN
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Abstract: Obsessive-Compulsive Disorder (OCD) is a debilitating condition affecting up to 3% of the population. Numerous brain regions have been implicated, but imaging studies suggest that striatal over-activity plays a central role. The presentation of repetitive, compulsive, habitual behaviors points to the involvement of the dorsolateral striatum (DLS), which encodes action selection and habit learning. Psychophysical models posit that the motor symptoms (compulsions) of OCD may be the result of pathologically reinforced attempts to alleviate distressful thoughts (obsessions). Consistent with this hypothesis, over-activity has also been observed in several limbic-associated structures in OCD patients, including the amygdala. We have recently shown that the basal and lateral nuclei of the amygdala (BLA) send functional projections directly to striatal spiny projection neurons (SPNs) in the DLS, and that these projections target direct pathway SPNs (dSPNs) and indirect pathway SPNs (iSPNs) similarly in wild type (WT) mice. Here we tested the hypothesis that repeated activation of the BLA-DLS pathway promotes the generation of compulsive motor movements. Repeated optogenetic stimulation of BLA axon terminals within the DLS (10 Hz, 10 minutes; design based on Ahmari

et al., 2013) for 5 consecutive days gradually induced compulsive grooming behavior in WT mice. This behavior was long-lasting, and persisted for at least a week following the last stimulation. Furthermore, the expression of compulsive grooming behavior was accompanied by long-lasting circuit alterations in the DLS: 1) an increase in the density of functional BLA inputs to dSPN dendrites, 2) an increase in the ratio of postsynaptic NMDA/AMPA receptor mediated responses at BLA inputs to dSPNs and 3) a general increase in dendritic spine density in dSPNs. These circuit alterations suggest elevated synaptic drive of the direct pathway, a phenomenon consistent with hyperkinetic behavior such as compulsive grooming. Taken together, these data describe how over-activation of a limbic-associated amygdala circuit can plastically modulate dorsolateral striatum output, leading to repetitive motor behavior.

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Poster

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Topic: E.03. Basal Ganglia

Title: The role of amygdala-striatal projections in instrumental learning

Authors: *A. VICENTE, P. BOTTA, R. M. COSTA
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Abstract: In a changing environment, animals have to learn new actions constantly. A key component in learning new goal-directed actions is the expected value of the outcome. Of particular interest to this question is the basolateral amygdala (BLA), which projects broadly to dorsomedial striatum (DMS), while mostly avoiding the dorsolateral striatum (DLS). Recordings in amygdala have shown the development of firing selectivity to stimuli associated with the valence of the reinforcer. Lesions in BLA, as well as disconnections to DMS, led to animals that are insensitive to changes in outcome value. Taken together, these experiments expose the possible role of BLA in updating the value of stimuli, and associating changes of value with learned behaviour.In this study, using a self-stimulation lever-pressing paradigm, we tested the sufficiency of DMS-projecting BLA neurons to reinforce specific novel actions. We trained mice injected with ChannelRhodopsin-2 (ChR2) or YFP in BLA to press an active lever to receive light stimulation in DMS, in the presence of an inactive lever leading to no outcome. We uncovered that stimulation of these projections is sufficient to reinforce a novel, self-paced action, with ChR2 injected animals rapidly acquired high rates of lever pressing for self-stimulation. The learned behavior was dependent on the contingency between the action and the outcome. Furthermore, stimulated animals could dynamically follow the stimulation, switching

promptly between levers when both contingencies were reversed. Therefore, the behavior of the animals has the hallmarks of a goal-directed action.To further elucidate the role of BLA in value updating during instrumental learning, we are currently training mice to press a lever to receive a natural reinforcer (sucrose solution), while recording calcium transients from BLA neurons.To the best of our knowledge, this study shows for the first time that DMS projecting BLA neurons can support the learning of novel action-outcome contingencies, in a flexible and dynamic manner that mimics a goal-directed strategy. These results, taken together with previous studies involving BLA in value tracking, suggest that the projections from BLA to DMS carry information necessary for the association between action and the current value of the outcome and could have important implications in our understanding of value association in action learning.

Disclosures: A. Vicente: None. **P. Botta:** None. **R.M. Costa:** None.

Poster

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Topic: E.03. Basal Ganglia

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Title: Isometric two action task for studying the effect of reward in action selection

Authors: *I. VAZ, D. S. PETERKA, R. M. COSTA
Zuckerman Inst., Columbia Univ., New York, NY

Abstract: Animals tend to perform actions that lead to a positive outcome or reward. Rewards reinforce specific behaviors that lead them. Recent work, including research from our lab, suggests that the reinforcement of neural and behavioral patterns leading to reward is dependent on plasticity in basal ganglia circuits. It has also been shown that the striatum encodes actions, outcomes and the overlap between action and outcome, which can guide action selection in basal ganglia circuitry. However, how rewards lead to an increase in the selection of a certain action over non-rewarded actions is not fully understood. It is also unknown what are the effects that the reinforcer has on the activity of the medium spiny neurons (MSNs) that eventually helps shape action selection and execution.
To address this problem, we have developed a head-fixed isometric force task in which animals are required to perform two different actions using the same manipulandum in a self-paced manner. The actions consist of either pulling or pushing an immobile, pressure sensitive joystick within an experimenter-defined duration and pressure range. The animals do not receive any

feedback besides their own proprioception and reward followed by the correct action. This makes the task totally trial and error based as animals will only know the correct and incorrect actions by the presence or absence of reward. After training, reward availability is alternated between the two actions in a block wise fashion within a single session. Hence, animals must match their action to the alternating contingencies in order to obtain rewards. This paradigm allows us to study the effect of the reinforcer on action selection. While animals executed the alternation between pull and push actions we performed one- and two-photon calcium imaging of MSNs in dorsolateral striatum using GRIN lenses to study task-related neuronal activity. We hope that this will allow us to understand how reward helps shape MSN activity and induce action selection according to the rules of the environment. Additionally, we hope this opens new windows to further understand how corticostriatal activity is necessary for action learning.

Disclosures: I. Vaz: None. **D.S. Peterka:** None. **R.M. Costa:** None.

Poster

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Topic: E.03. Basal Ganglia

Support: DA019473

Title: Sensory and motor encoding in the ventral pallidum and nucleus accumbens

Authors: *J. D. LEDERMAN[1], S. E. MORRISON[3], S. LARDEUX[2], S. M. NICOLA[4]
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Abstract: The ventral pallidum (VP) and nucleus accumbens (NAc) are components of the basal ganglia essential for translating limbic sensory, value and motivation signals into motor output, but how they do so is unknown. In this study we ask how neuronal firing in the VP and NAc relates to both spontaneous and reward-directed locomotor movements. Rats were food-restricted and trained on a discriminative stimulus (DS) task. The DS was an auditory cue, which directed the rat to approach and press a lever to obtain 10% sucrose reward. Trained animals were implanted with microelectrode arrays in either the VP or NAc, allowing multi-unit recording of neuronal activity while video tracking of head-mounted LEDs enabled detection and measurement of locomotion. Approximately half of the neurons in the VP and NAc were excited by DS presentation In both areas, an overlapping population of neurons showed changes in activity highly correlated with initiation and cessation of spontaneous locomotor movements (i.e., movements during the inter-trial interval that were typically not directed towards the lever)

in that neurons that showed inhibition during movement onset were excited during movement cessation, and vice versa. Ongoing analysis aims to compare and contrast the behavioral and locomotor parameters encoded in the cue-evoked and movement-related firing in these two areas. These results demonstrate a previously unknown role for the ventral pallidum in locomotor control, and provide a starting point for further interrogation of the NAc-VP connection in motivated behavior.

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Poster

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Program #/Poster #: 147.21/LL13

Topic: E.03. Basal Ganglia

Support: CONACyT-CB- 2013-01: 220412
CONACyT-Fronteras de la Ciencia: 2022
DGAPA-PAPIIT- UNAM: IA200815
DGAPA-PAPIIT- UNAM: IN226517
CONACyT PhD Fellowship: 584044
CONACyT PhD Fellowship: 597738

Title: Development of a task to study the shaping and switch between lever press sequences in a compulsive behavior model

Authors: *K.-I. RAMIREZ-ARMENTA, A. SANCHEZ-FUENTES, J. O. RAMÍREZ-JARQUÍN, F. TECUAPETLA
Natl. Autonomous Univ. of Mexico, Ciudad de Mexico, Mexico

Abstract: R-A, K and S-F, A contributed equally to this work.
The generation, development and modulation of sequences of movements are essential to the survival. Alterations in the mechanisms that control the selection, initiation and switching between actions has been observe in multiple motor pathologies as Parkinson's Disease, Huntington Disease and Obsessive-Compulsive Disorders.
The aim of this work was the development of a training program that leads animals to perform two consecutive sequences of lever presses forming a chain of sequences. In this task, the subjects were trained to respond in two different schemes in the same session: 1) Stimulus-response Chains: where the animals had a finite number of presses to do and a signal that guides them to move to the second sequence [FR4 on Lever 1→FR4 on Lever 2→ Reward] and, 2) Self-paced Chains: where the subjects are free to decided when to finish the first sequence and

when to start the second [>=4 Presses on Lever 1 → >=4 Presses on Lever 2→ Reward].
To probe this paradigm, we used the compulsive behavioral model Sapap3-KO mice. It has been reported that these subjects show repetitive behaviors that links them as an OCD-like syndrome model. In the first stages of the training, the Sapap3 KO mice were able to learn the action-outcome relationship as their littermates. During the first days of the Stimulus-Response scheme the Sapap3 KO mice have an increase in the number of unfinished chains, because them disrupts the second sequence to look for the reward. When both schemes of chains were presented in the same session, the Sapap3 KO mice only has disruptions after finishing the first sequence of the chain on the Self-Paced modality. Also, they showed an increase on the number of presses and the duration of the first sequence.
These findings will be discussed on the light of the interpretation of the SAPAP KO mice as a model for compulsions.
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Disclosures: K. Ramirez-Armenta: None. **A. Sanchez-Fuentes:** None. **J.O. Ramírez-Jarquín:** None. **F. Tecuapetla:** None.

Poster

147. Basal Ganglia Systems in Motivated Behaviors

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 147.22/LL14

Topic: E.03. Basal Ganglia

Support: NIH DA R01 027222

Title: The role of the ventral tegmental area in acute and chronic methylphenidate administration

Authors: S. A. IHEZIE, M. THOMAS, *N. DAFNY
Univ. of Texas Med. Sch. at Houston, Houston, TX

Abstract: INTRODUCTION: As a drug for narcolepsy and the treatment of choice for attention deficit hyperactivity disorder (ADHD), methylphenidate (MPD) is a potent psychostimulant. It increases the bioavailability of dopamine (DA). The ventral tegmental area (VTA) is a major source of DA in the brain. The aim of this study was to understand the role of the VTA in the acute and chronic MPD effect. METHODS: Forty-eight male Sprague-Dawley rats were divided into six random groups of eight: (1) control for electrical lesion (Control-1), (2) sham for electrical lesion (Sham-1), (3) nonspecific, electrical lesion, (4) control for chemical lesion (Control-2), (5) sham for chemical lesion (Sham-2), and (6) specific, chemical lesion with 6-OHDA. Baseline (BL) recording was obtained on experimental day 1 (ED1) followed by VTA

electrical or chemical lesion on ED 2. After 5 days recovery (ED3-7), the behavioral recordings were resumed on ED8 after saline injection. All six groups received daily intraperitoneal injections of 2.5 mg/kg MPD for six days (ED9-14) after which the animals received no treatment for 3 days (ED15-17). On ED 18, all six groups were given 2.5mg/kg MPD again to assess for the chronic effect of the psychostimulant. RESULTS: Three locomotor indices were used to measure the animals' behavioral response to the drug-- horizontal activity, number of stereotypy, and total distance. ED8 was compared to ED1 to assess the BL activity after chemical and electrical lesion; both types of lesions resulted in significant ($p<0.05$) increase in the animals' BL activity. Acute MPD exposure, comparing ED9 to ED8, elicited significant increase in all 3 locomotor indices. Comparing ED 14 to ED 9 for the induction phase, all groups responded to MPD with further significant ($p<0.05$) increase inlocomotion. Comparing ED18 to ED9 for the expression phase, the nonspecific, electrical lesion groups showed behavioral sensitization while the chemical, specific lesion group showed no change. CONCLUSION: The electrical and chemical lesion of the VTA did not modify the MPD acute effect while the chemical lesion prevents the chronic effects, suggesting that the VTA participates in the chronic MPD effect.

Disclosures: S.A. Ihezie: None. **M. Thomas:** None. **N. Dafny:** None.

Poster

147. Basal Ganglia Systems in Motivated Behaviors

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 147.23/MM1

Topic: E.03. Basal Ganglia

Support: NIH grant NS103226

Title: The function of the entopeduncular nucleus is action selection and evaluation

Authors: *M. WALLACE, B. L. SABATINI
Neurobio., Harvard Med. Sch., Boston, MA

Abstract: Animals select actions based on incoming sensory information and past experience to achieve goals. As a result of these experiences, they modify their behavioral selection to promote the repetition of actions associated with positive outcomes and suppress those associated with bad outcomes. Nevertheless, it is also advantageous to maintain behavioral flexibility in order to change behavior if the environment changes and to exploit new opportunities as they arise. The basal ganglia (BG) are an evolutionarily ancient group of nuclei in the brain conserved in all vertebrates and crucial for voluntary movements. The entopeduncular nucleus (EP) is a major output nucleus of the BG and has been suggested to have an important role in evaluating the outcome of recently performed actions and guiding the selection of future actions. We aim to

understand how genetically defined EP cell-types are involved in a motivated behavior that requires flexible relationships between sensory stimuli and motor action.

Disclosures: M. Wallace: None. **B.L. Sabatini:** None.

Poster

147. Basal Ganglia Systems in Motivated Behaviors

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 147.24/MM2

Topic: E.03. Basal Ganglia

Support: R01 NS103226

Title: Dissection of the parafascicular nucleus in mice reveals functional distinct cortico-thalamic-striatal circuits

Authors: *G. MANDELBAUM[1], B. L. SABATINI[2]
[1]Neurobio., [2]Harvard Med. Sch., Boston, MA

Abstract: The central-median/parafascicular nucleus (CM/Pf) of the thalamus forms synapses throughout the striatum. This implies significant divergence in the output of the CM/Pf and thus also, the potential to exert powerful control over the basal ganglia circuits. By integrating converging afferents from multiple cortical areas and the brain stem, the PF modulates the Striatum, the latter being important for normal behavior and action selection in humans. This is made clear from diseases with components of striatal dysfunction, such as Parkinson's, Huntington's, Obsessive Compulsive, and Tourette's Disorders. Conversely, both the healthy function and pathophysiology of CM/Pf is much less understood which raises the necessity for a better understanding of the CM/Pf mechanism of action.
In non-human primates, the CM/Pf is organized into multiple output channels. However, due to the limitations of genetic control in the primate, little is known about the function of each of these channels, of the circuitry that comprises the input and output of these channels, and of differential activity of these channels during behavior. Additionally, in the rodent, due to its small size (~350 µm in the anterior-posterior axis in mice), CM/Pf has been a challenge to study. We found that mouse CM/Pf can be functionally, anatomically, and molecularly divided into distinct zones. Each zone receives and sends specialized projections from cortex and to the Striatum, respectively. Guided by these findings we propose a model for CM/Pf function. In addition, we conducted in vivo loss of function experiments in both striatum and CM/Pf while the animals performed a novel behavior that requires them to switch between learned motor actions. Our data suggests that CM/Pf might play a central role in mediating action selection during changing environmental contingencies. We now seek to further establish a causal role for the cortical-CM/Pf-striatal- loops in both health and disease.

Disclosures: G. Mandelbaum: None. **B.L. Sabatini:** None.

Poster

147. Basal Ganglia Systems in Motivated Behaviors

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 147.25/MM3

Topic: E.03. Basal Ganglia

Title: Contribution of the rostral intralaminar thalamic nuclei to striatal physiology and behavior

Authors: *K. K. COVER, W. G. KERKHOFF, B. N. MATHUR
Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: The release of dopamine into the striatum is critical for action reinforcement and learning. As a modulatory neurotransmitter, dopamine directs signaling of striatal output medium spiny neurons and, consequently, behavior. We recently discovered that activation of glutamatergic projections arising from the rostral grouping of thalamic intralaminar nuclei (rILN) robustly elicits dopamine release in the dorsal striatum. Using fast-scan cyclic voltammetry, whole-cell electrophysiology, and optogenetics, we parse the specific mechanisms by which rILN projections evoke dopamine release and modulate striatal signaling. How the rILN contributes to striatal dopamine-dependent behaviors is unclear. To this end, we employ fiber photometry and chemogenetics to investigate the role of rILN-->dorsal striatum signaling in sensory-cued action selection and learning. The results of this study stand to inform how the thalamus leverages the striatal dopaminergic system to govern action and may have important implications for pathological states affecting striatal dopamine, including addiction and Parkinson’s disease.

Disclosures: K.K. Cover: None. **W.G. Kerkhoff:** None. **B.N. Mathur:** None.

Poster

148. Brain-Machine Interface: Neurophysiology: Non-Invasive Techniques: Ultrasound and Other

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 148.01/MM4

Topic: I.04. Physiological Methods

Support: NIH R01 MH111534

Brain Research Foundation
Kavli Institute of Brain and Mind
Salk Innovation Grants

Title: Sonogenetics- A non-invasive method to manipulate neurons

Authors: *C. LEE-KUBLI, U. MAGARAM[1], Y. TUFAIL[1], V. KO[1], R. SHIAO[1], T. GRIDER[2], E. EDSINGER[3], E. CALLAWAY[1], D. GIBBS[2], S. CHALASANI[1]
[1]Mol. Neurobio. Lab., Salk Inst. for Biol. Studies, La Jolla, CA; [2]UCSD, San Diego, CA; [3]Marine Biol. Lab., Woods Hole, MA

Abstract: A fundamental goal of neuroscience research is to understand the contributions of individual neurons to behavioral output, a task that requires an ability to manipulate specific neurons. Currently, optogenetic methods are used, where light of particular wavelength is delivered to target neurons expressing opsins (light-gated channels). While this approach has revealed many interesting insights, it suffers from a drawback - an inability to control targets that are deep within the brain without invasive surgery. We are developing a non-invasive method using ultrasound that can penetrate the full thickness of the brain and spinal cord and activate specific mechanosensory proteins ("Sonogenetics"). We have developed an *in vitro* assay to screen candidate mechanosensitive channels and identify optimized ultrasound characteristics. Candidate channels of invertebrate and bacterial origins are expressed and trafficked to the cell membrane of mammalian neurons. Consistently, we observe an increase in feeding behavior when mice expressing these ultrasound sensitive channels (USSCs) in agouti-gene related peptide (AGRP) neurons in the arcuate nucleus of the hypothalamus are exposed to ultrasound stimuli compared to unstimulated or GFP controls (n=6-7 per group). These preliminary data support the validity of sonogenetics as an approach for activating mammalian neurons and identify candidate channels that can be improved upon by increasing ultrasound-sensitivity and cation-selectivity.

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Poster

148. Brain-Machine Interface: Neurophysiology: Non-Invasive Techniques: Ultrasound and Other

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 148.02/MM5

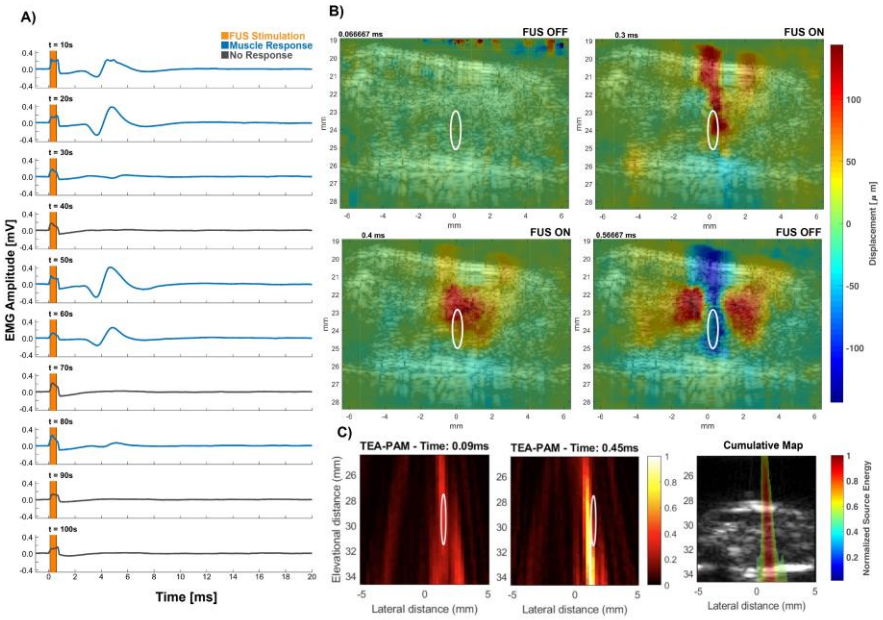
Topic: E.05. Brain-Machine Interface

Support: DARPA-GG012363

Title: Real-time displacement and cavitation imaging of non-invasive neuromodulation of the peripheral nervous system via focused ultrasound

Authors: *S. A. LEE[1], M. BURGESS[2], A. POULIOPOULOS[2], E. KONOFAGOU[2]
[1]Biomed. Engin., [2]Columbia Univ., New York, NY

Abstract: Focused ultrasound (FUS) neuromodulation could provide an important noninvasive surrogate with high specificity to chemical or electrical modulation techniques. The *in vivo* mechanism remains unknown with some theoretical models assuming approximations that may not reflect the physical response of the nerve. Previously, we demonstrated FUS activation of the sciatic nerve can be imaged and quantified using tissue displacement generated by the radiation force. We developed a real-time displacement and passive cavitation mapping methodology that further assists in targeting and studying the FUS neuromodulation mechanism. A 4 MHz single-element HIFU transducer, focal volume: 0.2 x 2 mm, (Sonic Concepts, WA) applied tone burst pulses of 0.5 ms, 0.01 Hz PRF, 30 MPa peak pressure to the nerve, recorded by electromyography (EMG). Real-time displacement images and passive acoustic mapping (PAM) were acquired using an 18-MHz linear array (Vermon, France). Displacement images were calculated through 1D cross correlation (30kHz frame rate). Cavitation was quantified using a robust Capon beamformer (RCB-PAM). Both imaging methods were temporally and spatially overlaid onto B-mode images of the mouse leg. EMG recordings shows time dependent relationships. In 10 sonications, successful activation was observed in 6 events. Targeting via real-time displacement images increased the rate of success of sonicating the nerve by 33%. Nerve tissue was tracked before, during, and after stimulation. Peak displacement for successful activation was 200 μm. RCB-PAM indicated cavitation was located within 1.0 ± 0.6 mm of the focus. Cavitation was spatially consistent with the location of the nerve and suggests that cavitation accompanies radiation force at these parameters. Simultaneous cavitation and displacement imaging during FUS modulation may be capable of providing solid evidence for mechano-electric nerve conduction as well as monitoring the safety and efficacy of the methodology, rendering it thus a viable method for clinical application of ultrasound in peripheral neuropathy.



Disclosures: S.A. Lee: None. **M. Burgess:** None. **A. Pouliopoulos:** None. **E. Konofagou:** None.

Poster

148. Brain-Machine Interface: Neurophysiology: Non-Invasive Techniques: Ultrasound and Other

Location: SDCC Halls B-H

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Program #/Poster #: 148.03/MM6

Topic: E.05. Brain-Machine Interface

Support: NIH BRAIN Grant U01NS099724
T &C Chen Brain-machine Interface Center at Caltech
Boswell Foundation

Title: Listening to the brain: Functional ultrasound imaging in the non-human primate posterior parietal cortex during a memory-guided saccade task

Authors: *V. N. CHRISTOPOULOS[1], D. MARESCA[2], S. NORMAN[1], C. DEMENE[3], T. DEFFIEUX[3], M. TANTER[3], M. SHAPIRO[2], R. ANDERSEN[1]
[1]Div. of Biol. and Biol. Engin., [2]Div. of Chem. & Chem. Engin., Caltech, Pasadena, CA; [3]Inst. Langevin, CNRS, ESPCI Paris, Inserm, PSL Res. Univ., Paris, France

Abstract: High-density neural interfaces require the ability to observe large-scale patterns of neural activity with high spatiotemporal resolution. Ideally, they should be non-invasive (or minimally invasive) to facilitate their applications in research and potential clinical studies. Current methods to monitor neural activity, including electrophysiology, optical imaging and functional magnetic resonance imaging (fMRI) fall short of these criteria. Recently, functional ultrasound (fUS) was introduced as a revolutionary technique that can non-invasively image neural activity by offering a unique combination of spatial coverage (several cm) and improved spatiotemporal resolution compared to fMRI (<100 μm and <10 ms), using probes that can be mounted on freely moving animals. However, most of the previous fUS studies have focused predominantly on small animals (e.g., rodents) leaving open the question of whether fUS is capable of monitoring brain activity in larger organisms such as non-human primates (NHPs). To address this question, we examined the capabilities of fUS in a NHP using an ultrasound probe that was inserted daily within a custom-designed square chamber (24 mm width) over the left intraparietal sulcus (*ips*). The chamber accommodated a 15 MHz ultrasound transducer (Verasonics Vantage™) that was attached to a stereotaxic frame. The animal was trained to perform memory-guided saccades to a single target presented either in the left or in the right visual field. Each trial started with the animal fixating at a central cue for 6 s before a single target was presented in the peripheral visual field for 300 ms. The animal had to memorize the location of the briefly flashed target for 10 s while maintaining eye fixation. When the central fixation cue was extinguished, the animal had to perform an eye movement to the remembered target location to receive a liquid reward. By acquiring fUS images with 1 s temporal resolution over the *ips*, we found that the Doppler signal in the lateral intraparietal area (LIP) - an area that is involved in planning of eye movements - was higher during the memory-period for contralateral than for ipsilateral eye movements. No changes on the Doppler signal were found during the eye-fixation period prior to the target flash. This result suggests that fUS is capable of capturing the preparatory motor signal in LIP that precedes the motor response (i.e., eye movement) in a memory-guided saccade task. Our findings provide direct evidence that fUS is capable of measuring an accurate readout of regional brain activity in cortical regions in awake and behaving NHPs, opening a new avenue in non-invasive functional neural imaging.

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Poster

148. Brain-Machine Interface: Neurophysiology: Non-Invasive Techniques: Ultrasound and Other

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 148.04/MM7

Topic: E.05. Brain-Machine Interface

Support: DARPA BTO HR0011-16-C-0014

Title: Noninvasive neuromodulation of the immune system using ultrasound stimulation of the spleen to treat autoimmune disease

Authors: *D. ZACHS[1], C. R. W. KAISER[1], A. HEILLER[1], S. OFFUTT[5], H. GUO[1], J. BASILE[1], T. LI[1], C. D. GLOECKNER[1], J. MUELLER[5], Y. KIM[5], R. DUTTA[2], J. L. AUGER[2], N. J. SCHULDT[2], E. J. PETERSON[3], J. K. ALFORD[5], B. A. BINSTADT[2], H. H. LIM[1,4]
[1]Biomed. Engin., [2]Ctr. for Immunol., [3]Rheumatic and Autoimmune Dis., [4]Dept. of Otolaryngology-Head and Neck Surgery, Univ. of Minnesota, Minneapolis, MN; [5]Medtronic, Minneapolis, MN

Abstract: Background: The cholinergic anti-inflammatory pathway is a unique tool to modulate the mammalian immune response. Electrical vagus nerve stimulation (VNS) triggers an inflammatory reflex that dampens the body's response to infection or tissue injury and has been shown to reduce in vivo cytokine production in animals during endotoxemia. Recently, it was discovered that ultrasound energy delivered noninvasively to the spleen of mice diminished inflammation and tissue damage during renal ischemic reperfusion injury (IRI). It was proposed that ultrasound initiates the same cholinergic anti-inflammatory pathway triggered by VNS, but how this pathway is activated remains unclear. Therefore, we pursued a multi-faceted investigation to determine how ultrasound stimulation can modulate this cholinergic anti-inflammatory pathway, how the neural activity of the system is altered, and whether or not this treatment can be translated to other diseases. We now report on the successful use of ultrasound stimulation of the spleen to treat a rodent model of rheumatoid arthritis, and quantify how alternative neuromodulation techniques including electrical stimulation compare to ultrasound.
Methods: For the initial phase of the study we used a mouse model of arthritis to determine if ultrasound-treatment would have therapeutic effects on disease severity or cytokine production. The K/BxN transgenic mouse line allows consistent transfer of arthritis when serum from host K/BxN animals is injected i.p. into recipient wildtype C57BL/6 (B6) mice. Recipient animals were treated noninvasively with spleen-targeted ultrasound for 7-14 days and monitored daily for arthritis severity. Follow-up experiments were also performed to determine if electrical stimulation using surface electrodes near the spleen or other body locations could achieve similar benefits to ultrasound stimulation.
Results: We tested many ultrasound stimulation parameters and durations and identified several key parameters that can significantly treat arthritis with reductions in ankle swelling and clinical scores. Electrical stimulation near the spleen with various parameters proved to be ineffective. Interestingly, we also identified one body location where electrical stimulation drove significant therapeutic effects.
Conclusions: Noninvasive ultrasound stimulation targeted towards the spleen can significantly reduce arthritis severity in a mouse model of the disease, which is not as readily possible with electrical stimulation. These findings have led to an ongoing clinical trial to translate the findings into patients with rheumatoid arthritis.

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Poster

148. Brain-Machine Interface: Neurophysiology: Non-Invasive Techniques: Ultrasound and Other

Location: SDCC Halls B-H

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Program #/Poster #: 148.05/MM8

Topic: E.05. Brain-Machine Interface

Support: SONIC Lab Discretionary Funds
MnDRIVE Brain Conditions Innovations Grant
DARPA HR0011-15-C-0155

Title: Ultrasound modulation of the brain and peripheral nerves

Authors: *H. GUO[1], S. OFFUTT[4], M. HAMILTON[1], Y. KIM[4], C. GLOECKNER[1], J. ALFORD[4], H. LIM[1,2,3]
[1]Biomed. Engin., [2]Otolaryngology, Head and Neck Surgery, [3]Inst. for Translational Neurosci., Univ. of Minnesota, Minneapolis, MN; [4]Res. and Core Technol. – Implantables – Restorative Therapies Group, Medtronic, Minneapolis, MN

Abstract: Introduction:
Ultrasound (US) stimulation is a new modality that has shown potential for noninvasively activating the brain in animals and humans. Recent *in vitro* or *in vivo* studies have shown that stimulation of nerves with US is also possible. However, little is known about the underlying mechanism(s) of US neural activation. In our study, we investigated if US can directly activate different brain regions and also the sciatic nerve of guinea pigs, and if there are parameters in which US stimulation can enhance or suppress neural activity within the central and peripheral nervous system.
Methods:
For the brain experiments, 32-site arrays were positioned within the right primary somatosensory cortex (SC1) or primary auditory cortex (A1) of ketamine-anesthetized guinea pigs. The US transducer was coupled to the SC1 or A1 with degassed agarose. We also performed US stimulation of SC1 and A1 of surgically deafened animals. For the nerve experiments, we applied US to the intact leg skin above the sciatic nerve, the exposed sciatic nerve lying in the muscle cavity, and an isolated sciatic nerve floating in the air coupled to the transducer with agar. Numerous parameters were used to evaluate if US could activate, enhance or suppress brain or nerve activity in different preparations.
Results:
US elicits extensive activation across cortical and subcortical brain regions with a wide range of

parameters. However, transection of the auditory nerves or removal of cochlear fluids eliminated the US-induced activity, revealing an indirect auditory mechanism for US brain activation. Additionally, we showed that ultrasound did not directly activate an intact sciatic nerve isolated from the surrounding tissue even at high pressures (up to 5 MPa) with various pulse patterns, but could activate skin and/or muscle receptors during noninvasive US stimulation. Moreover, reversible suppression of neural activity of both the nerve and brain were observed.
Conclusion:
Our finding of indirect US activation of neurons in the intact brain suggests that future studies will need to control for this US-induced auditory effect to reach reliable conclusions. Considering that we could not elicit any sciatic nerve activity with US once the skin and muscles were removed further reveals other confounding factors that can lead to indirect US-induced neural activity. Although US has immense potential for various clinical applications, especially with the ability to noninvasively suppress neurons, future studies need to carefully control for various confounding factors to properly characterize the neural activation or modulation capabilities of US.

Disclosures: H. Guo: None. **S. Offutt:** A. Employment/Salary (full or part-time):; Medtronic. **M. Hamilton:** None. **Y. Kim:** A. Employment/Salary (full or part-time):; Medtronic. **C. Gloeckner:** None. **J. Alford:** A. Employment/Salary (full or part-time):; Medtronic. **H. Lim:** None.

Poster

148. Brain-Machine Interface: Neurophysiology: Non-Invasive Techniques: Ultrasound and Other

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 148.06/MM9

Topic: E.05. Brain-Machine Interface

Support: NIH Grant R01-NS074980

Title: Computational modeling of low-intensity focused ultrasound pulsation for deep brain neuromodulation

Authors: *S. VISAGAN[1], M. M. MONTI[2], D. W. SHATTUCK[1]
[1]Neurol., [2]Psychology, UCLA, Los Angeles, CA

Abstract: Low-intensity focused ultrasound pulsation (LIFUP) provides a noninvasive, highly resolved (~mm^3) novel alternative to procedures such as deep brain stimulation (DBS) or trans-cranial magnetic stimulation (TMS) for treating neurological and psychiatric disorders. In contrast to high-intensity focused ultrasound (HIFU), a procedure used to ablate tissue by generating localized heat, LIFUP delivers much less harmful low-intensity acoustic energy to

targeted tissue to stimulate cells and produce non-necrotic cellular responses. Using simulations, we explored the effects of LIFUP on computational models of human tissue, namely those making up the human head, and analyzed the manner in which acoustic energy emitted from a simulated ultrasound (US) transducer affects bone and neural tissue. We applied numerical tools to perform a range of analyses, primarily to model the human head and brain, and ultimately to predict and simulate the trajectory of the emitted energy. We employed Finite Difference Time Domain (FDTD), k-space Pseudospectral Time Domain (PSTD), and Finite Element (FEM) formulations in MATLAB and Python to accurately reconstruct the sound wave and measure critical changes in pressure, density, and temperature. Modeling the head as concentric heterogeneous ellipsoids, we solved the Westervelt equation for wave propagation and Penne's bioheat transfer equation using nonlinear approximations. Our results showed that the temperature at the focus of a simulated single-element US transducer pulsed output increases by 0.01 K for frequencies between 0.25 to 0.5 MHz and intensities at 0.1 W/cm^2. At higher frequencies, from 0.75 MHz to 3 MHz, and at higher intensities, 2 W/cm^2 to 3 W/cm^2, temperature increases ranged from 1 K to 3 K. Changing the position of the focus from the center of the modeled skull to near the base of the modeled skull produced standing waves with nodes separated every 3 mm. Furthermore, we observed the predicted generation of higher harmonics, where the second harmonic was approximately 18 dB lower than the first. In both schemes, the final density was approximately the same as the initial density and the final pressure was approximately 0.2 Pa higher than the initial pressure. We are currently using optimized C code and graphical processing units (GPUs) to speed up wave reconstruction and to enable use of more accurate head and brain mesh models constructed from subject-specific MRI and CT data. Comprehensive simulations will provide the groundwork for future applications, may provide insights into the efficacy and safety of LIFUP, and may contribute to a better understanding of the mechanism by which LIFUP induces neuromodulation.

Disclosures: S. Visagan: None. **M.M. Monti:** None. **D.W. Shattuck:** None.

Poster

148. Brain-Machine Interface: Neurophysiology: Non-Invasive Techniques: Ultrasound and Other

Location: SDCC Halls B-H

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Program #/Poster #: 148.07/MM10

Topic: E.05. Brain-Machine Interface

Support: NIH/NIBIB Biomedical Technology Resource Center Grant P41EB018783.

Title: Cortico-muscular coupling during individuated finger movements

Authors: *D. J. MCFARLAND[1], W. A. SARNACKI[1], S. M. HECKMAN[1], S. L. NORMAN[2], E. T. WOLBRECHT[3], D. J. REINKENSMEYER[4], J. R. WOLPAW[1]

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Abstract: Brain-computer interface (BCI) technology can restore communication and control to people who are severely paralyzed. BCI technology may also be able to enhance rehabilitation of motor function (Lancet Neurology 7:1032-43, 2008). Toward this end, we seek to find features of cortico-muscular coupling that individuals might learn to control through feedback-based training.

Six individuals without disability performed a task in which they flexed the index finger or middle finger according to the position of targets on a video screen while a robotic device provided resistance. At the beginning of a trial, each of two blue targets appeared at different heights either at the right edge of the video screen or near the center. At the same time, two yellow cursors appeared on the right edge at the same two heights. The horizontal position of the top cursor was controlled by the index finger and that of the bottom cursor by the middle finger. The task was to move one cursor to the target that was near the center and hold the other on the target that was on the right edge. When both cursors were positioned within their targets, the targets turned from blue to green. After the person held the cursors in their targets for 4 s, both targets turned blue and appeared on the right edge; the person was then required to put each cursor in its target to end the trial. The screen went blank for 4 s before the next trial.

Scalp EEG was recorded from 58 locations according to the modified 10-20 system (minus 8 posterior locations); EMG activity was recorded from 4 pairs of electrodes over 4 right arm and hand muscles (brachioradialis, flexor carpi radialis, flexor digitorum superficialis, dorsal interosseous I). Finger position was monitored and resistance was provided by the FINGER robot (J Neuroeng Rehabil 11(1):10, 2014). EEG and EMG signals were band-pass filtered with 4^{th}-order Butterworth filters at 16, 20, 24, 28, and 32 Hz and Hilbert transforms were applied to derive phase locking values (PLV) and amplitude envelope correlations for the 4-s hold period of each trial.

The results show that both PLV and envelope correlations indicate which of the two fingers is flexed ($p < 0.0001$ for every person). The specific frequency bands and signal features providing the best indication varied across individuals. Thus, measures of cortico-muscular coupling reflect individuated finger movements. BCI-based protocols that teach people to control these measures might contribute to the rehabilitation of finger motor function for people with stroke or other neuromuscular disorders.

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Disclosures: D.J. McFarland: None. **W.A. Sarnacki:** None. **S.M. Heckman:** None. **S.L. Norman:** None. **E.T. Wolbrecht:** None. **D.J. Reinkensmeyer:** None. **J.R. Wolpaw:** None.

Poster

148. Brain-Machine Interface: Neurophysiology: Non-Invasive Techniques: Ultrasound and Other

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 148.08/MM11

Topic: E.05. Brain-Machine Interface

Support: NIH/NIBIB P41EB018783

Title: Sensorimotor rhythm control can improve finger movement after stroke

Authors: *D. J. REINKENSMEYER[1], S. L. NORMAN[2], D. J. MCFARLAND[3], A. MINER[1], S. C. CRAMER[1], E. T. WOLBRECHT[4], J. R. WOLPAW[3]
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Abstract: Brain-computer interface (BCI) technology is attracting increasing interest as a tool for enhancing recovery of motor function after stroke, yet the optimal way to apply this technology is unknown. Here, we studied the immediate and therapeutic effects of BCI-based training to control pre-movement sensorimotor rhythm (SMR) amplitude on robot-assisted finger extension in people with moderate to severe hand impairment due to chronic stroke.
Eight participants with a mean Box and Blocks Test (BBT) score of 14 (vs. a normal score of 71) completed a 4-week 3-phase protocol during which they practiced finger extension with assistance from the FINGER robotic exoskeleton. In Phase 1 (week 1), we identified for each person SMR features in the pre-movement EEG that correlated with the intent to extend the index and/or middle finger(s) during the robot-assisted finger extension task. In Phase 2 (weeks 2 and 3), the participants learned to increase or decrease their individual SMR features given visual feedback, without finger movement. Finally, in Phase 3 (week 4), the participants were cued to increase or decrease their SMR features, and when successful, were then cued to immediately attempt to extend the finger(s) with robot assistance.
Four of eight participants achieved SMR control in Phase 2. In Phase 3, three of these four initiated finger extensions with a reduced reaction time after decreasing (vs. increasing) pre-movement SMR amplitude. Two also extended at least one of their fingers more forcefully after decreasing pre-movement SMR amplitude. Across the course of training, hand function, measured by the BBT, showed modest improvement in those with SMR control (7.3 +/- 7.5 blocks) vs. those without control (3.5 +/- 3.1). Higher BBT scores at baseline correlated with larger change in BBT score (R^2=0.630, p=0.019). The strength of this effect was improved by limiting the model to the participants with BCI control (R^2=0.93, p=0.038).
These results suggest that learning to control person-specific pre-movement SMR features

associated with finger extension may improve finger extension ability after stroke. Thus, further investigation of BCI-based training in a rehabilitation context is merited.

Disclosures: D.J. Reinkensmeyer: None. **S.L. Norman:** None. **D.J. McFarland:** None. **A. Miner:** None. **S.C. Cramer:** None. **E.T. Wolbrecht:** None. **J.R. Wolpaw:** None.

Poster

148. Brain-Machine Interface: Neurophysiology: Non-Invasive Techniques: Ultrasound and Other

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 148.09/MM12

Topic: E.05. Brain-Machine Interface

Support: NIH/NIBIB P41EB018783

Title: Using targeted neuroplasticity to improve motor recovery after neurological injury: A computational model

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Abstract: People with motor deficits after neurological injuries often develop abnormal patterns of neural activity that can limit motor recovery. Here, we use a computational model of corticospinal cells eliciting finger movements to study: (1) the mechanisms and patterns of cortical reorganization after a stroke; and (2) how giving targeted feedback on underutilized cell populations can enhance neuroplasticity to improve motor recovery. The model employs a neural network of corticospinal cells projecting to motoneuronal pools. A biologically plausible reinforcement learning algorithm uses feedback about simulated finger force to guide a stochastic search that optimizes corticospinal cell activation patterns.
When it was not damaged, the network lateralized cortical activation for unilateral finger extension to the contralateral hemisphere. After a simulated stroke followed by continued learning, the surviving network exhibited bilateral activation for unilateral movements (a pattern often seen in clinical imaging data) and it failed to achieve its maximal possible force. To access the latent capacity for recovery, we interdigitated normal movement practice (I_A) with a targeted neuroplasticity intervention (I_B) that gave the network feedback based on a targeted population of underutilized ipsilesional cells. This intervention restored laterality of cortical activation and improved force recovery. Its effectiveness depended on which cells were targeted and the ratio of I_A and I_B trials. Targeting secondary motor areas in the contralateral hemisphere on 20% of trials maximized motor recovery.
The results elucidate mechanisms that may underlie suboptimal cortical organization after injury,

and they provide rationale and guidance for using targeted neuroplasticity interventions to improve functional recovery.

Disclosures: S.L. Norman: None. **D.J. Reinkensmeyer:** None.

Poster

148. Brain-Machine Interface: Neurophysiology: Non-Invasive Techniques: Ultrasound and Other

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 148.10/MM13

Topic: E.05. Brain-Machine Interface

Support: H2020 SME Phase II ComaWare

Title: Vibro-tactile brain-computer interface tools in patients with unresponsive wakefulness syndrome

Authors: *C. GUGER[1], R. SPATARO[2], G. EDLINGER[3]
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Abstract: Persons diagnosed with disorders of consciousness (DOC) might suffer from motor disablities, and thus assessing their spared cognitive abilities can be difficult. Recent research from several groups has shown that non-invasive brain-computer interface (BCI) technology can provide assessments of these patients' cognitive function that can supplement information provided through conventional behavioral assessment methods. In rare cases, BCIs may provide a binary communication mechanism. Here, we present results from a vibrotactile BCI assessment aiming at detecting command-following and communication in 12 unresponsive wakefulness syndrome (UWS) patients. Two different paradigms were administered at least once for every patient: (i) VT2 with two vibro-tactile stimulators fixed on the patient's left and right wrists and (ii) VT3 with three vibro-tactile stimulators fixed on both wrists and on the back. The patients were instructed to mentally count either the stimuli on the left or right wrist, which elicits a robust P300 for the target wrist only. The EEG data around each stimulus was extracted and subdivided into 8 averages. This data was classified with linear discriminant analysis and used to calibrate a brain-computer interface to test YES/NO communication abilities. The grand average VT2 accuracy was 38.3 % and the VT3 accuracy 26.3 %. Two patients achieved a VT3 accuracy ≥80% and went through communication testing (one answered 4 out of 5 questions correctly in session 1, whereas the other could answer 6/10 and 7/10 questions correctly in sessions 2 and 4). In 6 other patients, the VT2 or VT3 accuracy was above the significance threshold of 23% for at least one run, while in 4 patients the accuracy was always below this threshold. The study highlights the importance of repeating EEG assessments to increase the chance of detecting

command-following in patients with severe brain injury. Furthermore, the study shows that BCI technology can be useful to test command following in chronic UWS patients and can allow patients to answer YES/NO questions. Beside UWS patients, the principle can be used in locked-in/completely locked in and minimal consciousness patients for assessment and communication.

Disclosures: C. Guger: A. Employment/Salary (full or part-time):; g.tec neurotechnology GmbH. **R. Spataro:** None. **G. Edlinger:** A. Employment/Salary (full or part-time):; g.tec Guger Technologies OG.

Poster

148. Brain-Machine Interface: Neurophysiology: Non-Invasive Techniques: Ultrasound and Other

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 148.11/MM14

Topic: E.05. Brain-Machine Interface

Support: Grant DPI2014-58431-C4-2-R, funded by the Spanish Ministry of Economy and Competitiveness and by the European Union through the European Regional Development Fund (ERDF), "A way to build Europe".

Title: Motor imagery tasks coherence while controlling lower limb exoskeleton through real time BMI

Authors: *J. M. AZORIN, M. RODRÍGUEZ-UGARTE, E. IÁÑEZ, M. ORTIZ
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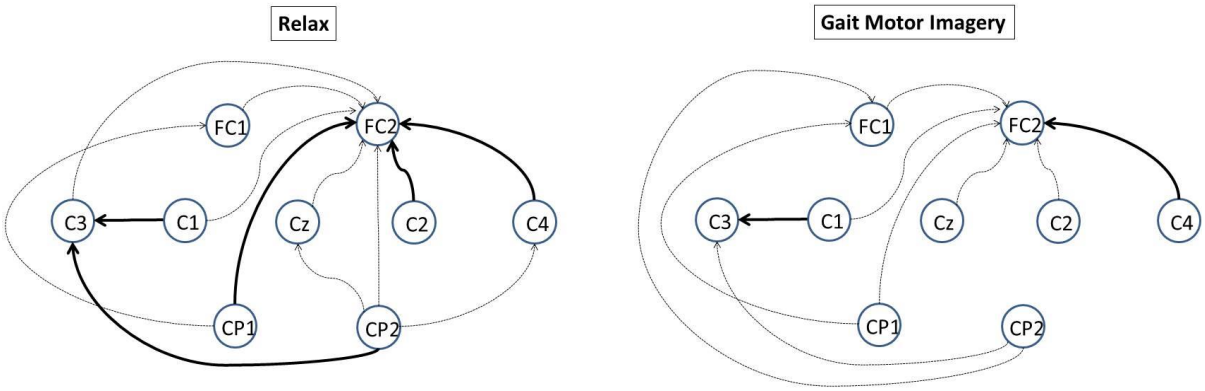
Abstract: Coherence measures the interaction between different cortical structures for analyzing brain anatomical connections and information exchange. This works studies the coherence of 2 motor imagery tasks, relax and gait motor imagery, while 2 subjects are controlling a lower limb exoskeleton through a real time brain-machine interface (BMI) based on electroencephalographic (EEG) signals.
The experimental protocol consisted on 80 trials composed by: 2 Relax periods of 8 s each, separated by 1 gait motor imagery (GMI) period of 14 s, where subjects had to imagine they were walking. An auditory cue indicated transitions. First 40 trials trained a support vector machine (SVM) classifier with kernel radial basis function and the latter 40 tested it.
Data analysis of the 30 channels recorded was in epochs of 1 s. each 0.2 s. Each epoch was preprocessed with: Notch (50 Hz), BPF (0.05-45 Hz) and a Laplacian filter. Then, the feature vector was computed as the power at the optimal frequency of 9 electrodes associated with MI tasks: Cz, CP1, CP2, C1, C2, C3, C4, FC1 and FC2. Training trials determined the optimal frequency and it corresponded to the normalized frequency that represents the maximum difference between the 2 cognitive states. The exoskeleton moved by itself during GMI train

periods to provide a realistic feeling. However, users' EEG signals activated the lower limb exoskeleton during GMI test periods.
Partial directed coherence (PDC) was calculated as in Baccalá and Sameshima (1996) for the test trials. The PDC was computed as the mean of each PDC frequency belonging to mu band (8-12 Hz and resolution of 1 Hz).
Figure 1 shows the PDC graphs for relax and GMI periods. During relax periods there are a strong interaction between sensorymotor area to motor and premotor area (PDC ≥ 0.4, represented by thick lines); while in imagine periods this interaction drops(0.3<PDC<0.4, thin lines).



Disclosures: J.M. Azorin: None. **M. Rodríguez-Ugarte:** None. **E. Iáñez:** None. **M. Ortiz:** None.

Poster

148. Brain-Machine Interface: Neurophysiology: Non-Invasive Techniques: Ultrasound and Other

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 148.12/NN1

Topic: E.05. Brain-Machine Interface

Support: FAPEMIG Grant APQ-00476-14

Title: Transcranial Direct Current Stimulation (tDCS): Molecular and behavioral evoked alterations

Authors: *E. S. NICOLAU, H. TENZA-FERRER[1], F. D. REZENDE[1], N. F. NICOLAU[1], M. F. BARROS[1], K. A. F. ALVARENGA[1], L. A. V. MAGNO[1], M. A. ROMANO-SILVA[2]
[2]Mental Hlth., [1]Univ. Federal De Minas Gerais (FM), Belo Horizonte, Brazil

Abstract: Transcranial Direct Current Stimulation (tDCS) is a non-invasive, low-cost, therapeutic technique, that focuses on neuronal modulation through the use of low-intensity (0,10 - 2,0 mA) continuous currents. tDCS has been proposed in clinical studies with the attempt of offering an alternative or complementary treatment for several neurological and neuropsychiatric disorders, such as epilepsy, bipolar disorder, stroke, major depression, Alzheimer's disease and Parkinson's disease. Despite growing interest and its use in clinical trials, detailed cellular and molecular evoked alterations, short and long-lasting effects as well as side effects are yet to be deeply investigated. Therefore, this study aims at investigating tDCS' gene expression and behavioral (learning and memory) mechanisms in an animal tDCS model. A total of 4 groups were studied (Groups: 5/1, 5/5, 10/1 and Task Paired-Barnes Maze 5/1 - meaning ex: 5/1 Five days of stimulation, collecting the tissue 24h after the last stimulation), comprising of treatment (tDCS) and control (Sham). Each group underwent differential chronic stimulation, varying mainly on days of treatment, time of tissue (Brain Hippocampus and Cortex) extraction after stimulation for gene expression analysis and paired task stimulation. All groups received anodal stimulation at 0,35 mA for 10 min. Among eight genes analyzed, we found that tDCS evoked significantly higher levels of *BDNF* (brain derived neurotrophic factor) and *GFAP* (glial fibrillary acidic protein) gene expression in the tDCS stimulated 5/1 group, but no differences were found in the 5/5 and 10/1 groups. We also assessed total cortical glutamate levels through a redox system. There were no significant differences in levels for 5/1, 5/5 and 10/1 related to control groups. Task paired tDCS animals presented no significant differences in *GFAP* gene expression, while BDNF levels were considerably higher in the tDCS group, no statistical significance was observed compared to the control group. Moreover, an enhanced performance (latency, errors and distance to execute task as well as adopted strategy) in the barnes maze task stimulated (tDCS) group was observed compared to task control (Sham) group. The task paired tDCS group presented no difference in glutamate quantification related to control, but in general presented higher levels compared to the 5/1, 5/5 and 10/1 groups. In conclusion, tDCS is capable of evoking gene expression alterations and enhance learning and memory performance in a treatment dependent manner, which, furthermore, may help in the understanding of treatment protocol selection and in the understanding of tDCS treatable diseases.

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Poster

148. Brain-Machine Interface: Neurophysiology: Non-Invasive Techniques: Ultrasound and Other

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 148.13/NN2

Topic: E.05. Brain-Machine Interface

Support: NSF IIS 1219200
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FISP G2171
FISP G3155

Title: Investigating feedback-related brain activity in electroencephalography

Authors: *M. MOUSAVI, V. R. DE SA
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Abstract: Electroencephalography (EEG) is one popular means of collecting brain signals due to its high temporal resolution and non-invasiveness. EEG-based neurofeedback (NFB) and brain-computer interface (BCI) systems interpret neural signals collected from EEG and translate them into meaningful signals for the user. One challenge in these systems is the non-stationarity of the brain signals and one major source of such non-stationarity is the effect of feedback provided by the system itself. In recent work, we studied the feedback-related brain activity in a motor imagery task [Mousavi et al., 2017]. In that study, participants participated in a task where they were imagining movement of their right/left hand to move a cursor on the monitor in front of them to the right/left. The goal of each trial was for the cursor to hit the target on the monitor. We trained filter-bank Common Spatial Patterns (CSP) [Ramoser et al., 2000] followed by a linear discriminant analysis (LDA) classifier to detect whether each participant was satisfied/dissatisfied with the last cursor movement - i.e., if the cursor moved towards/away from the target respectively. This was called a Good/Bad (G/B) classifier. We showed classification results well above chance for most of the participants in various frequency bands ranging from delta to beta and in some low gamma.
In the current study, we further investigate the underlying information used by these classifiers. To do so, we trained similar filter-bank CSP+LDA G/B classifiers on the last cursor movement, but this time, conditioned on the previous cursor movement being a change in direction or a continued movement. The classifiers were trained separately for each condition (continued or change in direction), trials were balanced among classes, and results are reported with 10-fold cross-validation. Our preliminary results show that within the frequency range in which we found G/B classifiable above chance, a G/B classifier following a change in direction generally tends to rely more on information in lower frequency components and a G/B classifier following a continued movement seems to rely more on higher frequency components. These are preliminary results of ongoing work; however, we believe that this study has the potential to shed light on the unknown aspects of the feedback-related brain activity in EEG-based NFB and BCI systems.

Disclosures: M. Mousavi: None. **V.R. de Sa:** None.

Poster

149. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 149.01/NN3

Topic: E.06. Posture and Gait

Title: Stabilization of the mediolateral trajectory of the swing foot during a wide range of gait speeds

Authors: *T. FUJINO[1,4], N. KANEMURA[2], K. HIRATA[5], H. HANAWA[6], K. TAKAYANAGI[3]
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Abstract: Bipedal gait is passively stabilized in the sagittal plane; however, active control is necessary to ensure stability in the frontal plane. The result of this control appears as the trajectory of mediolateral foot placement, and it is expressed as step width at the time of the double support phase. In previous studies, the kinematic synergy to stabilize the mediolateral trajectory of a swing foot was clear; however, the kinematic synergy under a wide range of gait speed conditions was not clear. This study was performed to clarify the kinematic synergy of the swing foot at different gait speeds. Ten healthy young volunteers (five males and five females) participated in this study. The preferred gait speed was defined as 100%, and five gait speed conditions of 60%, 80%, 100%, 120%, and 140% were randomized for each subject. From marker data, the segmental angles of the geometric model were calculated. The model was used to derive an analytic expression for the mediolateral trajectory of the ankle joint center of the swing limb. The model consisted of four segments: stance limb, pelvis, swing-limb thigh, and swing-limb shank. To extract kinematic synergy, an uncontrolled manifold (UCM) hypothesis was used to analyze important performance variables. Swing-foot mediolateral trajectories were used as performance variables in the UCM analysis. The seven lower-limb and pelvis angles were set to elemental variables; "good variance" (V_{UCM}), which does not affect performance variables, and "bad variance" (V_{ORT}), which affects task performance variables, were calculated. ΔV_z, which is an index of synergy, was obtained from V_{UCM} and V_{ORT}. V_{UCM} at 140% gait speed was significantly lower than those for the 60%, 80%, and 100% gait speed conditions, whereas V_{ORT} showed no significant differences between the conditions. The total variance(V_{TOT}) was significantly lower at the 140% gait speed condition. Also, ΔV_z did not produce any significant

differences between the conditions. This suggests that the kinematic synergy that stabilizes the mediolateral foot trajectory in the swing foot is similar irrespective of the gait speed. However, in the fast gait speed condition (140%), the dispersion in the UCM direction tended to be lower. Because the angular velocity in the sagittal plane of each joint increases at a fast gait speed, the segment posture in the mediolateral direction is not easily disturbed. This result suggests the possibility of maintaining mechanical stability at a fast speed when the synergy of the mediolateral direction is reduced.

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Poster

149. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 149.03/NN5

Topic: E.06. Posture and Gait

Support: JSPS Grant 17H00874

Title: Sensorimotor adaptation to alteration in postural dynamics induced by a novel electrical muscle stimulation system

Authors: *A. A. AZAT[1], S. HAGIO[2], D. NOZAKI[1]
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Abstract: The human postural control system translates sensory information into appropriate motor command to maintain the balance (Lockhart & Ting 2007). As compared to the understanding of reaching movement control, it has not been well investigated how the postural control system possesses flexibility to the change in the body dynamics. Here, we propose a new experimental paradigm to artificially alter the body dynamics using the electrical stimulation (ES). This system enabled to impose stiffness and/or viscosity to the body dynamics by modulating the ES to the tibialis anterior muscle (TA) with the position and velocity of the center of body mass (COM).
Five participants were asked to stand on a force platform with their eyes closed and maintain the COM position to a reference position throughout the experiment. The COM position was approximately obtained from a movement of the lumbar part using a laser displacement sensor (LK500, Keyence, Japan). The experiment consisted of 1 min baseline, 7 min stimulation and 7 min washout phases. During the stimulation phase, the ES was applied to TA (Biphasic asymmetric pulse with 1 ms width, 20 Hz) and the intensity was modulated so that the ankle dorsiflexion torque was linearly increased with the forward COM shift from the reference

position (the relationship between the ES intensity and dorsiflexion torque was obtained for each participant beforehand). Thus, this setting required the postural control system to generate stronger plantarflexion torque to a certain amount of forward COM shift.
We found that the exposure to this ES intervention changed the COM fluctuation pattern during the following washout phase in which the ES was turned off. The magnitude of COM velocity was significantly increased, indicating that the postural control system was likely to modify the control parameters of how they translated the sensory information (i.e., forward COM shift) into motor command to generate ankle joint torque.

Disclosures: A.A. Azat: None. **S. Hagio:** None. **D. nozaki:** None.

Poster

149. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 149.04/NN6

Topic: E.06. Posture and Gait

Support: Michael J. Fox Foundation (9605)
NINDS Grant 5 R21 NS096398-02
Robert and Ruth Halperin Foundation
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Helen M. Cahill Award for Research in Parkinson's Disease
Stanford Bio-X Bowes Graduate Student Fellowship

Title: Detecting neural and kinematic features of different forward walking tasks in Parkinson's disease

Authors: *J. O'DAY[1], C. ANIDI[2], J. SYRKIN-NIKOLAU[3], R. W. ANDERSON[6], M. AFZAL[2], A. VELISAR[4], H. BRONTE-STEWART[5]
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Abstract: Objective: The neural mechanisms of freezing of gait (FOG) in Parkinson’s disease (PD) are unknown due to the difficulty of recording neural signals in freely moving people in environments that elicit FOG. We successfully elicited FOG during novel gait tasks, from which we report neural and kinematic features of PD freezers; we used these to model optimal features to objectively classify FOG.
Methods: Beta band fluctuations (beta bursts) from synchronized subthalamic (STN) local field

potentials (LFPs) and gait kinematics were recorded in 12 PD (6 Freezers) subjects, off medication, off deep brain stimulation, who performed forward walking (FW), walking in ellipses and figures of eight through narrow openings, in a turning and barrier course (TBC). STN LFPs were recorded bilaterally from electrodes 0-2 or 1-3 of the left and right STN DBS leads (model 3389, Medtronic, Inc.) to an investigational sensing neurostimulator (Activa © PC + S, Medtronic Inc., IDE-, IRB-approved). Patients wore inertial measurement units (IMUs) on each shank, foot, lumbar (L5) and chest. The shank IMU was used to calculate peak shank angular velocity and stride time over each gait cycle for each leg. A neurologist (HBS) labelled freezing behavior by video. We input gait parameters to a binary logistic regression model to classify FOG and conducted a leave-one-out cross validation.
Results: The average area under the receiver operator curve (AUC) was calculated to evaluate models using (A) peak shank angular velocity and (B) stride time. The AUC was highest (0.746) for the model that included A + A from the previous step. The model with A only (0.727) was better than A + B (0.725). The TBC elicited the most pathological neural and kinematic features of gait impairment in Freezers (F): beta burst durations were longest and shank angular velocity slowest during figures eights, then ellipses, then FW ($p < 0.05$). Gait was more arrhythmic for F and non-freezers (NF) during TBC than FW ($p < 0.05$). F and NF had slower shank angular velocity during ellipses than FW ($p = 0.008$, $p < 0.001$), and in figure eights compared to ellipses ($p = 0.035$, $p < 0.001$). F were more arrhythmic and asymmetric in FW ($p < 0.05$) and more arrhythmic in figure eights ($p = 0.009$) than NF.
Conclusions: We demonstrate that kinematic data from TBC can be used to create a FOG classifier. The novel TBC elicited more pathological neural and kinematic features of gait impairment and FOG than FW, namely longer STN beta burst durations and slower shank angular velocity in F. Within the TBC walking in figure eights exhibited longer beta burst durations and freezing behavior than ellipses.

Disclosures: J. O'Day: None. **C. Anidi:** None. **J. Syrkin-Nikolau:** None. **R.W. Anderson:** None. **M. Afzal:** None. **A. Velisar:** None. **H. Bronte-Stewart:** None.

Poster

149. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 149.05/NN7

Topic: E.06. Posture and Gait

Support: Israel Ministry of Science and Technology Grant #3-12072

Title: Assessing recovery time from unexpected loss of balance during walking in young adults

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Abstract: Introduction: Behavioral evidence from dual tasking (DT) gait studies has demonstrated that higher functions are involved in locomotion. An exploratory study was performed to test the **hypothesis** that the recovery time (RT) from unexpected loss of balance (i.e., perturbation) while performing DT will be longer than without a concurrent cognitive task (single task - ST).
Methods: 12 young healthy adults (five females, average age 26.8±3.4) participated in the study. The participants were fully immersed in a virtual reality system (CAREN High End, Motek Medical, The Netherlands). They were exposed to unexpected perturbations while walking on a level treadmill (self-paced mode) synchronized by a motion capture system (Vicon, Oxford, UK). Unexpected perturbations were introduced under DT and ST conditions and varied in direction (backward (AP), right and left (ML)) in a random order.
Balance RT was calculated using a customized algorithm that detected the time point (after perturbation presentation) in which gait parameters (step width & length, calculated based on the Vicon data) returned to steady state (based on statistical moments).
Results: No significant differences (Wilcoxon signed rank test, $p \geq 0.091$) were found when comparing RT between perturbation types (AP vs. ML), condition (DT vs. ST) and the type of stepping strategy responses (i.e., cross over- vs. side - stepping; see table 1). Longer RT were found for restoring regular step length (median= 6.67 seconds) as compared to step width (median = 5.77 seconds; p=0.05).
Conclusions: An algorithm that determined RT, which is based on statistical moments, is efficient and provide comparable results to subjective assessments (data not shown). The results refute the hypothesis regarding significant differences in balance RT between ST and DT conditions, probably due to relative low perturbations magnitude for this cohort. Further, no differences were found in terms of RT related to perturbation types (AP vs. ML) and balance recovery strategies (cross over stepping vs. side stepping). The data suggest that recovery of regular step width precedes the recovery of step length.
Table 1: Group median recovery time from unexpected loss of balance based on step width and step length.

	ST †	DT†	ML‡	AP‡	Cross over step₸	Side step₸
RT-SW	5.53(3.63-15.46)	6.05(4.21-11.64)	5.46(3.63-8.54)	6.95(4-11.42)	5.40(3.64-12.35)	5.25(3.43-8.53)
RT-SL	7.13(4.22-16.87)	6.35(4.63-16.73)	5.957(4.22-16.30)	7.26(4.45-13.65)	7.07(4.52-16.78)	6.59(4.10-12.30)

No significant differences in recovery time from unexpected loss of balance were found between ST-DT conditions, ML-AP perturbation types and Cross over-side stepping strategies;

† For this comparison data were pulled across all perturbation types; ‡ For this comparison data were pulled across all conditions; ₮ Only for ML perturbations pulled from both conditions. Abbreviations: ST – Single task; DT – Dual task; ML – Medio-Lateral; AP – Anterior-Posterior; RT – Recovery time; SW – Step width; SL – Step length.

Disclosures: U.D. Rosenblum: None. **I. Melzer:** None. **L. Kribus-Shmiel:** None. **Y. Bahat:** None. **G. Zeilig:** None. **M. Plotnik:** None.

Poster

149. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 149.06/NN8

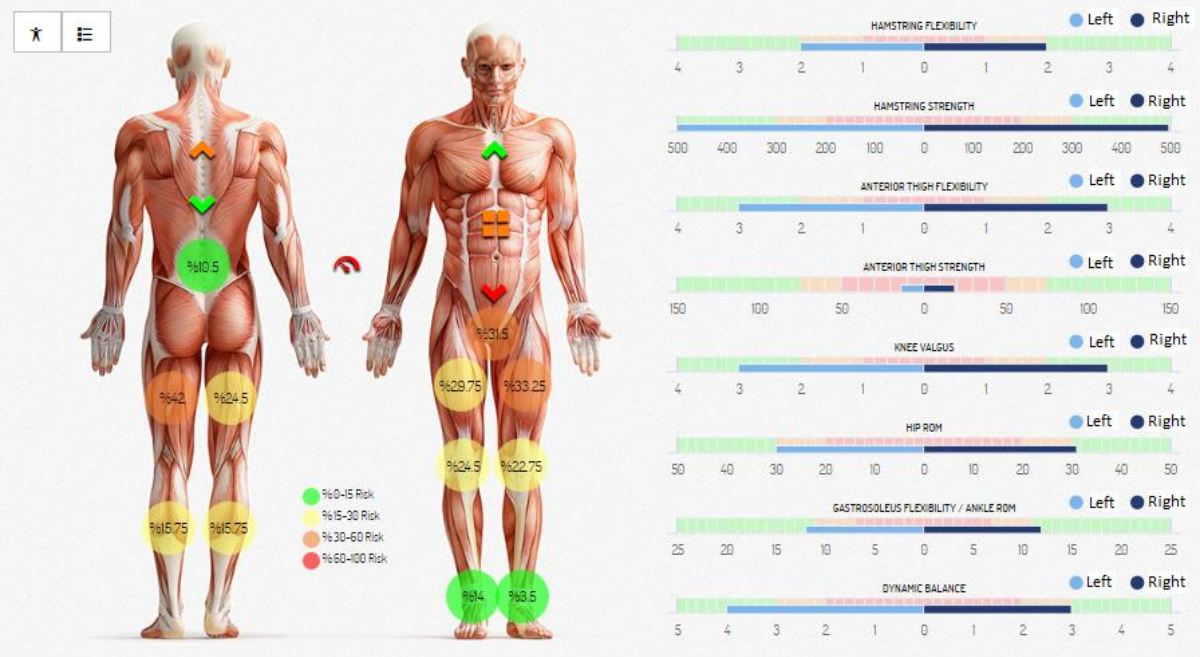
Topic: E.06. Posture and Gait

Title: A novel algorithm for individual injury risk mapping with preselected background assessment factors may avoid non-contact injuries with personalized training program

Authors: *A. M. OZMEN[1], A. ISIK[2], B. YALCIN[1], M. B. KILICOGLU[1], H. ARGUNSAH BAYRAM[1], M. B. BAYRAM[1]
[1]Med. Engin., Acibadem Mehmet Ali Aydinlar Univ., Istanbul, Turkey; [2]Acibadem Sports, Istanbul, Turkey

Abstract: INTRODUCTION: The Functional Movement Screen (FMS™) has gained popularity in recent years. However, most of the research on stress and athletic injury has been conducted on a narrow scope, without the benefit of an adequate framework to explain the relationships between training pattern and injury. The purpose of this research is to create an innovative, simple and accurate test for prediction of mobility, stability, and flexibility. By using these metrics create an injury profiling risk map for specific segments of the body and develop individual specific training programs.
METHODS: 80 (12 female) Division 1 athletes (age=27.0±4.5 years, height=186.4±6.1 cm, body mass=85.3±12.0 kg; mean±SD) from 4 different professions (soccer, basketball, volleyball, handball) participated. Two set of test batteries (Injury Risk profiling and Fitness Profiling) were conducted in a professional environment. Based on the parameters of each test a final prediction of injury rate was founded for 7 different segments of the body: sacrum, hamstrings, calves, pelvis, quadriceps, patellae and tali. 5 different factors besides the tests were used for estimating the injury profile: premorbidity, age, kinanthropometry, lower extremity dimensions, oral health. The final injury prediction was evaluated as percentage scores and named “Grade 1” (0-15%), “Grade 2” (15-30%), “Grade 3” (30-60%) and “Critical” (60-90%).
RESULTS: The results showed that %51.3 of the athletes who had attended for injury risk profiling had non-contact injuries (41/80 athletes). The injury occurrences in parts of the body

were estimated true positively with 75.6% for Grade 2,3 and Critical combined (31/41 athletes) whereas for Grade 3 and Critical, the estimation reliability was %51.2 (21/41 athletes). CONCLUSION: Preliminary data showed that our set of test batteries and the underlying risk mapping algorithm could predict the injury risk and its location significantly. This marker could be used to lower the injury risk by personalized and focused training.



Disclosures: A.M. Ozmen: None. **A. Isik:** None. **B. Yalcin:** None. **M.B. Kilicoglu:** None. **H. Argunsah Bayram:** None. **M.B. Bayram:** None.

Poster

149. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 149.07/NN9

Topic: E.06. Posture and Gait

Title: Correlation of hand-eye coordination and successful free shot rate of elite basketball players

Authors: *M. B. KILICOGLU[1], A. ISIK[2], B. YALCIN[1], A. M. OZMEN[1], M. B. BAYRAM[1], H. ARGUNSAH BAYRAM[1]
[1]Med. Engin., Acibadem Mehmet Ali Aydinlar Univ., Istanbul, Turkey; [2]Acibadem Sports, Istanbul, Turkey

Abstract: Objective: The goal of this study was to investigate the correlation between the hand-eye coordination and successful free throws in elite basketball players by using eye tracking and wearable motion capture (WMC) systems. This study hypothesizes that different skill levels of the players will result in versatile movement pattern in the upper extremity kinematics and center of mass profiles; and there will be a strong correlation between the success rate and hand-body coordination of the players. **Methods:** 10 male elite basketball players were chosen with the exclusion of the ones that had prior surgical history and have recent injury which has effect on their routine performance. Players were informed about the study protocol and signed the informed consent form that has been approved by the Institutional Review Board. The kinematic data was collected with Xsens MVN (Xsens Technologies BV ® (Netherlands)), which allows an unrestricted, 3D and spontaneous gait analysis. Real time pupil movements of the players were monitored wirelessly with Tobii Pro (Tobii AB (Sweden)) Glasses 2. WMC sensors were placed on player's body as described by the MVC manufacturer. After calibrating the eye tracking and WMC systems, ten successful and ten unsuccessful free throw data was collected from each player. **Results:** Preliminary data has been collected using Tobii Pro Glasses 2 from an amateur basketball player, who had no previous injury and/or surgery history. According to our preliminary results strong correlation was found between the subject's hand-eye correlation and free-throw success rate ($r=0.97$, $P<0.001$). **Conclusions:** Based on the preliminary data, we have found that the hand-eye coordination is critical for increasing the success rate of free shots. Poor hand-eye coordination results in weak body positioning, upper extremity control and a rapid elevation of center of mass.

Disclosures: M.B. Kilicoglu: None. **A. Isik:** None. **B. Yalcin:** None. **A.M. Ozmen:** None. **M.B. Bayram:** None. **H. Argunsah Bayram:** None.

Poster

149. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 149.08/NN10

Topic: E.06. Posture and Gait

Title: Correlation of hand-eye coordination and successful free shot rate of elite basketball players

Authors: *H. ARGUNSAH BAYRAM[1], A. ISIK[3], B. YALCIN[2], A. M. OZMEN[2], M. B. BAYRAM[4]
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Abstract: The goal of this study was to investigate the correlation between the hand-eye coordination and successful free throws in elite basketball players by using eye tracking and wearable motion capture (WMC) systems.
10 male elite basketball players were chosen with the exclusion of the ones that had prior surgical history and have recent injury which has effect on their routine performance. Players were informed about the study protocol and signed the informed consent form that has been approved by the Institutional Review Board. The kinematic data was collected with XSENS MVN (Xsens Technologies BV ® (Netherlands)), which allows an unrestricted, 3D and spontaneous gait analysis. Real time pupil movements of the players were monitored wirelessly with Tobii Pro (Tobii AB (Sweden)) Glasses 2.
10 members of elite basketball team, who did not have prior surgery history were participated in the study. A major exclusion criterion was having surgery that put the player to the bench more than one game. WMC sensors were placed on player's body as described by the MVC manufacturer. After calibrating the eye tracking and WMC systems, ten successful and ten unsuccessful free throw data was collected from each player.
This study hypothesized that different skill levels of the players will result in versatile movement pattern in the upper extremity kinematics and center of mass profiles; and there will be a strong correlation between the success rate and hand-body coordination of the players.
Preliminary data has been collected using Tobii Pro Glasses 2 from an amateur basketball player, who had no previous injury and/or surgery history. Based on this data, we found that the hand-eye coordination of the subject was poor, which result in weak body positioning and upper extremity control. Rather than using wrist and hand effectively and energy efficiently, the subject tried to match the planned ball trajectory by jumping and causing a rapid elevation in center of mass.

Disclosures: H. Argunsah Bayram: None. **A. Isik:** None. **B. Yalcin:** None. **A.M. Ozmen:** None. **M.B. Bayram:** None.

Poster

149. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 149.09/NN11

Topic: E.06. Posture and Gait

Title: Real time balance feedback mechanism during orthopedic and neuromuscular disease rehabilitation

Authors: *B. YALCIN[1], M. B. KILICOGLU[1], C. KURTOGLU[2], A. M. OZMEN[1], H. ARGUNSAH BAYRAM[1]
[1]Med. Engin., [2]Biodesign Ctr., Acibadem Mehmet Ali Aydinlar Univ., Istanbul, Turkey

Abstract: Introduction: Biological feedback, which favors motor control in static and dynamic tasks via augmenting motor information for improving balance and performance, has been used for many years to assist patients and clinicians during rehabilitation. Biological feedback mechanisms provide continuous real time physiological information to the participant and facilitate self-regulation; as a result expedite the repetitive capacity of the activity. The success of the rehabilitation program depends on its patient- specific design, the ability to keep the patient's performance at the optimal level during therapy, the ability of the patient to adapt to treatment and the team's ability to monitor the physical development of the patient instantly and accurately. Therefore, it is very important to support conventional orthopedic rehabilitation practices with innovative engineering applications.
Methods: We developed a tactile and visual biological feedback mechanism intended to be used during the orthopedic and neuromuscular disease rehabilitation. Four vibration motors -for front-back-left-right directions (corresponding two motors run when patient leans on combination of two directions), were located between the patient`s under bust and natural waist line with an adjustable strap designed with the loop side of the Velcro material. Accurate positioning of the motors for each patient were obtained by using ergonomic 3D printed cases (using stereolithography) attached with the hook side of the Velcro. Patient`s real-time balance was monitored with Motion Processor Unit (MPU) 9250 (9-axis sensor with 3-axis accelerometer, 3-axis gyroscope and 3-axis magnetometer), which was placed on patient’s sternum. All motors and the MPU were controlled by Raspberry Pi.
Results: Real-time balance feedback mechanism was validated with Xsens MVN (Xsens Technologies BV® (Netherlands)) on 2 subjects and a strong correlation was found between the subject’s center of mass profile monitored with the balance sensor and Xsens MVN analysis ($r=0.92$, $P<0.001$).
Conclusions: Lack of postural control and balance has the greatest impact on patients’ mobilization and independent performance during the activities of daily living. Although an individual’s "static" balance is acceptable while standing still or performing a single task at a time; “dynamic” balance problems may become apparent during mobilization or while doing multiple tasks at a time. Therefore, being aware of the real-time dynamic balance of the patient is critical for both the patient and the physical therapist.

Disclosures: B. Yalcin: None. **M.B. Kilicoglu:** None. **C. Kurtoglu:** None. **A.M. Ozmen:** None. **H. Argunsah Bayram:** None.

Poster

149. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 149.10/NN12

Topic: E.06. Posture and Gait

Support: T32 AG052375 from the National Institute on Aging

Title: Self-paced omnidirectional locomotion in virtual reality using a split-belt treadmill

Authors: *M. T. BOOTS[1], S. YAKOVENKO[2]
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Abstract: Trauma to the nervous and/or musculoskeletal systems can result in asymmetric gait that may lead to maladaptation with further long-term health consequences. The locomotor training on a regular or split-belt treadmill is the common gait rehabilitation approach. However, the problems with treadmill locomotion associated with an incongruent visual flow and an imposed speed have been implicated in impeding the generalization of skills to over-ground locomotion. Our goal was to overcome these problems using virtual reality (VR) with an integrated self-paced treadmill to allow changes in heading direction. The self-paced algorithm tracks foot contact locations and timing to calculate the leg speed as the distance traveled over the last step (measured between step onsets) divided by the step cycle duration. An additional correction to the middle was added to the belt speed control and not to the visual flow control in VR. This allowed the user to adjust the belt speeds while remaining in the middle of the treadmill without the disruption of flow in VR. The foot contact spatial and temporal information was calculated from ground reaction forces during the threshold crossing events in vertical forces on each belt (50 N or 18% body weight). The leg speeds controlled the body velocity and heading direction in the VR environment. The heading direction (gamma) was calculated as gamma=atan(Tc*(VL-VR)/W), where Tc - cycle duration, V - leg velocities, and W - stride width. Healthy volunteers performed 3 locomotor tasks to test the ability to walk symmetrically with the decoupled treadmill belts (task1) and to recover from asymmetric gait perturbation without (task 2) and with (task 3) congruent visual flow in VR. Overall, we found that subjects can walk symmetrically or at requested asymmetric belt speeds (RMS<10% normalized to speed). The convergence on the desired speed was 4 times faster with the visual flow in VR than without it (about 6s and 25s, respectively). We demonstrated a novel algorithm for the control of omnidirectional locomotion using the leg speed differential as a turning command. This supports our previous observations that heading direction may be represented by the leg speed differential.

Disclosures: M.T. Boots: None. **S. Yakovenko:** None.

Poster

149. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 149.11/NN13

Topic: E.06. Posture and Gait

Title: Patterns of acceleration during walking are altered in adults with autism spectrum disorder

Authors: *S. MORRISON[1], C. N. ARMITANO[1], H. J. BENNETT[2], J. A. HAEGELE[2]
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Abstract: Individuals with autism spectrum disorder (ASD) can exhibit a range of movement issues, which are often characterized by a slowing of movement responses including declines in reaction time, finger tapping speed and walking speed. While this general pattern of slowing reveals something of the global impact this neurodevelopmental disorder has on motor function, less is known about any residual differences in how a person with ASD performs a given movement. For walking in particular, stabilization of the head is critical for ensuring optimal visual and vestibular function. For healthy persons, one function of the trunk and neck is to attenuate gait-related oscillations in order to ensure head stability. However, it is unclear whether adults with ASD exhibit a similar pattern of responses during walking. This study was designed to examine the pattern of acceleration for the trunk, neck and head during walking for a cohort of adults diagnosed with ASD compared to neurotypical controls. Twenty young adults with ASD and 20 age-matched neurotypical adults participated in this study. Participants performed five trials across a 20ft Protokinetics pressure sensitive surface while walking at their preferred walking speed. Accelerations were collected using three triaxial accelerometers affixed to the head, neck, and lower trunk. Comparisons of amplitude (i.e., RMS) and signal regularity (i.e., ApEn) of the gait-related acceleration data were performed. Results revealed that the adults with ASD walked slower than the neurotypical controls. Further, even when walking at a slower speed, the acceleration pattern about the trunk, neck and head segments for the adults with ASD was greater in amplitude and more irregular (i.e. higher ApEn) compared to the controls. These results indicate that persons with ASD demonstrated a reduced ability to dampen gait-related accelerations, even when walking at a slower speed. Overall, these findings suggest that adults with ASD exhibited an inability to accommodate and control dampen those oscillations related to walking. As declines in gait speed are often linked with loss of head control, one suggestion is that the inability to appropriately compensate for gait-related oscillations may, in part, explain why persons with ASD walk slower.

Disclosures: S. Morrison: None. **C.N. Armitano:** None. **H.J. Bennett:** None. **J.A. Haegele:** None.

Poster

149. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 149.12/NN14

Topic: E.06. Posture and Gait

Support: ERC Grant ERC-2014-CoG

Title: Use of EMG and accelerometry to assess changes in gait during 10m Walk following a high intensity exercise therapy in Parkinson's disease

Authors: *B. P. O'CALLAGHAN, M. FLOOD, P. DIAMOND, M. M. LOWERY
Univ. Col. Dublin, Dublin, Ireland

Abstract: BACKGROUND AND AIM:High intensity exercise interventions that focus on increasing the amplitude of movements in bradykinetic and dyskinetic Parkinson’s patients have shown clinical improvements in UPDRS and motor scores with respect to other exercise therapies. LSVT BIG, a derivative of LSVT LOUD, is one such therapy that consists of 16 one hour sessions over a four week period. To determine factors that influence the changes in clinical and motor scores following LSVT BIG, surface EMG and accelerometer data were recorded from a group of volunteers participating in the LSVT BIG therapy program during a number of clinical tests including a 10m walk at a self-selected fast pace. METHODS: EMG and tri-axial accelerometer data were recorded using wireless surface electrodes (Trigno® Delsys Inc.) from seven older adults with Parkinson's disease (age = 74.28 yrs ± 6.89) and seven healthy age matched volunteers (age = 68.71 yrs ± 3.15). Sensors were placed on the surface of the skin over the rectus femoris, vastus medialis, vastus lateralis, tibialis anterior, gastrocnemius lateralis, biceps femoris and semitendinosus muscles using SENIAM guidelines. An accelerometer was placed over the fifth lumbar vertebrae (L5). Data were recorded for the healthy control group during a single recording session, while data for the PD group were recorded at 2 weeks pre-therapy, once per week during therapy and at 2, 5 and 13 weeks post therapy. Accelerometer data were used to determine timings of heel strike and toe off from which stance, swing and stride times were calculated. Intermuscular coherence between quadriceps muscle pairs was also calculated during the stance phase of gait. Wilcoxon-Mann-Whitney tests were conducted to quantify whether the changes in stance, swing or stride times or asymmetry in those measures were significant between the healthy control group and the PD group. Likewise, a Wilcoxon signed rank test was used to determine the significance of the changes between the pre and post therapy condition in the PD group. RESULTS: Mean stance times for the left leg pre-therapy were correlated with UPDRS scores (rho = 0.706, $p < 0.05$). Significant decreases in the stance (10.3%), swing (14.4%) and stride (12.9%) times for both left and right legs were observed in the

PD group ($p<0.05$). There was also a significant decrease in the asymmetry of swing time for the PD group post therapy ($p<0.05$). No changes in EMG coherence were observed in any of the frequency bands examined. CONCLUSIONS: Significant improvements in gait measures pre and post therapy were observed using accelerometer data. No changes were observed in EMG coherence, however further analysis is necessary.

Disclosures: B.P. O'Callaghan: None. **M. Flood:** None. **P. Diamond:** None. **M.M. Lowery:** None.

Poster

149. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 149.13/NN15

Topic: E.06. Posture and Gait

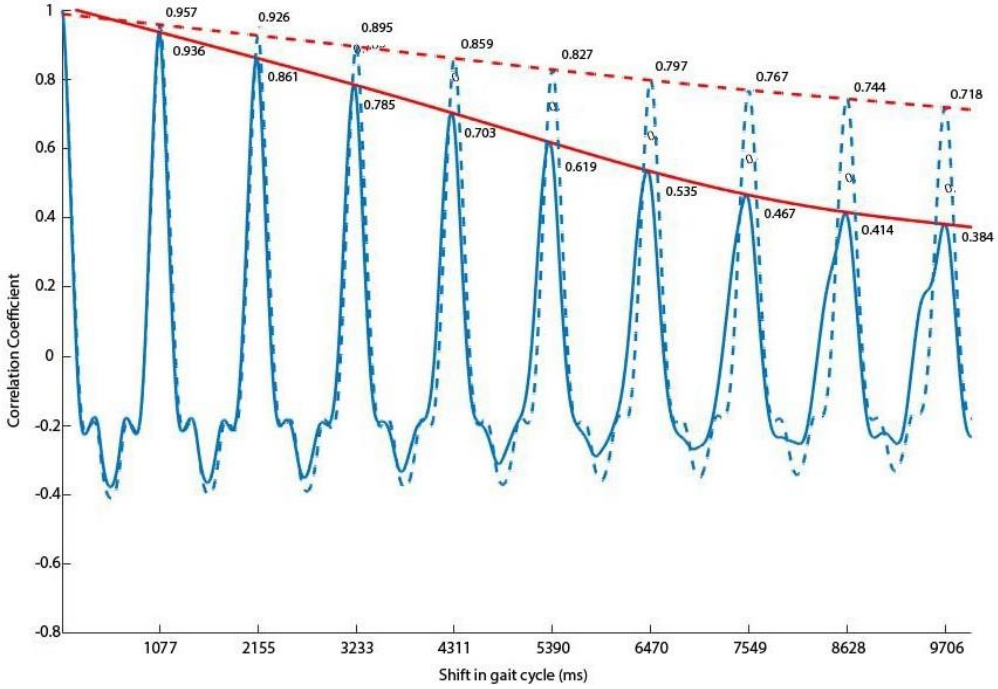
Support: Partial funded by HD Human Biology Project

Title: The temporal reproducibility of the gait cycle in overground and treadmill walking in young and old adults

Authors: *A. M. PHIPPS[1,2], K. KITANO[1], D. M. KOCEJA[1,2]
[1]Kinesiology, [2]Program in Neurosci., Indiana Univ., Bloomington, IN

Abstract: Motorized treadmills (TM) are widely utilized in both rehabilitation and research. TMs have many benefits over overground (OG) walkways. These benefits include less space requirements, the ability to easily secure individuals to prevent falls, and the ability to maintain a constant speed and thus a more rhythmic, repeatable gait cycle. Past studies have explored autocorrelations with regard to several gait parameters during different walking conditions. However, it is not clear to what extent temporal reproducibility is intact in terms of the gait cycle between young and old adults. Therefore, the purpose of this study was to examine the temporal reproducibility of the gait cycle in young and old adults in two walking conditions. Participants ($n=10$) were recruited from two age groups: 1) 5 young subjects (37.8 ± 14.75 yrs) and 2) 5 old subjects (62.6 ± 2.88 yrs). Subjects performed two walking tasks (OG and TM) while wearing custom-made shoe insoles to detect ground contacts from the toe and heel during gait. The OG condition consisted of hallway walking for a distance of 96 meters at a self-selected speed. Next, subjects walked on a TM for approximately 2 minutes at the same speed calculated from the OG condition. Autocorrelation coefficients for ground contacts were calculated for 60 seconds of gait. The first 3 peaks of the correlation coefficients were evaluated by calculating the slope of the line of best fit. Figure 1 shows a young subject's data. The solid line represents OG walking and the dotted line TM walking. The correlation coefficients are displayed at each peak. Both

age groups showed a decreased slope for the OG condition compared to TM (Old: -0.085 vs -0.035; Young: -0.066 vs -0.036). An ANOVA with split plot design showed a significant interaction of the slopes F(1,8)=6.43). This finding suggest the reduction in temporal reproducibility from TM to OG is greater in the old group compared to the young group.



Disclosures: A.M. Phipps: None. **K. Kitano:** None. **D.M. Koceja:** None.

Poster

149. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 149.14/NN16

Topic: E.06. Posture and Gait

Title: Contributions of arm swing to real-world gait coordination in humans

Authors: C. WANG[1], P. SHAH[2], S. A. SISTO[4], *E. V. VASUDEVAN[3]
[1]Physiol. and Biophysics, [3]Neurobio. and Behavior, [2]Stony Brook Univ., Stony Brook, NY; [4]Rehabil. Sci., Univ. of Buffalo, Buffalo, NY

Abstract: Gait retraining after neurological damage tends to focus on lower-limb stepping while seldom emphasizing full-body locomotion and posture. However, coordination between the arms

and legs is an important feature of bipedal gait in humans, just as coordination between the fore- and hind-limbs is essential for quadrupedal gait in animals. Both human and animal studies have shown an interlinked neuronal circuitry that connects all four limbs to coordinate rhythmic movement patterns. These connections bring up the exciting possibility that activity in the arms may augment activity in the legs; however, this hypothesis has primarily been tested on devices like treadmills and other exercise machines which limit the natural variability of walking. We hypothesized that muscle activity and range of motion in the arms would impact leg muscle activity and coordination during real-world walking (over the ground). Human adults without neurological or orthopedic disorders walked over the ground while kinetic, kinematic, and electromyographic data were recorded. Each subject was tested during seven arm swing conditions, in random order: (1) No arm swing with arms held by sides, (2) No arm swing while holding hand weights (1 kg), (3) Natural arm swing, (4) Natural arm swing with hand weights, (5) Exaggerated arm swing (increased range of motion), (6) Exaggerated arm swing with hand weights, and (7) Passive arm swing. During the last condition, the subject was asked to relax, while an experimenter walked behind the subject, using long poles to move the arms back and forth. We found few differences between self-generated arm swing conditions (conditions 1-6). However, the passive arm swing condition was associated with reduced leg muscle activity, stride length, and speed compared to the other conditions. We concluded that slight disruptions in arm-leg coordination induced by the experimenter in the passive arm swing condition were sufficient to interfere with normal gait control. If extended to rehabilitation, this would suggest that externally-driven arm swing must be precisely coordinated with the legs to obtain the benefits of full-body locomotor training.

Disclosures: C. Wang: None. **P. Shah:** None. **S.A. Sisto:** None. **E.V. Vasudevan:** None.

Poster

149. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 149.15/OO1

Topic: E.06. Posture and Gait

Title: Characterization of compensatory stepping for balance recovery in response to falling

Authors: *J. YOO[1,4], J. LEE[1,4], K. CHAN[2,4], J. UNGER[2,4], K. MUSSELMAN[2,3,4], K. MASANI[1,4]
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Abstract: Individuals with incomplete spinal cord injuries (iSCI) often experience falls due to an impaired ability to react to sudden losses of balance. While able-bodied (AB) individuals are usually able to recover with one step, individuals with iSCI often require multiple steps or cannot recover at all. We hypothesized that 1) a multiple-step response occurs when the first step does not sufficiently adjust the base of support to account for the change in the center of mass, i.e., the step is not far enough in the fall direction, and 2) the ground reaction forces (GRF) generated during the step response will be more variable in individuals with iSCI than in AB individuals due to their impaired control. Using a lean-and-release perturbation system, we investigated the biomechanical differences in the compensatory stepping response between individuals with iSCI and AB individuals, and between single- and multi-step responses across both groups of individuals. The study involved 7 individuals with iSCI (5 females and 2 males; age 57.3±17.8) and 7 age- and sex-matched AB individuals (5 female and 2 male; age 56.7±16.8). The participants were attached to a tether at their lower back and instructed to lean forward, from their ankles with their upper body and legs in a straight line, until the tension on the tether was at 10±2% of their body weight. The tether was then released at a random time unknown to the participants, causing them to fall forward. For each participant, 10 such lean-and-release trials were conducted along with 3 sham trials where no tether release occurred; the 13 trials in total were performed in a randomized order of which the participant had no advance knowledge. Kinetic data for the participants were recorded using 4 force plates (2 left, 2 right), and force data from the tether was recorded using a load cell. The average step length measured by the COP was shorter in individuals with iSCI (38.3 ± 10.7 cm) than in AB individuals (41.4 ± 12.7 cm). The variability measured by the standard deviation of vertical GRF was larger in individuals with iSCI (15.8 ± 5.5 N) than AB individuals (11.8 ± 4.8 N), while that of anterior-posterior GRF was not statistically different. We conclude that individuals with iSCI tend to make shorter and more variable compensatory steps due to impaired control of their legs, resulting in reduced reactive balance in this population.

Disclosures: J. Yoo: None. **J. Lee:** None. **K. Chan:** None. **J. Unger:** None. **K. Musselman:** None. **K. Masani:** None.

Poster

149. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 149.16/OO2

Topic: E.06. Posture and Gait

Support: NIH NINDS NS054894
NIH NINDS NS072651
The Craig Neilsen Foundation

Title: Alterations to local and global kinematics during locomotor rehabilitation in the neonatally spinalized rat

Authors: *J. VANLOOZEN, S. F. GISZTER
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Abstract: Rats spinalized at T9/10 as neonates (P5/6) show recovery of hindlimb alternation as adults (NTX). Around 20% of animals show autonomous weight-supporting locomotion without therapeutic intervention. Utilizing robot-assisted treadmill-based rehabilitation based at the pelvis, some NTX animals increase the amount of weight-supported stepping (WSS) across a 30-day training period. This rehabilitation framework simultaneously trains the adult NTX rats lumbar spinal cord and cortical regions, which developed independently. Trunk motor cortex is likely of particular importance in spinal animals undergoing locomotor rehabilitation because it serves as a mechanical link between cortically-controlled upper trunk muscles and spinally-controlled areas under the lesion site. Kinematics were recorded from animals throughout robot-assisted treadmill training at the hip and ankle using OPTOTRAK 3020. Pelvic roll, pitch, yaw and ankle translation and position across the step-cycle were used to correlate changes in biomechanics to changes in WSS.

In animals that transition from low levels of weight-supported stepping (<50%WSS) to higher levels of weight-support (>50%WSS) (TWS NTX) we demonstrate a significant reduction in pelvic roll, pitch, and yaw across the entire step cycle at the end of the robotic rehabilitation training. Changes to pelvic rotation are critical to the development of WSS in these animals, increases in %WSS and limb patterning are not observed until late in the training paradigm after changes to the pelvic rotations have been established. In animals that do not increase their WSS using robot-assisted treadmill training (NWS NTX) we see no changes to pelvic roll or yaw, but an increase in pelvic pitch across the step cycle late in training; demonstrating that these animals are affected by the training, but do not expand limb task space or learn how to use the robot appropriately for pelvic stabilization leading to WSS. Animals that begin training at a high level of weight-supported stepping (WS NTX) demonstrate a significant reduction in pelvic roll across the entire step cycle, swing specific reduction to pelvic yaw, and stance specific reduction to pelvic pitch while improving limb parameters and coordination.

The role of pelvic control through trunk musculature has a clear influence on WSS in NTX animals that are successfully rehabilitated. Understanding the development of coordination between lumbar spinal cord controlled stepping behavior and trunk motor cortex controlled pelvic stabilization in this group of animals informs rehabilitation of complete spinal cord injury and increases our understanding of motor systems.

Disclosures: J. Vanloozen: None. **S.F. Giszter:** None.

Poster

149. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 149.17/OO3

Topic: E.06. Posture and Gait

Support: SDSU Summer Undergraduate Research Program

Title: Direction and modality sensitivity of walking balance to visual perturbations

Authors: P. S. BALUYUT, *S. M. O'CONNOR
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Abstract: Three-dimensional biomechanical walking models are passively stable in the sagittal plane but unstable in the frontal plane, indicating that visual information pertaining to frontal plane movement may be more useful for controlling balance than information about sagittal plane movement. Since walking is a pendular motion, we also hypothesized that pendulum-like visual perturbations would have greater impact on walking balance compared to linear perturbations. To test these hypotheses, we used a virtual reality environment to induce visual perturbations and measured balance corrections during walking. Nine healthy subjects (aged 22 ± 1.7 yrs., mean ± s.d.) received visual information through a head-mounted display while walking on a large instrumented treadmill at a comfortable speed. The display consisted of a virtual dark hallway tiled with randomly placed white rectangles synchronized with the walking speed. Subjects were exposed to sinusoidal visual perturbations that were categorized as frontal or sagittal plane, linear or pendular modality, and at four amplitudes. The effect of the perturbations was assessed by measuring variability in foot placement (step length and step width) and muscle activity (EMG) of 3 hip muscles recorded from at least 300 steps. To measure the subject sensitivity to the visual perturbations, we calculated the slopes of the step placement and EMG variability vs perturbation amplitude trends. A two-way analysis of covariance (ANOCOVA) test revealed that subjects were 9.3 times more sensitive to frontal than sagittal plane perturbations ($P<1e-10$), indicating that greater emphasis is placed on side-to-side visual information than front-to-back for controlling walking balance. The ANOCOVA also revealed no significant main effect ($P=0.54$) of modality (linear vs. pendular), suggesting both modalities equally induced balance corrections in walking subjects. We also found that variations in the hip muscle activity (Gluteus Medius, Adductor Longus, Gracilis) were sensitive to the roll perturbations. A one-way ANCOVA test revealed a significant main effect of roll perturbation amplitude on hip EMG variability ($P=7.0e-6$), suggesting that variations in the activation of these muscles produce observed variations in step width. The ANCOVA also revealed no significant main effect ($P=0.30$) of hip muscle, suggesting both adductors and abductors contribute to step

width adjustment. These findings establish normative balance control behavior and may lead to improvements in clinical assessments for detecting balance impairment.

Disclosures: P.S. Baluyut: None. **S.M. O'Connor:** None.

Poster

149. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 149.18/OO4

Topic: E.06. Posture and Gait

Title: Interdependence of balance mechanisms during walking

Authors: *T. D. FETTROW[1], H. REIMANN[1], E. THOMPSON[1,2], D. GRENET[1], J. JEKA[1]
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Abstract: Currently there is not a well-defined, comprehensive theory for how the healthy human nervous system maintains balance during walking. Unlike quiet standing balance, the gait cycle creates a system that requires different strategies to achieve the task of balance. We have discovered multiple mechanisms of balance control during walking, in a young healthy cohort (n=20), that are temporally coordinated to explain the maintenance of upright balance in the medial-lateral direction. The protocol for determining such a balance response involved inducing a visually perceived fall to the side on heel strike. The subjects walked on a self-paced treadmill surrounded by a 3D virtual environment. The stimulus consisted of a rotation of the visual scene at 60°/sec² for 600ms. Initial interpretation of the results allowed for identification of three major balance responses within the first step: 1) Foot placement shift: Active control of swing foot placement in direction of perceived fall. In the event of a sensory perturbation and a perceived shift in the CoM, a correction must be made, and the most obvious way to do this is take a step. 2) Lateral ankle roll: Active control of center of pressure under the stance foot in the direction of the perceived fall. The active control of ankle inversion/eversion angle modulates the CoP under the stance foot during sustained locomotion. 3) Push-off modulation: Active shift of weight between two legs in double stance. During double stance, the feet are not directly in front of one another, but are offset laterally, so a weight shift between legs would have effects on the lateral balance. We are currently unaware of any reports of a push-off mechanism being used to aid in the maintenance of balance in the medial-lateral direction. The foot placement response (difference from control) is strongly dependent on the degree to which the lateral ankle mechanism was used during stance (R^2 = .49, p = <.05). Interestingly, the push-off modulation does not correspond to the magnitude of foot placement response (R^2 = .002), nor the lateral ankle mechanism (R^2 = 0). This finding suggests that the lateral ankle mechanism and the foot placement mechanism are coordinated to produce a balance response in the medial-lateral

direction, but the push-off modulation may be a byproduct of the balance response, or serve a different functional role. Future work includes the investigation of the role of cadence in relation to the basic balance mechanisms for maintenance of balance in the medial-lateral direction.

Disclosures: T.D. Fettrow: None. **H. Reimann:** None. **E. Thompson:** None. **D. Grenet:** None. **J. Jeka:** None.

Poster

149. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 149.19/OO5

Topic: E.06. Posture and Gait

Title: Merging enriched environments and assistive technology to enhance early exploratory and motor activity in young children

Authors: *E. KOKKONI[1], J. C. GALLOWAY[2,1]
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Abstract: Background. Exploration of Enriched environments (EEs) early in life leads to plastic changes in motor ability of animals. Young humans may be benefited from early exploration of EEs; like infants with Down syndrome (DS) that present significant motor delays compared to their typically developing (TD) peers. In this study, TD and DS infants performed various exploratory activities in an EE with the assistance of an open-area body weight support device. The goal was to assess if the addition of the device in a short-term exposure to the EE would lead to increased motor performance in both populations. **Methods.** Subjects were 11 TD infants (M=9.9±1.2 months) and 5 DS toddlers (M=17±3 months). All subjects participated in two 2-hour sessions where they performed the following tasks in the EE: Spontaneous Movement Task (SMT), Platform Ascending Task (PAT), and Staircase Ascending Task (SAT). Subjects went through the tasks 3 times: without (Pre), with, and without (Post) the assistance from the support device. Results are presented from comparing Pre of session 1 to Post of session 2 to evaluate change in the beginning and by the end of the short-term exposure. Overall performance (*success rate of task completion*) and spatiotemporal measures of movement (*movement path, distance, speed*) were derived from video based behavioral and motion analyses. **Results.** TD group: Length of *movement path* was significantly larger in Post compared to Pre (z=-2.223, p=0.026) in SMT. *Success rate of task completion* was larger for SAT than PAT in Pre but both increased reaching similar levels in Post (PAT_{Pre} = 63.6%, PAT_{Post} = 90.9%, SAT_{Pre} = 81.8%, SAT_{Post} = 90.9%). For the subjects that completed the ascending task in both conditions, *speed of ascending* was greater (approaching significance) in Post compared to Pre in PAT (z=-1.859, p=0.063) but not in SAT ((z=-0.533, p=0.594). The remaining subjects

that failed to complete the ascending task in Pre covered greater *distance* on the inclined platform (approaching significance) in Post compared to Pre (z=-1.841, p=0.066). DS group: *Success rate of task completion* was overall lower compared to the TD group but showed similar patterns of change. Subjects had greater rates in SAT than PAT and both increased from Pre to Post (PAT_{Pre} = 40%, PAT_{Post} = 60%, SAT_{Pre} = 60%, SAT_{Post} = 80%). **Discussion.** The addition of the device allowed for training of motor activities beyond the current level of ability that translated into improvement in performance for both groups within just 2 sessions. This study provides preliminary support for inclusion of this paradigm (EE + support device) in future longitudinal training studies to assess long-term changes.

Disclosures: E. Kokkoni: None. **J.C. Galloway:** None.

Poster

149. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 149.20/OO6

Topic: E.06. Posture and Gait

Support: NIH NIAMS Grant R01-AR050520
NIH NIAMS Grant R01-AR052345
US DoD CDMRP Grant MR150091

Title: A NeuRoBotic experimental system to study muscle function

Authors: *D. URBINA-MELÉNDEZ[1], J. A. BERRY[2], H. ZHAO[1], F. VALERO-CUEVAS[1,3]
[1]Dept. of Biomed. Engin., [2]Dept. of Computer Sci., [3]Div. of Biokinesiology and Physical Therapy, USC, Los Angeles, CA

Abstract: We designed a planar robotic tendon-driven leg with three muscles and 2 joints which walks on a miniature treadmill. The electric motors that pull on the tendons are programed to behave as muscles. Here we use one control strategies to control the leg while implementing different muscle models.
Our goal is to characterize how different muscle models (e.g., low-pass filter of muscle activation, one and two element Hill-type, Virtual Muscle [Song et al. 2008]) affect the behavior and control of the robotic leg. It is important to note that we tested each muscle model both in a purely feed-forward mode, as well as when afferented with Golgi-Tendon organs and muscle spindles [Nagamori et al. 2018].
We find there is no “best model” as they each trade-off simplicity of control and implementation vs. physiological fidelity. While all models produce reasonable mechanical function, the user must choose a model to use based on the nature of the question asked. In particular, we find that

models considering recruitment and rate coding can best produce both realistic eccentric and concentric contractions when afferented. Thus, they are best able to inform the study of neurological conditions and stroke.
This research reported in this work is partially funded by National Institutes of Health grants NIAMS R01-AR050520 and NIAMS R01-AR052345, and US Department of Defense CDMRP grant MR150091 awarded to Dr. Francisco J. Valero-Cuevas. The content of this endeavor 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 Defense.

Disclosures: D. Urbina-Meléndez: None. **J.A. Berry:** None. **H. Zhao:** None. **F. Valero-Cuevas:** None.

Poster

149. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 149.21/OO7

Topic: E.06. Posture and Gait

Support: University Committee on Research and Creative Activity (UCRCA)

Title: The presence of a 1/f structure in our walking can enable us to withstand falls

Authors: *A. SKIADOPOULOS, J. KENT, J. WICKSTROM, C. SLOAN, N. STERGIOU
Univ. of Nebraska At Omaha, Omaha, NE

Abstract: The structure revealed in the stride-to-stride time series of healthy walking patterns resembles pink noise - namely a 1/f pattern. We compared the response to, and recovery time following, a completely unannounced perturbation delivered during treadmill walking in four groups of young unimpaired adults instructed to walk either without a stimulus or to walk matching their foot contacts with the beat of one of three auditory stimuli: 1/f, random or isochronous. We hypothesized that healthy individuals walking with no stimulus (i.e. with their normal stride dynamics allowed to unfold naturally) and with the 1/f stimulus would exhibit a less severe perturbation response than those synchronizing with the random and isochronous stimuli. Thirty-three healthy young adults undertook a 25-minute baseline walk with no stimulus at their self-selected comfortable speed on a dual belt treadmill (Bertec Corp., Columbus, OH). After 20 minutes break, they completed a 45-minute trial on a treadmill walking to a 1/f, random or isochronous auditory stimuli, or with no stimulus (None). At minute 25, one treadmill belt was arrested for ½ second at the instance of heel strike, delivering a perturbation. Belt movement resumed and the participant continued to walk for a further 20 minutes. Reflective markers were attached to participants and tracked at 100 Hz using an 8-camera, 3D motion analysis Vicon

system. Static trials were used to define a nine-segment mechanical model of the human body using Visual3D software for post process. The extrapolated center of mass was used to determine the margin of stability in the anteroposterior direction during treadmill walking at heel strike. We identified the period before perturbation (PRE) and after the recovery from perturbation took place (POST) and we determined the effect on margin of stability of the auditory stimuli and PRE - POST periods using ANOVA and Tukey HSD tests. The results showed that the random group had significantly higher margin of stability than the other three auditory stimuli conditions before the perturbation indicating the usage of a more cautious gait to maintain stability. The margin of stability significantly decreased after the perturbation but only for the random group indicating an increased vulnerability to the effect of the perturbation. The variable 1/f based auditory stimuli as well as the None and the isochronous were not affected by the perturbation in terms of their margin of stability. These results allow us to further hypothesize that in the case of older adults who actually exhibit more random fluctuations in the stride-to-stride walking patterns, perturbations can seriously affect their stability.

Disclosures: A. Skiadopoulos: None. **J. Kent:** None. **J. Wickstrom:** None. **C. Sloan:** None. **N. Stergiou:** None.

Poster

149. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 149.22/OO8

Topic: E.06. Posture and Gait

Support: HD32571

Title: Altered medial gastrocnemius activation after cross-reinnervation with an antagonist during single session percutaneous recordings in the cat

Authors: *T. NICHOLS[1], E. KAJTAZ[2], H. ANDERSON[2], H. MAAS[3], M. A. LYLE[4]
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Abstract: Surgical cross-union of antagonist to agonist muscle nerves can be performed to restore motor function in cases of lost innervation from injury, similar to tendon transfer procedures. After nerve transection and repair, a period of muscle denervation is followed by recovery of motor activation around 6-8 weeks. We have performed cross-union of the medial gastrocnemius muscle nerve with the deep peroneal nerve in 4 cats with the goal to evaluate the effects of this procedure on locomotor behavior and spinal reflex pathways. Recently, we

reported behavioral adaptations that persist for at least 20 weeks after the cross-unions that were characterized by increased ankle extension in 2 cats during the stance to swing transition and increased MTP extension in all cats through midswing, along with a tendency for increased knee flexion. An additional observation was increased ankle flexion during the stance phase. The behavioral changes during swing could be explained by activation of the medial gastrocnemius by the pretibial flexor motor pool, and increased ankle flexion during stance could be explained by activation of the pretibial flexors and lack of medial gastrocnemius excitatory length feedback onto its motoneuron pool. To evaluate whether the cross-union resulted in altered muscle activation patterns, we attempted percutaneous recordings during a single recording session. We sedated 3 cats and then inserted fine wire electrodes into the cross-reinnervated medial gastrocnemius and tibialis anterior muscles, as well as the intact lateral gastrocnemius. Medial gastrocnemius and tibialis anterior muscles were implanted from the non-surgical limb. Upon reversing sedation, we recorded EMG, joint kinematics, and ground reaction forces during level walking. We were able to record 10-15 walking trials on 2 of the 3 cats. In general, we observed normal activation patterns from the medial gastrocnemius of the non-surgical limb. In contrast, the cross-reinnervated medial gastrocnemius was co-active with the tibialis anterior during the swing phase in 1 cat and in the other cat the medial gastrocnemius was most active during the stance to swing transition and tibialis anterior most active late swing to early stance similar to lateral gastrocnemius. The altered muscle activations in cross-reinnervated muscles is consistent with a prior report (Gordon et al. 1986) and provide an explanation for the persistent behavioral adaptations. Future work will evaluate whether the mismatch between altered activation patterns and mechanical activation of sensory feedback during locomotion results in spinal reflex circuit plasticity.

Disclosures: T. Nichols: None. **E. Kajtaz:** None. **H. Anderson:** None. **H. Maas:** None. **M.A. Lyle:** None.

Poster

149. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 149.23/OO9

Topic: E.06. Posture and Gait

Support: Programme 4.2.1 Partenariat de recherche clinique en physiothérapie (OPPQ-REPAR): $14855
2017-2018 New Initiatives Program (CRIR): $ 7000

Title: Real-time avatar-based feedback to enhance gait symmetry after stroke: Instantaneous effects of different avatar views

Authors: *L. Y. LIU[1], S. SANGANI[3], K. PATTERSON[4], J. FUNG[2], A. LAMONTAGNE[5]
[1]McGill Univ., Laval, QC, Canada; [2]McGill Univ., Montreal, QC, Canada; [3]Feil/Oberfeld/CRIR Res. Ctr., Jewish Rehabil. Hosp., Laval, QC, Canada; [4]Univ. of Toronto, Toronto, ON, Canada; [5]Feil and Oberfeld Res. Centre, Jewish Rehabili, Laval, QC, Canada

Abstract: Introduction: Gait asymmetry often persists after stroke despite rehabilitation, negatively affecting functional mobility. Providing appropriate feedback to correct the gait pattern can be challenging for clinicians. Interventions aiming at improving gait symmetry have so far provided mitigated outcomes. **Objectives:** The objectives of this ongoing study are to: (1) determine the feasibility of using virtual avatars to provide real-time visual feedback on the symmetry of gait in people with stroke and (2) examine any difference between frontal, side and back views in changing symmetry outcomes. **Methodology:** Participants with post-stroke gait asymmetry were assessed during self-paced treadmill walking which included trials of 30s of walking without the avatar, followed by 1 min of walking while visualizing the avatar replicating the walking pattern of the participant in real time, and finally 1 min of walking without the avatar. Three avatar views were randomly presented, which included viewing from (1) the back, (2) the paretic side; and (3) the front. In each trial, ratios (paretic/nonparetic) of step length (SLR) and swing time (SWR) as well as gait speed were examined. **Results:** In two stroke participants analyzed so far, improvement in SLR (Δ =0.07 and 0.09) and SWR (Δ=0.05 and 0.11) were observed in the adaptation phase with the back and paretic side views, respectively, while the front view yielded mixed results. Modest increments in gait speed ($\Delta \leq 0.10$ m/s) were present for all avatar views. Gains in symmetry and gait speed were not maintained once the avatar was removed. **Conclusion:** These preliminary results suggest that back and paretic side views lead to larger improvement compared to the front view. The results also support the feasibility of using virtual avatars as a source of visual feedback to promote gait symmetry in stroke survivors.

Disclosures: L.Y. Liu: None. **S. Sangani:** None. **K. Patterson:** None. **J. Fung:** None. **A. Lamontagne:** None.

Poster

149. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

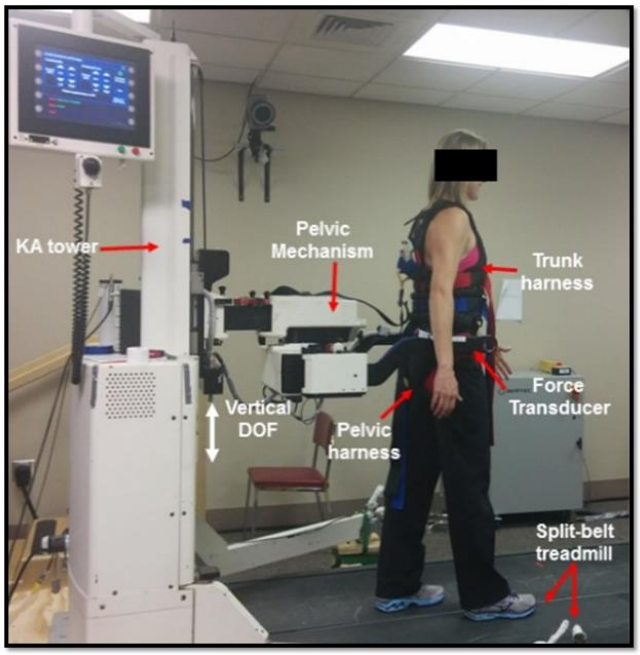
Program #/Poster #: 149.24/OO10

Topic: E.06. Posture and Gait

Title: Neuromuscular adaptationsof walking against fore-aft resistance applied at the center of mass

Authors: *A. NAIDU[1], C. HURT[2], D. A. BROWN[3]
[1]Rehabil. Sci., [3]Dept. of Physical Therapy and Occup. Therapy, [2]Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Purpose: Poststroke hemiparetic weakness impairs the ability to generate appropriate fore-aft ground reaction force i.e., braking and propulsive forces, during walking. The paretic (P) limb is unable to generate sufficient propulsive forces, and relies on increased propulsive force generation by the non-paretic (NP) limb to maintain walking speed. In this study, we explored neuromuscular adaptations of walking against fore-aft resistance (FAR) applied at the center of mass (COM) in both nonimpaired (NI) individuals and individuals poststroke (PS). We hypothesized that to maintain a constant speed; at higher levels of FAR NI individuals will symmetrically increase interlimb propulsion, while individuals PS will asymmetrically increase interlimb propulsion with the NP limb producing the majority of additional propulsive forces required. Methods: We used a novel interface consisting of the KineAssist (KA) robot synced to a Bertec split-belt treadmill. To drive each treadmill belt, individuals walking inside the interface must overcome a set FAR at their COM and produce additional propulsive forces to maintain an intended target speed. Using different percentages of FAR demands equivalent to vertical body weight levels, we compared kinetics and kinematic data of NI and PS participants. Results: To date we have analyzed data of 14 NI (Mean age 55 yr(15), 8 Female) individuals and 1 individual PS poststroke (Left hemiparesis, 55 yr, Male). Average propulsive impulse for NI individuals increased with different levels of FAR, with no increase in propulsion of either lower-limb (slope 1.95, R square=0.99, $p<0.05$). PS interlimb symmetry decreased from 38 to 34%, with increase in propulsion of NP limb (slope 2.58, R square =0.99) and P limb (slope =1.02, R square =0.95). Discussion/conclusion: NI individuals maintain force symmetry with greater FAR, indicating sharing global strategy for force generation. In the one individual PS, our data indicates preference for increasing NP contribution compared to the P limb.



Disclosures: A. Naidu: None. **C. Hurt:** None. **D.A. Brown:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); HDT Robotics.

Poster

150. Posture and Gait: Afferent Control

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 150.01/OO11

Topic: E.06. Posture and Gait

Title: Simulation study of bipedal walking based on motor modules of synergistic muscle activations

Authors: *D. ICHIMURA[1,2], T. YAMAZAKI[1]
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Abstract: Bipedal waking is a basic motor activity that requires to control many muscles simultaneously. Physiological experiments have suggested that the nervous system controls bipedal walking efficiently using motor modules of synergistic muscle activations. If these modules were merged, abnormal walking patterns would be realized as observed in post-stroke patients. To examine the role of motor modules in bipedal walking, several neuro-musculoskeletal models based on anatomical and physiological knowledge have been proposed. These models employ central pattern generators (CPGs) to produce walking rhythm. A CPG has a hierarchical structure composed of a rhythm generator (RG) and a pattern formation (PF) network. An RG is a simple oscillator that drives units in a PF. A PF network contains various units that emit pulses to stimulate muscles. In other words, PF units act as motor modules. It is essential for robust bipedal walking to drive PF units appropriately.Previous studies determined the parameters for the CPGs by hand. It remains unclear how such PFs or motor modules are organized through the interaction with the environment. In this study, we used the Matsuoka oscillator model to model CPGs. An RG is composed of four neurons mutually inhibited to generate rhythm, whereas a PF network contains five neurons with mutual and self inhibition that correspond to five motor modules. In response to rhythmic activation of the RG, the five modules are activated one by one sequentially with different phases. The phase and duration are controlled by the level of excitation from RG to PF and various feedback signals. We adopted this CPG model in a two-dimensional bipedal walking model with seven segments and nine muscles for each leg. Parameters were tuned by a genetic algorithm (GA). After 3000 generations of GA, the model acquired stable robust bipedal walking. Furthermore, by changing the level of excitation, the walking pattern was changed. These results imply that temporal

coordination of motor modules controlled by top-down excitation from RG to and mutual inhibition of motor modules are indispensable for robust bipedal walking.

Disclosures: D. Ichimura: None. **T. Yamazaki:** None.

Poster

150. Posture and Gait: Afferent Control

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 150.02/OO12

Topic: E.06. Posture and Gait

Title: Neural correlates of neck proprioceptive inputs integration for spatial orientation

Authors: *L. BONZANO[1], L. PEDULLÀ[1], A. BISIO[1], G. BRICHETTO[2], M. BOVE[1]
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Abstract: Neck muscle vibration can affect the generation of an egocentric body-centered coordinate system. It elicits apparent motion of a stationary visual target and deviation of the perceived “straight ahead”. In standing subjects, neck muscle vibration induces body tilt and increased sway, suggesting that posture is organized with respect to a “body schema”, to the construction of which neck input contributes together with eye and skeletal muscle. Up to know there is no demonstration of this kind of neural processing in a spatial orientation task during walking or stepping-in-place. The aim of this study was to describe the neural correlates of the effect of an asymmetric neck muscle vibration on body orientation during an extended stepping task. Five healthy blindfolded subjects were asked to stand in quiet upright position or to step in place for 30s with or without the vibration of the right/left sternocleidomastoid muscle at 80Hz. Each condition was repeated four times, with a rest interval of 2 min to reposition the subject and to avoid vibration post-effects. By means of an optoelectronic motion analysis system we measured possible disorientation induced by neck muscle vibration during stepping-in-place. The reflective markers were attached to the skin on the forehead, vertex of the head, left and right acromions. The markers’ position was at a frequency of 100 Hz. Further, by means of functional near infrared spectroscopy (fNIRS) we measured concomitant cortical activity in frontal, sensorimotor and parietal associative areas of both the hemispheres (16 sources and 16 detectors, 45 channels). We measured changes in oxyhemoglobin concentration in the three conditions during stepping-in-place (no vibration, right vibration, left vibration) with respect to the standing position without vibration, and evaluated the statistical contrasts between conditions. Neck vibration caused clear-cut whole body rotation toward the side opposite to vibration. The body rotated around a vertical axis placed at about arm's length from the body. The rotation did not begin immediately on switching on the vibrator. The delay varied from subject to subject from a few seconds to about 10 s. We found significant activations in bilateral frontal and sensorimotor areas both during no vibration and vibration conditions. However, contrast analysis revealed

increased frontoparietal activity contralateral to the vibration side. The results confirm and extend the notion that the neck proprioceptive input plays a major role in body orientation during navigation through the activation of a frontoparietal network.

Disclosures: L. Bonzano: None. **L. Pedullà:** None. **A. Bisio:** None. **G. Brichetto:** None. **M. Bove:** None.

Poster

150. Posture and Gait: Afferent Control

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 150.03/OO13

Topic: E.06. Posture and Gait

Support: GRACA grant

Title: Passive exoskeleton assistance during a split-belt adaption task alters both spatial and temporal patterns of gait coordination

Authors: *T. SADO[1], J. NIELSEN[2], B. GLAISTER[4], K. TAKAHASHI[2], P. MALCOLM[2], M. MUKHERJEE[3]
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Abstract: Unilateral sensorimotor pathologies like stroke can lead to gait asymmetry. One way to counter this challenge is by using assistive devices such as exoskeletons. An active (powered by external sources) exoskeleton is known to be more efficient and accurate in its assistance, but it is expensive, less portable, and may require technical expertise to operate. A passive exoskeleton (self-powered) can be a favorable choice for local or home rehabilitation settings because it is cheap, light weight, and less complex to utilize. While there is research that investigates the effects of exoskeleton on gait patterns, research examining the effects of such devices on gait adaptation, is rare. This is important because diseases like stroke, substantially impair adaptation, such that recovery becomes difficult. In this study young healthy subjects were recruited with the objective of determining characteristic gait adaptation patterns that result from exoskeleton usage during a split-belt adaptation task. Healthy young participants were randomly assigned to an exoskeleton (EXO) or a no-exoskeleton (NO EXO) group. Individuals in EXO group wore a passive exoskeleton on their right leg. Each participant performed the split-belt adaptation task on the treadmill, where the speed of each belt was controlled independently. Participants self-selected their preferred walking speed (PWS) and fast walking speed (FWS). Slow walking speed (SWS) was calculated as half of the FWS. Baseline, split-belt adaptation, and post-adaptation trials were sequentially performed. Spatiotemporal variables, including step length, step time, limb excursion, stride time, stance time, and double support time were

calculated to quantify gait adaptation. For each variable, inter-limb symmetry was quantified using symmetry indices (SI). To analyze the adaptation, trials were divided into early adaptation (EA), late adaptation (LA), degree of adaptation/catch effect (DA), and transfer effect (TR). *Spatial Variables:* Significant condition effects were observed for EA, LA, DA, and TR for step length and limb excursion. DA showed significant interaction effects for limb excursion. *Temporal Variables:* Group, condition and interaction effects were significant for EA and DA for stance time. Use of a passive (unpowered) exoskeleton device altered gait adaption during a split-belt treadmill task changes compared to NO EXO. An unpowered device assisting the swing phase of gait can provide a unique solution for coordinating the lower limbs during different gait tasks. Such a solution may be useful for enhancing paretic propulsion in stroke survivors during gait coordination tasks.

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Poster

150. Posture and Gait: Afferent Control

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 150.04/OO14

Topic: E.06. Posture and Gait

Title: Sample entropy and wavelet transform suggest automaticity of postural control in young and older adults in cognitive task conditions

Authors: *N. RICHER, Y. LAJOIE
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Abstract: It has been suggested that improvements in stability in certain dual-task conditions are due to a shift to a more automatic postural control. External focus conditions have led to reductions in sway area and variability compared to baseline standing and internal focus conditions, and cognitive tasks that attempt to distract individuals from postural control altogether have yielded even greater improvements than the external focus in young and older adults [1-2]. To provide evidence that these changes in sway are due to automaticity, sample entropy and wavelet transform analyses were used to analyze postural control in attentional focus and cognitive task conditions in young and older adults.
The center of pressure data from 22 young adults (20.8 ± 2.82 years, 9 male) and 20 older adults (69.2 ± 3.47 years, 15 male) was analyzed using sample entropy and the wavelet transform. Participants stood with feet together in 5 conditions: baseline standing, internal focus on minimizing movements of the ankles, external focus on minimizing movements of markers extending from the ankle joint, an easy cognitive task of counting one digit in an string of numbers, and a difficult cognitive task of counting two digits in a string of numbers.

Results of sample entropy revealed no main effect of group, while the main effect of condition demonstrated higher sample entropy in cognitive task conditions compared to other conditions. Increases in sample entropy suggest a more irregular and unpredictable sway, which is thought to reflect automaticity [3-4].
Results of the wavelet transform revealed no main effect of group, but demonstrated a shift towards higher frequencies in cognitive task conditions compared to other conditions. Results have suggested that contributions from different systems to sway changed across conditions. Specifically, in cognitive task conditions there was an increase in the frequency band thought to represent contributions from the cerebellum compared to the rest, an increase in the band thought to represent the vestibular system compared to focus conditions, and a decrease in the band thought to represent contributions from the visual system compared to the rest. This shift towards higher frequency ranges and towards the use of the cerebellum and vestibular system is thought to represent automaticity.
Altogether, results suggest that improvements in postural control in cognitive task conditions are due to automaticity.
References: [1] Richer *et al*. (2018). Submitted for publication. [2] Richer *et al*. (2017). Gait & Posture, 54, 45-49. [3] Donker *et al*. (2007). Experimental Brain Research, 181, 1-11. [4] Potvin-Desrochers *et al*. (2017). Gait & Posture, 57, 40-45.

Disclosures: N. Richer: None. **Y. Lajoie:** None.

Poster

150. Posture and Gait: Afferent Control

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 150.05/OO15

Topic: E.06. Posture and Gait

Support: NSERC DG 321007

Title: Skin input from the dorsal ankle joint is used differently by lead and trail limbs during obstacle crossing

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Abstract: Skin input is critical for proprioception. Our work has provided evidence that skin from the dorsal ankle joint is capable of evoking whole limb kinematic changes during level gait [1]. To date, much of the human obstacle avoidance literature has focused on contributions from vision [2] while alluding to the role of kinaesthetic input if the limb is out of sight. The contribution from skin during obstacle crossing remains unknown. The purpose of this study was

to investigate skin input from the ankle dorsum in the lead and trail limb in obstacle crossing. Additionally, the interaction between vision and skin was examined.Eleven healthy adults crossed an obstacle with 4 different sensory feedback conditions; i) complete sensory input (control) ii) reduced skin input (anesthesia), iii) reduced visual input (partial vision) or iv) simultaneous impedance of skin and vision (paired). Anesthesia (EMLA cream) was applied to a $30cm^2$ area on the ankle dorsum. Vision was reduced using goggles that occluded the bottom half of the visual field. Kinematic data (OPTOTRAK 3020; NDI, Waterloo, Canada) were recorded from the lower limbs during obstacle crossing with the anesthetised foot as either the lead or trail limb. Crossing trajectory parameters such as toe clearance, toe peak, and time-to-peak were calculated. Additionally, angles of the ankle, knee and hip were examined. It was hypothesized that lead and trail limbs would be influenced by visual and skin input respectively. Regardless of sensory decrement, increases in the lead limb's margin of safety (increased toe clearance) and upward bias of the swing limb (increased toe peak) were observed. However *how* these toe trajectories were facilitated was dependent on the source of sensory loss. With partial vision, subjects increased hip flexion ($p=0.009$), whereas with anaesthesia, increased hip roll was observed ($p=0.026$). When paired, both hip strategies were employed ($p=0.0002$). The trail limb toe trajectory was affected only by vision, with no apparent influence from reduced skin input. Similar to previous findings of increased knee flexion with increased obstacle heights [3], visual interference generated greater safety margins through knee flexion. The absence of skin effects may suggest a reliance on sensory cues other than skin for trail limb placement. Importantly, our work provides evidence that we use skin from the ankle dorsum of the lead limb to help control swing limb trajectory over an obstacle even in the presence of visual availability. [1] Howe, E et al. (2015). *Exp Brain Res* 233(8): 2477-87. [2] Mohagheghi et al. (2004) *Exp Brain Res* 155(4): 459-68. [3] Patla AE et al (1996) *J Mot Behav* 28(1): 35-4

Disclosures: E.E. Howe: None. **A.J. Toth:** None. **L.R. Bent:** None.

Poster

150. Posture and Gait: Afferent Control

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 150.06/OO16

Topic: E.06. Posture and Gait

Support: NIH Grant HD-032571

Title: Stretch reflex removal from ipsilateral hamstrings, quadriceps and sartorius reduces joint ranges of motion and increases leg length bilaterally during level walking in the cat

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Movement Studies, [4]Dept. of Mechanical Engineeting, Georgia Inst. of Technol., Atlanta, GA; [5]Dept Cell Biol., Emory Univ. Sch. of Med., Atlanta, GA; [6]USC, Valencia, CA

Abstract: Injuries to peripheral nerves with subsequent nerve repair and muscle self-reinnervation lead to stretch reflex loss due to failure of muscle spindle Ia afferents to provide length feedback to the nervous system (Bullinger et al., 2011). Muscle length feedback contributes to limb position sense (Proske, Gandevia, 2015), and patients with lost proprioception poorly coordinate interactive moments (Sainburg et al. 1995). On the other hand, no locomotor deficits were reported during level walking in cats after self-reinnervation of ankle extensors (Gregor et al 2018). This could be explained by the minor effects of errors in ankle angle on paw position due to the relatively short length of the foot. Similar angular errors in the more proximal knee joint should lead to greater errors in paw position and thus greater changes in walking kinematics. The goal of this study was to determine the effects of stretch reflex removal from major knee muscles on walking kinematics. We hypothesized that (1) self-reinnervation of knee muscles would cause substantially greater changes in joint kinematics during level walking than those after self-reinnervation of ankle extensors and (2) the precision of paw position at stance onset and offset would be reduced. Muscle nerves to the right hamstrings, quadriceps and sartorius muscles of 4 cats were surgically transected and repaired using fibrin glue. Mechanics and EMG activity of hindlimb muscles during level walking were recorded before and 6-9 months after surgery. Following self-reinnervation, the affected muscles recovered their EMG activity; however, hindlimb kinematics of both hindlimbs significantly changed ($p<0.05$). Range of motion decreased, bilaterally, during swing and stance at the ankle and hip, and at the knee during swing and the push-off phase of stance. Knee yield during stance increased in the affected and decreased in the sound hindlimb. Leg length during mid-swing and at stance onset (but not stance offset) increased, bilaterally. The distribution area of paw positions at stance onset and offset decreased for both hindlimbs (precision increased). These results supported hypothesis 1 but not hypothesis 2. Given stabilization of paw position at stance onset in intact cats (Klishko et al 2014) and greater radial endpoint precision at longer limb lengths (Oh, Prilutsky, 2015), we suggest that the observed kinematic adaptations improve precision of paw position of the affected hindlimb at stance onset. More extended hindlimb and greater knee flexor-extensor co-activation during swing help reduce knee interactive moments. Similar kinematic changes in the intact hindlimb permit symmetric walking.

Disclosures: B.I. Prilutsky: None. **K. Oh:** None. **A.N. Klishko:** None. **D. Zuniga:** None. **A.W. English:** None. **R.J. Gregor:** None.

Poster

150. Posture and Gait: Afferent Control

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 150.07/OO17

Topic: E.06. Posture and Gait

Title: Soleus H-reflex modulation by visual perturbation in humans

Authors: *K. KITANO[1], A. M. PHIPPS[1,2], D. M. KOCEJA[1,2]
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Abstract: An H-reflex method and related techniques have been employed in order to study spinal neural mechanisms and it is known that soleus H-reflex modulation is related to human postural control. Human posture is considered to be controlled by tactile sensory, proprioceptive, vestibular, and visual systems. Previous studies have revealed that soleus H-reflex is modulated through changes in ankle angle (proprioception) and body orientation (vestibular system). The purpose of the study was to evaluate whether manipulation of visual input has effects on human soleus H-reflex amplitudes. Four subjects participated. None of subjects had a history of neuromuscular disease and were of a normal fitness level. Subjects stood on a force plate. An EMG signal was collected from the right soleus and stimulating electrodes were prepared at the right popliteal fossa. Control H-reflex amplitudes were set at 20% of Mmax. In order to administer visual perturbation, moving walls were set within 0.5 m of subjects. The upper body of subjects were enclosed by a custom made box and the frontal visual field was limited. The wall was manually moved away from the subject at approximately 0.1 meter / sec for 1sec. Stimulation was delivered at 5 cm displacement of the wall from initial position. Soleus H-reflex amplitudes were compared with/without visual perturbation (repeated t-test). H-reflex amplitudes were significantly facilitated from 20.5% at control to 28.2% with visual perturbation ($p < 0.05$). COP data from the force plate showed that subjects did not initiate postural adjustment in response to the moving wall at stimulation administration. Results suggest that spinal activities are modulated by visual information before postural adjustment.

Disclosures: K. Kitano: None. **A.M. Phipps:** None. **D.M. Koceja:** None.

Poster

150. Posture and Gait: Afferent Control

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Program #/Poster #: 150.08/OO18

Topic: E.06. Posture and Gait

Support: NIH grant number NS086973 (MCT)

Title: Restoration of global, but not local, kinematics after denervation of vastus lateralis in rats

Authors: *C. ALESSANDRO[1], B. A. RELLINGER[2], F. BARROSO[2], M. C. TRESCH[3]
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Abstract: What aspects of motor output are controlled by the central nervous system (CNS)? This issue has often been investigated by examining how the CNS adapts to perturbations or injuries. Because of the redundancy of the musculoskeletal system, the CNS can compensate for perturbations by either restoring the original joint angle trajectories or establishing novel kinematic patterns. In previous experiments, investigators denervated ankle extensor muscles in rats and cats and observed alterations at individual joints but minimal changes in hip-to-toe limb length and angle. These results suggested that the CNS adapts to peripheral nerve injuries by coordinating changes at individual joints in order to maintain global limb kinematics. Here we evaluated the generality of this finding, examining whether the CNS uses a similar adaptation strategy after selective paralysis of a muscle that acts at a more proximal joint, the knee.
We denervated the knee extensor muscle vastus lateralis (VL) in rats (n=6), and examined the subsequent locomotor adaptation over the following 7 weeks. We found that the CNS compensated for the loss of VL by preferentially increasing the activity of rectus femoris (RF). Since RF acts both as a knee extensor and as a hip flexor, this strategy either requires compensation from other muscles or causes alterations in joint kinematics. We found no change in hip extensor muscle activity 7 weeks after denervation. Consistent with this lack of compensation in muscle activation, we observed extra flexion in hip angle kinematics and more extension at the ankle. On the other hand, both limb length and angle were not significantly different from pre-paralysis angles at 7 weeks, although they were altered immediately after the paralysis.
In summary, the CNS adopted a muscle coordination strategy that modified individual joint kinematics but restored global limb kinematics after the initial deficits induced by the peripheral nerve injury. These results demonstrate that this strategy of prioritizing recovery of global kinematics is general across perturbations applied to different joints and muscles, suggesting that it might reflect an important organizing principle of the neural control of movement.

Disclosures: C. Alessandro: None. **B.A. Rellinger:** None. **F. Barroso:** None. **M.C. Tresch:** None.

Poster

150. Posture and Gait: Afferent Control

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 150.09/PP1

Topic: E.06. Posture and Gait

Title: Kinematic and neuromuscular adaptation to unloaded walking

Authors: *R. KABBALIGERE[1], B.-C. LEE[2], C. S. LAYNE[1]
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Abstract: Lower body positive pressure (LBPP) treadmills can be used to unload the body by providing body weight support. Changes in sensory input and segmental kinematics associated with unloaded walking can lead to recalibration of the body schema and result in aftereffects. The main objective of this study was to identify the adaptive changes in gait produced by 30 minutes of walking at reduced body weight.
Treadmill walking at normal body weight was assessed in 12 healthy, young participants before and after 30 minutes of unloaded walking at 38% body weight in the AlterG® Anti-Gravity Treadmill®. Lower limb kinematics and temporal gait measures were obtained before and after unloaded walking. EMG of lower limb muscles was recorded. A customized weight perception scale was used to assess perception of body weight during and post unloading.
The results showed that post unloaded walking, 100% of the participants perceived their body weight to be heavier than normal. There were no changes in temporal gait parameters when compared to baseline. The ROM of ankle and knee joints was significantly reduced in the first three minutes post unloaded walking ($p<0.05$). This was accompanied by a significant reduction of EMG activity in rectus femoris (RF), more specifically during pre-swing, mid swing and terminal stance phases of the gait cycle ($p<0.05$). This pattern of reduced neuromuscular activity and kinematic ROM relative to baseline, was similar to that observed during unloaded waking and trended towards returning to baseline, although they never fully recovered at the end of 10 minutes post unloading.
Taken together, these findings suggest that walking at reduced body weight results in alterations in segmental kinematics, neuromuscular activity and perception of body weight which persists up to 10 minutes post unloading. These alterations are the aftereffects of the adaptation of the sensorimotor system to altered load-related afferent information during unloaded walking. During this process, the movement control mechanisms and body schema was updated to generate new movement patterns that represent new relationships between sensory and motor elements. These arrangements continue to persist post unloading as the sensorimotor system recalibrates back to being fully loaded. Understanding the adaptive responses of gait to unloading and the time course of the aftereffects that persist, as a result of this adaptation, has important implications for practitioners who administer gait rehabilitation using LBPP treadmills and will aid in the development of effective countermeasures against the deleterious effects of spaceflight.

Disclosures: R. Kabbaligere: None. **B. Lee:** None. **C.S. Layne:** None.

Poster

150. Posture and Gait: Afferent Control

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 150.10/PP2

Topic: E.06. Posture and Gait

Title: Gravitational unloading delays adaptation to support surface translations

Authors: *C. S. LAYNE[1], B.-C. LEE[2], R. KABBALIGERE[3]
[1]Dept Hlth. & Human Performance, Univ. Houston, Houston, TX; [3]Hlth. and Human Performance, [2]Univ. of Houston, Houston, TX

Abstract: Introduction: Lower body positive pressure (LBPP) treadmills serve to alter the sensory input and segmental kinematics associated with walking and may lead to a recalibration of the body schema associated with bipedal postural control. In addition to altered limb proprioception, it has been reported that cutaneous sensation of the soles is modified in astronauts and similar effects may occur in participants walking on LBPP treadmills. Changes in cutaneous sole sensory perception may be related to postural control aftereffects. Identifying the adaptive responses to unloading, and the time course of these aftereffects, has important implications for patient populations using LBPP treadmills for rehabilitation as well as for space scientists tasked with developing countermeasures to the negative effects of prolonged exposure to microgravity. **Methods**: Participants included 13 young, healthy individuals. The responses to a series of forward support surface translations were assessed before and after walking for 30 minutes in an AlterG® Anti-Gravity Treadmill®. Body weight for the experimental condition was 38% with the control body weight set to 100%. Posture testing consisted of 4 blocks of 5 translations; the first block performed prior to walking. Experimental conditions were randomized. On a different day, vibration perception thresholds (VPT) and touch detection thresholds (TDT) of the right sole were collected prior to and after walking. Range, RMS, and velocity of center of pressure (COP) displacement for each trial and participant were used to calculate grand means for each block. Repeated measures ANOVA and post-hoc tests were used to test for potential differences between conditions. Pearson r correlations were developed to explore potential relationships between changes in cutaneous sensation and COP measures. **Results**: Unloading walking resulted in delays in the reduction of the COP measures compared to loaded walking. After loaded walking, participants reduced their COP measures within the first block of tests while after unloaded walking, the reductions were not present until the 2nd post walking testing block. There were no changes in any foot sensitivity measure as a result of either loaded or unloaded walking and no significant relationships between sensitivity measures and COP measures. **Discussion**: The data indicate there was an aftereffect of unloaded walking that negatively influenced adaption to repeated exposure to support surface translations. The aftereffect does not result from reduced sole sensitivity input but rather from a reweighting of proprioceptive lower limb inputs that requires time to readapt to being fully loaded.

Disclosures: C.S. Layne: None. **B. Lee:** None. **R. Kabbaligere:** None.

Poster

150. Posture and Gait: Afferent Control

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 150.11/PP3

Topic: E.06. Posture and Gait

Support: NSERC Discovery Grant RGPIN 250348
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Canadian Foundation for Innovation
Ontario Innovation Fund

Title: The role of plantar-surface mechanoreception during unexpected slips

Authors: *S. D. PERRY, J. BERRIGAN, R. BILLO
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Abstract: The role of plantar-surface sensation has been shown to play a role during compensatory stepping, gait and gait perturbations. One of the main roles of sensation from the plantar-surface is to detect the distribution of pressure under each foot to maintain balance. There has been little research done on the role of plantar-surface sensation during slips. Thus the focus of this study is to determine the influence of plantar-surface sensation loss on modulation of lower limb muscle activity during the slip. There were 20 young adult participants in this study. Ten were control participants (2 males, 22 yrs old, 71 kg and 1.68 m) and 10 (3 males, 22 yrs old, 67 kg, 1.69 m) were test group participants who had reduced plantar-surface sensation (via hypothermia ice submersion). Reduced sensation was confirmed using Semmes Weinstein monofilaments to determine touch thresholds measured in grams (g) of force (control 0.98 g versus test group 32.9 g, $p<0.01$). Unexpected slips were induced by placing waxed paper beneath the high friction sand paper that was typically secured to the force platform surface. Two consecutive slips were randomly presented during multiple walking trials (at least 7 trials occurred prior to the slip trials) were used for analysis. Muscle activity was recorded from the tibialis anterior (TA), medial gastrocnemius (MG)*, rectus femoris (RF)*, biceps femoris (BF)* and peroneus longus (PL) of the slipped limb (*-indicates muscle also recorded from trial limb) to determine activation timing. Kinematic markers were placed on the participants to track foot displacement during slip. Both groups had similar number of slips (10 in the control group and 9 in the test group); however, only 3 of 10 in the control group were considered severe slips (foot displacement > 0.1 m) whereas the test group had 6 of 9 severe slips. The reduced sensation group had multiple bursts of tibialis anterior muscle activity during the midstance of the slipped foot compared to a single burst in the control group. The control group's tibialis anterior activation duration was significantly smaller than that of the test group (19.8% (SD 5.4%) of the right stance phase versus 48.7% (SD 38.6), $p<0.05$). Co-contraction, in TA-PL and RF-BF, was

also found more often in reduced sensation slips. These findings indicate that the plantar-surface sensation may play a role in detecting the severity of the slip and also providing critical sensory feedback in order to produce appropriately scaled balance responses from the lower limb muscles.

Disclosures: S.D. Perry: Other; CEO, Balancepro, Inc.. **J. Berrigan:** None. **R. Billo:** None.

Poster

151. Rhythmic Motor Pattern Generation: Connectivity

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 151.01/PP4

Topic: E.07. Rhythmic Motor Pattern Generation

Support: VR(M) Grant 2017-02944 (TD)
VR(M) Grant 21076 (PZ)
NINDs NS 090919 (OK)

Title: Role of V0 commissural interneurons in control of basic motor behaviours

Authors: M. D. G. VEMULA, V. F. LYALKA, A. E. TALPALAR, O. KIEHN, T. G. DELIAGINA, *P. V. ZELENIN
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Abstract: Commissural interneurons (CINs) are critically important for the left-right coordination in context of different motor behaviors. Two groups of V0 CINs (excitatory $V0_V$ and inhibitory $V0_D$), characterized by the early expression of the transcription factor Dbx1/Evx1, represent a major class of CINs in the spinal cord. Their role in left-right coordination during forward locomotion was demonstrated earlier. *The aim of the present study was to reveal the role of $V0_D$ and $V0_V$ neurons in control of various behaviors:* backward walking (BW), scratching, righting reflex, and postural corrections. For this purpose, wild-type mice (control) and two types of mutant mice were used: $Vglut2^{Cre};Dbx1^{DTA}$ mice with $V0_V$ CINs ablated in the entire CNS, and $Hoxb8^{Cre};Dbx1^{DTA}$ mice with all V0 CINs ablated in the spinal cord caudal to C4. Mice performing a particular task were video recorded and kinematics of movements were analyzed. In control mice performing BW the hindlimbs moved in alternation, while in both types of mutants they moved in phase similar to hopping. In control mice, unilateral stimulation of an ear elicited scratching by a hindlimb ipsilateral to the stimulation side, while in both mutants the scratching involved synchronous movements of both limbs. These results suggest that the functional effect of excitatory $V0_V$ CINs during BW and scratching is inhibitory, and that execution of scratching involves active inhibition of the contralateral scratching CPG mediated by the $V0_V$ CINs with little contribution from the $V0_D$ CINs.
After release of mice positioned upside down, both mutants and control mice exhibited two

stages of the righting reflex (assuming the dorsal side up orientation first by the fore- and then by hindquarters). In both mutants, the durations of both stages were significantly longer than in control, and in all V0-ablated mutants they were significantly longer than in $V0_V$-ones. Thus, most likely both $V0_D$ and $V0_V$ CINs contribute to generation of righting reflex.
We found that ablation of $V0_V$ CINs did not affect efficacy of postural corrections suggesting that $V0_V$ CINs do not contribute to their generation. By contrast, ablation of all V0 neurons resulted in a significant decrease in efficacy of postural corrections in hindquarters, while postural corrections in the forequarters were similar to those observed in control. These results suggest that $V0_D$ CINs transmit posture-related sensory information from the contralateral hindlimb contributing to generation of the corrective movement, and thus $V0_D$ CINs represent important elements of the spinal postural network.
Our study shows the differential contribution of V0 neuron subpopulations to diverse motor acts.

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Poster

151. Rhythmic Motor Pattern Generation: Connectivity

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 151.02/PP5

Topic: E.07. Rhythmic Motor Pattern Generation

Support: KAKENHI grantnumber 15H01587
RIKEN-DOSHISHA developmental disorder project

Title: Serotonin modulates interaction between respiration and body movement in the pons

Authors: *O. HIROTAKA, C. UCHIDA, S. TONOMURA, A. ARATA
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Abstract: The co-ordination between respiration and repetitive upper-limb movement had been studied in the medicine for physical fitness field. In the neural circuit level, respiratory rhythm is belonging to the medullary rhythm generator and body movement is belonging to the spinal rhythm generator. The co-ordination mechanism of these rhythmic centers is still unknown. On the other hand, it is known that a group of serotonergic neurons are strongly activated during rhythmic activities and modulates the rhythm generator like respiratory and/or body movement. Parabrachial nucleus (PBN) is known as the conjunctive system of medulla and spinal cord; and also known as the inspiratory-expiratory phase switching system. However, the relationship between respiratory rhythm and body movement in the pons level has not been well investigated. In this study, we analyzed the relationship between respiratory rhythm and body movement in the pons using rat pons-medulla-spinal cord preparation and medulla-spinal cord preparation;

and examined the effect of serotonin on the respiratory rhythm and body movement. In the case of the pons-medulla-spinal cord preparation, the respiratory rhythm was synchronized to body movement when serotonin was applied. On the other hand, the respiratory rhythm was not synchronized to body movement under serotonin application when pons was removed. Consequently, the relationship between respiratory rhythm and body movement was not synchronized without pons. Moreover, we examined the developmental changes of this correlation. This correlation between respiration and body movement became strong along with development. When we add the 5-HT1A blocker to the preparation with pons, respiratory-body movement correlation weakened; Therefore, the respiratory-body movement coupling was mediated by 5-HT1A receptor. In addition, we examined the distribution of optical signals in the pons triggered by body movement. We found the optical signals in the dorsal pons (probably PBN). These results suggested that body movement projected to the dorsal pons which related to inspiratory-expiratory phase switching system; and that serotonin activated the synchronization between respiratory rhythm and body movement through 5-HT1A receptor in the dorsal pons.

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Poster

151. Rhythmic Motor Pattern Generation: Connectivity

Location: SDCC Halls B-H

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Program #/Poster #: 151.03/PP6

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH Grant R01-NS26539
NIH Fellowship F32-NS083099

Title: Development of hindbrain respiratory motor circuits in larval zebrafish

Authors: *K. L. MCARTHUR, J. R. FETCHO
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Abstract: The hindbrain generates many behaviors that are critical for an animal's survival, such as feeding and breathing. These behaviors depend on neural circuits that arise early in life and are refined during development. What are the fundamental principles guiding the development of these circuits? We previously demonstrated that respiratory motor circuits in larval zebrafish (*Danio rerio*) are surprisingly robust to a dramatic disruption of normal motor neuron migration, indicating that the absolute position of hindbrain motor neurons may not be critical for their ability to receive appropriate synaptic inputs. However, we still do not know if this functional robustness is achieved by preserving an identical network structure, or by establishing a modified network still capable of generating similar behavioral output under certain conditions. Addressing this question will reveal how the developing hindbrain responds to disruption, with

implications for our understanding of both neurological disease and evolutionary change. Here, we present an exploratory study of normal respiratory circuit development in larval zebrafish, between 3 and 5 days post-fertilization. Using high speed behavioral recordings, we chart the early development and refinement of coordinated respiratory behaviors. We show that both cranial and pectoral fin activity arise by day 3, become highly coordinated by day 4, and refine their temporal patterning by day 5. Using calcium imaging to monitor neural activity of motor neuron populations, we show that facial motor neurons exhibit both rhythmic and large burst respiratory patterns by day 3, indicating that they already receive early synaptic input from premotor respiratory networks. Finally, using a combination of calcium imaging and neural tracing, we present preliminary evidence for potential premotor respiratory areas in larval zebrafish. Future studies will investigate which of these regions contribute to respiratory motor activity via direct synaptic input to facial motor neurons, and how these synaptic connections change or persist in response to early developmental disruptions.

Disclosures: K.L. McArthur: None. **J.R. Fetcho:** None.

Poster

151. Rhythmic Motor Pattern Generation: Connectivity

Location: SDCC Halls B-H

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Program #/Poster #: 151.04/PP7

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH Brain Initiative Grant UH3NS095553

Title: Thalamocortical modulation though phase-amplitude coupling in humans affected by essential tremor

Authors: *E. OPRI[1], C. G. REDDY[2], M. S. OKUN[3], K. D. FOOTE[2], A. GUNDUZ[1]
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Abstract: Coupling of neural oscillations between distal brain regions may play an important role in their communication. Low frequency oscillations (LFO) propagate more easily through brain tissue and are thought to be communication mechanism for cognitive processing during visual and motor tasks, attention, and sleep. Current evidence links the origins of LFO to deep subcortical regions (e.g., thalamus). On the other hand higher frequencies are associated with spatially localized cortical processing. High broadband frequency activity (HBFA), also known as broadband or high gamma activity, has been shown to be related to local synaptic firing. Therefore, our hypothesis is that phase amplitude coupling (PAC) between LFO and HBFA is the manifestation of how slow oscillations influence the firing rate of distal neurons by modulating their membrane potential. Previous research into thalamocortical connectivity has shown low-to-high frequency coupling during rest, but the spectral range has been limited to

frequencies lower than 40 Hz, with no investigation into behavioral related changes. Considering high gamma cortical activity to be a local modulator of a motor node, we propose that the thalamus is a cortical modulator through LFO, facilitating or inhibiting its excitability. This study is the first to show LFO-HBFA thalamocortical coupling between the thalamic ventral intermediate nucleus (VIM) and the primary motor cortex (M1). Eight patients with essential tremor (ET) and undergoing deep brain stimulation (DBS) surgery were enrolled in our study. After obtaining informed consent, as approved by University of Florida Institutional Review Board (IRB), the patients were implanted with a subdural intraoperative monitoring strip over their hand motor cortex area. In addition to the standard DBS procedure, the cortical strip placement was planned with the use of pre-operative scan (CT and MRI) and verified through functional mapping. Once the DBS lead was also in place, we proceeded to record data from both structures simultaneously. Our current results suggest that thalamocortical coupling mirrors the patient state (rest vs movement), with higher coupling during rest. Hence this suggests that PAC is a manifestation of an gating mechanism of the thalamocortical loop. These results have major implications for understanding the disease mechanisms underlying ET, which is believed to involve a wide distribution of brain regions across the motor network and to shed light on general motor behavior in humans.

Disclosures: E. Opri: None. **C.G. Reddy:** None. **M.S. Okun:** None. **K.D. Foote:** None. **A. Gunduz:** None.

Poster

151. Rhythmic Motor Pattern Generation: Connectivity

Location: SDCC Halls B-H

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Program #/Poster #: 151.05/PP8

Topic: E.07. Rhythmic Motor Pattern Generation

Support: CIHR MOP 86470
CFI AET
Edmonton University Hospital Foundation

Title: Identification of locomotor-related spinal interneurons receiving glutamatergic input from hindlimb motoneurons

Authors: V. RANCIC[1], F. HAQUE[2], K. BALLANYI[1], *S. GOSGNACH[1]
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Abstract: Historically motoneurons (MNs) have been considered to be an exclusively cholinergic population of cells which activate effector organs (i.e. muscles) as well as Renshaw cells (RCs), an interneuronal population which is responsible for recurrent inhibition of the motor pools. Recent work has demonstrated that MNs can also release the neurotransmitter

glutamate onto RCs as well as at other central synapses, and that activation or inhibition of MNs during stepping results in a disruption of the locomotor pattern. These findings have led to the suggestion that some of the interneurons receiving glutamatergic input from MNs are components of the neural network responsible for locomotor activity (i.e. the locomotor CPG). Here we use a combination of electrophysiological and imaging approaches to identify and characterize the spinal interneurons which receive glutamatergic input from motoneurons. Initial findings indicate that these cells are located in the ventral region of the spinal cord, can be either excitatory or inhibitory, and have diverse axonal projections. Finally we show that these cells are rhythmically active during fictive locomotion suggestive of a role in integrating information from hindlimb motor pools into the ongoing locomotor task. Given the fact that antidromic activation of motoneurons has been shown to initiate locomotor activity, the identification of these interneurons and their connectivity may provide key information regarding the network structure and mechanism of function of the mammalian locomotor CPG, and may also provide a means of accessing this neural network after spinal cord injury.

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Poster

151. Rhythmic Motor Pattern Generation: Connectivity

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Program #/Poster #: 151.06/PP9

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH Grant RO1 NS095366
Wings for Life

Title: Intrinsic properties and connectivity of spinal flexor and extensor rhythm generating neurons

Authors: *N. HA, L. YAO, K. DOUGHERTY
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Abstract: The central pattern generator (CPG) controlling hindlimb locomotion is located in the thoracolumbar region of the spinal cord. This CPG receives an activation signal from the brainstem but spinal CPG neurons generate the rhythm (or timing) and control the pattern of muscle activation, even in the absence of supraspinal input and sensory feedback. At the top of the CPG hierarchy are the rhythm-generating (RG) interneurons, which can convert tonic input into rhythmic output, ultimately providing drive to other downstream neurons. The mechanisms contributing to rhythmogenesis in these RG interneurons (INs) are still largely unknown, partly due to the difficulty in identifying these neurons. Findings in other systems suggest that rhythmogenesis can result from activities of individual neurons (so-called pacemaker neurons),

from recurrent excitatory connections between neurons (the emergence of network properties), or from both. Recently, Shox2 INs have been identified to be one of the cellular components of the rhythm generator for locomotion. Ablating these neurons led to a reduction of the locomotor rhythm and no change in the flexor and extensor alternation. During drug-evoked locomotion in vitro, these Shox2 INs are rhythmically active and can fire action potentials and/or have membrane oscillation in phase with the flexor-dominant ventral root bursting (flexor RG Shox2 INs) or with extensor-dominant root bursting (extensor RG Shox2 INs). The goal of the present study is to determine the underlying mechanisms contributing to the rhythmic activity of the flexor and extensor RG Shox2 INs, with focus on connectivity and intrinsic properties related to excitability and rhythmogenesis. We performed whole cell patch clamp recordings from identified Shox2 INs in reduced isolated spinal cord preparations from neonatal Shox2:Cre; tdTomato mice. We have previously demonstrated that excitatory synaptic connections and electrical coupling are present between Shox2 INs. Our data suggests that there is preferential interconnectivity within flexor and extensor RG populations, with a higher degree of connectivity between flexor Shox2 INs. Potential rhythmogenic currents such as the persistent inward current, which has been implicated in several systems as a strong contributor to rhythm generation due to its subthreshold activation and slow inactivation characteristic, are present in both the flexor and extensor Shox2 INs. Together, this suggests that both rhythmogenic currents and the interconnectivity within the flexor and within the extensor Shox2 IN populations likely contribute to rhythmic activity of spinal flexor and extensor Shox2 INs.

Disclosures: N. Ha: None. **L. Yao:** None. **K. Dougherty:** None.

Poster

151. Rhythmic Motor Pattern Generation: Connectivity

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Program #/Poster #: 151.07/PP10

Topic: E.07. Rhythmic Motor Pattern Generation

Support: CIHR
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Title: The potential roles of mouse lumbar v3 interneurons for fore hind limb coordination

Authors: *H. ZHANG, D. DESKA-GAUTHIER, Y. ZHANG
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Abstract: Trotting is the major and most stable running gaits for quadruped animals. However, the understanding of neural circuits that regulate trotting behaviours is still lacking. V3 interneurons are a major group of glutamatergic commissural neurons in the spinal cord. They

directly innervate motor neurons as well as other ventral interneurons. When we specifically deleted the expression of Vesicular Glutamate Transporter 2 (VGLUT2) in V3 INs in $Sim1^{cre/+}$;$VGluT2^{flox/flox}$ ($V3^{OFF}$)mice, we found that these mutant mice couldn't run faster than 40 cm/s on the treadmill, at which speed wildtype mice trot constantly. However, $V3^{OFF}$ mice couldn't trot properly due to the lack of the capacity to precisely synchronize their diagonal limbs while running. Optical stimulation of V3 INs in the lumbar segments of isolated spinal cord of the neonatal $Sim1^{cre/+}$;$Rosa26^{ChR2/+}$ mouse could simultaneously evoke activities at lumbar and cervical ventral roots. Blocking glutamatergic synapses exclusively in the lumbar region didn't not prevent this light-induced cervical activity, suggesting that lumbar V3 interneurons could directly innervate cervical motor circuits. Using retrograde tracer, cholera toxin subunit B, injected in the cervical motor region, we then identified clusters of V3 INs within dorsal and intermediate spinal laminae in higher lumbar segments that had long ascending projections to cervical region. Next, we observed that the number of c-Fos protein expressing lumbocervical projecting V3 INs significantly increased after running at 40cm/s on the treadmill, compared to that after walking at low speeds, indicating that these long projecting V3 INs were highly active during trotting. Taken together, we propose that lumbocervical projecting V3 interneurons provide a direct excitatory drive to cervical locomotor networks during medium locomotor speeds essential for the transition from a walking to a trotting gait.

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Poster

151. Rhythmic Motor Pattern Generation: Connectivity

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Program #/Poster #: 151.08/PP11

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH Grant R35 NS097343

Title: Inference of receptor distribution on single neurons from focal glutamate uncaging and synaptic immunohistochemistry

Authors: *J. PIPKIN[1], A. G. OTOPALIK[2], E. E. MARDER[3]
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Abstract: Synaptic communication is thought to be spatially confined to the specific sites where the presynaptic cells vesicles release their content onto the postsynaptic cell. The area of the postsynaptic membrane opposite the presynaptic cell is thought to have a higher density of neurotransmitter receptors. However, this does not mean receptors cannot be found extrasynaptically. In the course of performing focal glutamate uncaging experiments on dye-

filled single neurons in the stomatogastric ganglion of the crab Cancer borealis, we observed that nearly every uncaging site on a given neuron's arbor was responsive, producing a hyperpolarization measured in the soma. Were postsynaptic sites so numerous that each uncaging location overlapped with one or more? Or do some uncaging sites appear to produce a response even in the absence of any obvious nearby location for the neuron to have concentrated glutamate receptors? To address these questions, we post-stained preparations used for glutamate uncaging for a marker of synapsin, and manually reconstructed the branches, nearby presynaptic puncta, and uncaging sites for 185 uncaging sites across 8 preparations. Across the 35 branches reconstructed (each branch had 4-9 uncaging sites), we found 210 presynaptic puncta within 1.5 microns and 61 within 250 nanometers. We found no relationship between the magnitude of the uncaging-evoked response and the presence of nearby presynaptic puncta. Many sites even responded despite the absence of any puncta nearby the branch (42/48 sites where there were 0 nearby puncta produced a measurable response). We infer that glutamate receptors must be broadly distributed throughout the arbors of these neurons and not concentrated solely at synaptic sites. Further evidence, implications, and alternate explanations are discussed.

Disclosures: J. Pipkin: None. **A.G. Otopalik:** None. **E.E. Marder:** None.

Poster

151. Rhythmic Motor Pattern Generation: Connectivity

Location: SDCC Halls B-H

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Topic: E.07. Rhythmic Motor Pattern Generation

Support: R01 NS 077986
R21 NS101441

Title: Anatomical and molecular deconstruction of the whisking central pattern generator and sensory feedback premotor circuits

Authors: *J. TAKATOH[1], V. PREVOSTO[2], J. LU[3], S. ZHAO[1], B.-X. HAN[1], F. WANG[4]
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Abstract: Animals actively move their sensory organs to probe the external environment. The movements of sensory organs are continuously updated by online sensory feedback and influenced by animal’s internal state. Rodents actively and repetitively sweep their whiskers (whisking) to explorer their nearby physical environment. Depending on contexts, they modulate the patterns of whisking, in terms of frequency, amplitude and setpoint. However, the neural circuits through which the external and internal states shape whisking kinetics and patterns remains unclear.Here we use a split-Cre based genetic-viral intersectional strategy to characterize

whisking premotor neurons in two brainstem regions: in the trigeminal nucleus interpolaris and in the vibrissal intermediate reticular nucleus (vIRt) which contains rhythm generator neurons for whisking. Chemicogenetic and/or optogenetic silencing of each group of premotor neurons revealed their critical roles in transmitting sensory feedback, or in rhythm generation, respectively. Optogenetic-tagged in vivo recording in awake behaving animals further show the in vivo activity patterns of those two groups of whisker premotor neurons. Using monosynaptic rabies virus based transsynaptic tracing, we also uncovered the input sources to these premotor neurons. Our results revealed the function and presynaptic circuits of whisking sensory feedback and central rhythm generator premotor neurons and lay the foundation for understanding how behavior tasks and contexts modulate movements of whiskers.

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Poster

151. Rhythmic Motor Pattern Generation: Connectivity

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Topic: E.07. Rhythmic Motor Pattern Generation

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Title: Dynamical invariants underlying robustness and flexibility in sequential neural dynamics

Authors: *I. ELICES[1], M. REYES-SANCHEZ[1], R. AMADUCCI[1], R. LEVI[2], F. B. RODRIGUEZ[1], P. VARONA[1]
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Abstract: Experimental and theoretical studies show that the generation of precise temporal sequences is a fundamental computational phenomenon in the nervous system. Therefore discovering general principles in the coordination and execution of robust neural sequences is an important step to relate neural activity to function and has a large potential impact in other fields such as robotics, control theory or rehabilitation technology.
In this experimental and model work, we used a novel time reference frame to analyze in a cycle-by-cycle basis regular and irregular pyloric central pattern generator (CPG) rhythms. We report two dynamical invariants in the form of linear correlations that are solidly conserved within intrinsic and ethanol induced variability in long intracellular recordings. We characterize these invariants and relate them to the balance between the robustness of the sequential neuron

activations and the flexibility of key phases arising from the asymmetric closed-loop interactions as well as the rich intrinsic dynamics of the neurons. Our work on cycle-by-cycle rhythm negotiation goes beyond previous studies on approximate CPG phase maintenance based in cross-preparation average analysis where important aspects of transient dynamics are typically disregarded. We hypothesize that the found robust dynamical CPG invariants participate in the instantaneous muscle coordination, and therefore can be related to the efficient cycle-by-cycle performance of motor activity in different contexts.
Standard conductance-based models of the pyloric circuit mimicking the connection asymmetry cannot reproduce the reported dynamical invariants. To address the missing model dynamics shaping the invariants, we have designed and built hybrid circuits by connecting neuron models to the living CPG circuit using real-time dynamic clamp technology. Connections to/from the CPG neurons were implemented with realistic models of fast and slow graded inhibitory synapses. The analysis of these hybrid interactions indicate that the dynamical invariants arise between living and model neurons as a function of the synaptic parameters. Dynamical invariants can be propagated through the artificial synapses but only in bidirectional configurations or in monodirectional connections from biological to model neurons. This work opens new perspectives for ongoing efforts in understanding the mechanisms for generation and real-time coordination of sequences of neural activations, from simple invertebrate circuits to vertebrate nervous systems.

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Poster

151. Rhythmic Motor Pattern Generation: Connectivity

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Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH grant NS101356
CNPq Science without Borders Scholarship 203059/2014-0

Title: Opto-anatomical neuron identification in the buccal ganglia of *Aplysia*

Authors: *R. M. COSTA, C. L. NEVEU[1], R. HOMMA[2], S. NAGAYAMA[2], D. A. BAXTER[2], J. H. BYRNE[2]
[1]Physiol., Northwestern Univ., Chicago, IL; [2]Neurobio. and Anat., The Univ. of Texas Hlth. Sci. Ctr. at H, Houston, TX

Abstract: Recently, voltage-sensitive dye (VSD) recordings have been used to monitor population dynamics underlying motor pattern generation in *Aplysia* (Neveu et al. 2017; Bruno et

al. 2017). However, to fully leverage the advantages of VSD recordings it is necessary to gather sufficient information to identify specific neuronal elements across preparations. But capturing such correspondences is challenging. Here, we show that combining VSD recordings with stimulation of peripheral nerves can aid in identifying specific cells. VSD imaging was used to monitor neural activity in the caudal surface of the buccal ganglia following stimulation of peripheral nerves. An average of 171.0 ± 9.7 (mean ± standard error; n = 10) neurons were distinguishable per hemiganglion. Nerve stimulation elicited short latency (≤ 10 ms) spike activity in 44.2 ± 5.2 cells, which suggests that these 44 cells have peripheral projections. Other neurons exhibited longer latency, presumably synaptically mediated, responses. We compared size, position, and nerve projections of the 44 cells to the properties of previously characterized neurons. Previous studies identified 18 cells with peripheral projections and with somata on the caudal surface. Because VSD recordings indicated that 44 cells respond to nerve stimulation, the data suggest that ~26 neurons have yet to be characterized by conventional electrophysiological approaches. To further characterize stimulation-responsive cells, we are analyzing their activity during motor pattern generation. In addition, we are blocking synaptic transmission with a high-Mg^{2+}/low-Ca^{2+} solution in order to differentiate direct projections from synaptic input. Ultimately, this approach will allow neurons from each preparation to be represented in a feature space comprising anatomical and functional features such as size, position, nerve projections, synaptic input from nerve stimulation, firing frequency during motor patterns, and timing of activity. We anticipate that this rich feature space, alone or when processed with machine learning approaches (e.g., Frady et al. 2016), will increase the accuracy and extent of neuron identification, thereby making a significant stride toward a more complete characterization of this system.

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Poster

151. Rhythmic Motor Pattern Generation: Connectivity

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Program #/Poster #: 151.12/PP15

Topic: E.08. Respiratory Regulation

Support: NIH R01 104127 (PI: Del Negro)

Title: Burstlet hypothesis for inspiratory rhythm generation: Are rhythm and pattern generation separate mechanisms?

Authors: *P. KALLURKAR, M. C. D. PICARDO, C. A. DEL NEGRO
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Abstract: Breathing is a vital rhythmic behavior that originates due to a central pattern generator network in the lower brainstem. The core oscillator for inspiration is the preBötzinger complex (preBötC), which consists of a cluster of excitatory (glutamatergic) interneurons that generate the neural rhythm and basic motor pattern for inspiratory breathing movements in mammals. Neural bursts in the preBötC are critical for the inspiratory motor output pattern, but their role in rhythmogenesis is less clear. Here we test 'burstlet' theory, first proposed by Kam & Feldman (*J Neurosci* 33: 9235, 2013) which hypothesizes that the neural mechanisms of inspiratory rhythm generation depend on a lower level of neural activity dubbed a 'burstlet', which results from recurrent synaptic excitation among preBötC neurons but is not a bona fide 'burst' per se. Under normal circumstances, burstlets putatively underlie the preinspiratory phase of activity that triggers a suprathreshold process to transform burstlets into bursts as well as drive motor output. Here we tested these basic tenets of burstlet theory. We isolated the preBötC in slice preparations that remain rhythmically active and generate inspiratory breathing-related motor output that is measurable via the hypoglossal cranial nerve (XII, also retained in slices). Under standard conditions *in vitro* (where the Ringer solution contains 9 mM K^+), the field recordings in the preBötC neural activity consist of bursts and concurrent XII motor output, intermingled with lower amplitude preBötC burstlets, that do not produce XII motor output. At the lowest levels of excitability (where the Ringer solution contains 3 mM K^+) the rhythm consists mainly of burstlets, with few bursts and concurrent XII motor output. The relative fraction of bursts and XII motor output increases monotonically as K^+ concentration is raised from 3 to 9 mM. We found that burst and burstlet rhythms were voltage-dependent, graded increases in external K^+ concentration increased the frequency of bursts and burstlets in parallel. These data show that Kam & Feldman's burstlet theory is a viable explanation for inspiratory rhythm, which may be independent of full amplitude inspiratory neural bursts that drive motor output. Our data further demonstrate that burstlet rhythms are voltage dependent, which will help define the cellular and synaptic mechanisms that underlie burstlet rhythmogenesis.

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Poster

151. Rhythmic Motor Pattern Generation: Connectivity

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Program #/Poster #: 151.13/PP16

Topic: E.07. Rhythmic Motor Pattern Generation

Support: SCI.CHE.10.HFSP

Title: Mapping connectivity of sensory neurons linking cerebrospinal fluid to motor circuits in vertebrates

Authors: *M.-Y. WU, K. FIDELIN, M. CARBÓ-TANO, A. PRENDERGAST, B. P.-E. TSENG, P. GARNERET, C. WYART
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Abstract: The cerebrospinal fluid (CSF) is a complex solution circulating around the brain and spinal cord. Although development and function of the nervous system are influenced by the content and flow of the CSF, the underlying mechanisms are elusive. CSF-contacting neurons (CSF-cN) by their location at the interface between the CSF and the nervous system are in ideal position to sense CSF cues and to relay information to local networks. We previously demonstrated that the CSF-cNs detect local bending of the spinal cord and in turn, feedback GABAergic inhibition to excitatory interneurons driving slow locomotion and motor neurons controlling posture in the ventral spinal cord. Here we performed high throughput quantitative behavior analysis in animals where the output of these cells is silenced to show that CSF-cNs perform a speed-dependent modulation of locomotion. While these sensory cells decrease locomotor frequency in the slow locomotor regime, they increase locomotor frequency in the fast locomotor regime. The neuronal targets we previously identified in the spinal cord cannot explain such effects on locomotor speed. By taking advantage of the transparency of zebrafish larva, we investigated here CSF-cN projections in the hindbrain and found their ascending axons arborize onto the occipital and pectoral motor neurons, involved in the control of fins and posture. In addition, we discovered that CSF-cNs form axo-axonic varicosities onto descending fibers from reticulospinal neurons, revealing that these sensory neurons can provide a feedback on the motor command transmitted by the brain to the spinal cord. Within the ventral spinal cord, we identified that CSF-cNs form axo-axonic varicosities on descending axons from V2a interneurons and somatic varicosities on V3 interneurons, enabling a local modulation of premotor excitation. To investigate the physiology of these putative connections, we are now combining optogenetics, patterned light for cell-targeted photostimulation, in vivo whole cell recording of CSF-cN targets and CSF-cN cell-specific ablation. Altogether, this body of work sheds light on the cellular and network mechanisms enabling sensorimotor integration of mechanical and chemical cues from the CSF onto distributed motor circuits controlling locomotion and posture in hindbrain and spinal cord.

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Poster

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Program #/Poster #: 151.14/PP17

Topic: E.07. Rhythmic Motor Pattern Generation

Support: Wellcome Trust Institutional Strategic Support Fund
Kaunas Industrial Water Supply
Howard Hughes Medical Institute, Janelia Research Campus

Title: Neural control of direction of movement in *Drosophila melanogaster* larvae

Authors: *J. JONAITIS[1], A. HIRAMOTO[2], J. MACLEOD[1], K. HIBBARD[3], A. CARDONA[3], J. W. TRUMAN[4], A. NOSE[2], S. R. PULVER[1]
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Abstract: Animals transition from one type of behavior to another using shared neuromuscular components. Neural networks responsible for these transitions and the cellular mechanisms underlying transitions are not fully understood. The *Drosophila* larval locomotor system is an attractive model to address these problems. Here we explore the role that a group of ascending interneurons play in triggering motor programme transitions. A19f interneurons have cell bodies in all abdominal and 2 thoracic segments of the ventral nerve cord (VNC). Each cell has neurites in the corresponding hemi-segment, and ipsilateral projections which terminate in thoracic segment 1. Reconstruction of synaptic partners within an existing EM database revealed that A19f neurons are not directly connected to motor or sensory neurons, are highly connected to each other, and have a variety of postsynaptic partners that project to both the ventral nerve cord and brain. We used the split gal4 system to create a construct that drives gal4 expression in A19f cells. We imaged spontaneous activity of motorneurons and A19f cells in the isolated central nervous system using the genetically encoded calcium indicator GCAMP6f. A19f neurons are recruited into both forward and backward fictive locomotion and they follow motorneuron activity within each segment. Optogenetic activation of A19f cells with CsChrimson in behaving animals triggered a short pause during both forward and backwards locomotion. Next we activated random subsets of A19f cells using a construct in which CsChrimson expression is controlled by heatshock mediated flippase expression. We found that pauses in locomotion can be triggered by as little as two A19f cells. A19f cells labeled positive for Choline Acetyl transferase (ChAT) and negative for gamma-Aminobutyric acid (Gaba) in immunocytochemical experiments, suggesting that they are cholinergic excitatory interneurons. Current work is focused on dissecting the role of inhibitory premotor neurons downstream from A19f that may be inhibiting motor neuron activity. Overall, this work provides a basis for uncovering circuit motifs that facilitate transitions amongst motor programs.

Disclosures: J. Jonaitis: A. Employment/Salary (full or part-time):; University of St Andrews. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Wellcome Trust Institutional Strategic Support Fund, Kaunas Industrial Water Supply. **A. Hiramoto:** None. **J. MacLeod:** None. **K. Hibbard:** None. **A. Cardona:** None. **J.W. Truman:** None. **A. Nose:** None. **S.R. Pulver:** None.

Poster

151. Rhythmic Motor Pattern Generation: Connectivity

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 151.15/PP18

Topic: E.07. Rhythmic Motor Pattern Generation

Title: Endogenously oscillating excitatory motoneurons produce undulatory output in a model of C. elegans without proprioception

Authors: *G. HASPEL[1], H. ANWAR[1], L. DENG[1], J. E. DENHAM[3], T. RANNER[3], N. COHEN[3], C. O. DIEKMAN[2]
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Abstract: Neuronal oscillators underlie rhythmic behavior, and particularly locomotion, in all animals in which the neural mechanism has been determined. However, despite the availability of a connectome, the neuronal mechanism underlying undulatory locomotion in the nematode *Caenorhabditis elegans* is not known. Hypotheses have included sensory feedback and neuronal oscillators. We took a computational approach to find a minimal set of conditions for central pattern generation by a chain of oscillators along the body, in the absence of proprioceptive feedback.
We recently described how the existing *C. elegans* connectivity data can be extrapolated into a complete neuromuscular network by identifying connectivity rules. Here we use an extrapolated network that spans the full length of an animal and includes seven classes of motoneurons, muscle cells, and synaptic connections, both chemical and electrical. We populate our network model with two kinds of motoneurons and muscle cells: leaky (passive) and endogenously oscillating (pacemaker) and systematically screened all 2^7=128 combinations of leaky vs pacemaker motoneuron classes. Within each combination, we screened parameter space and used an evolutionary simulation approach to search for synaptic weights that produce a propagating dorsoventral alternation of muscular activity in forward or backward directions. The opposing directions of locomotion are induced by adding a tonic current to targets of the forward or backward pools of premotor interneurons targets. Successful fictive patterns were fed into a neuromechanical model to interpret the locomotion behavior.
An undulatory pattern in both forward and backward directions was not generated when all motoneuron classes were passive or when all motoneuron classes were endogenous oscillators. Several combinations in which some excitatory motoneurons are oscillators produced undulatory-like motor programs in both forward and backward directions. Notably, a ventral excitatory class of motoneurons and a dorsal excitatory class are represented in all successful combinations.

Disclosures: G. Haspel: None. **H. Anwar:** None. **L. Deng:** None. **J.E. Denham:** None. **T. Ranner:** None. **N. Cohen:** None. **C.O. Diekman:** None.

Poster

151. Rhythmic Motor Pattern Generation: Connectivity

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 151.16/PP19

Topic: E.07. Rhythmic Motor Pattern Generation

Support: Killam Level 2 Predoctoral Scholarship
The Natural Sciences and Engineering Research Council of Canada
Dalhousie University

Title: Discrete spinal V3 interneuron subpopulations are specified through temporally and molecularly distinct developmental pathways

Authors: *D. A. DESKA-GAUTHIER[1], H. ZHANG[1], L. BENNETT[1], C. JONES[1], J. B. BIKOFF[2], Y. ZHANG[1]
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Abstract: Coordinated motor control relies on the appropriate recruitment of distinct interneuron populations within the spinal cord. The cardinal class of excitatory V3 spinal interneurons are crucial for generating robust and stable locomotion, yet their molecular heterogeneity, circuit organization, and functional outputs have remained elusive. Here we define three distinct V3 subpopulations that diversify along temporally and molecularly unique developmental pathways. Nr3b3- and Onecut2-expressing V3 interneurons emerge together during an early wave of V3 neurogenesis and require Sim1 for molecular, anatomical and electrophysiological specification at post-mitotic stages. In contrast, Prox1-expressing V3 interneurons emerge later and post-mitotically differentiate independent of Sim1. Respective Nr3b3-, Onecut2- and Prox1-expressing V3 subpopulations display distinct laminar settling positions, axon projection profiles, sensory innervations and task-specific recruitment patterns during motor control. Taken together, this work is the first to reveal distinct V3 subpopulations and the developmental logic guiding their diversification.

Disclosures: D.A. Deska-Gauthier: None. **H. Zhang:** None. **L. Bennett:** None. **C. Jones:** None. **J.B. Bikoff:** None. **Y. Zhang:** None.

Poster

151. Rhythmic Motor Pattern Generation: Connectivity

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 151.17/PP20

Topic: E.07. Rhythmic Motor Pattern Generation

Title: Using Fos-TRAP model to compare mice lumbar spinal cord neuronal activations when stepping with and without incline

Authors: *J. LUO[1], H. ANAND[3], B. N. PHAM, JR[2], V. EDGERTON[4]
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Abstract: Using c-fos as an activity dependent marker, more interneuron activation was seen when rats stepped on 25-degree incline than without incline (Tillakaratne et al., 2014). The objective of our study is to validate a new transgenic mouse model, Targeted Recombination in Activated Populations (TRAP), by comparing with this past study. The TRAP model distinguishes two separate c-fos activation patterns from two time points in the same mouse. The first activity is characterized by tdTomato expression that labels activated neurons and their projection throughout the animal's lifetime. The later activity is characterized by c-fos expression, labelling the neuronal cell body 1 hour after activity. The TRAP model has previously been used in the brain but has not been applied to the spinal cord (Guenthner et al., 2013). In this experiment, we randomly divided TRAP mice into two groups. The first group (n = 3) stepped on 0 incline for the first session and 15-degree incline for the second, with both session lasting 30 minutes and the speed being 25 cm/s. The second group (n = 10) completed the same task in reversed order. Mice were perfused after the second session and had their spinal cord dissected for immunohistochemical staining for c-fos and tdTomato expression. Most tdTomato expressing neurons were in dorsal horn, and the amount of neuronal labelling in mice running with or without incline were not significantly different in L4 and L5 spinal segments. The amount of c-Fos expressions for both activities were similar comparing to the corresponding tdTomato expressions. However, c-Fos expressing neurons located mostly in lamina IV to VII. One possibility is that the spatial distribution of neurons showing tdTomato expression may differ. Another interpretation is that the sensitivity of the two techniques in identifying a given level of neuronal activity differs substantially. Using specific interneuron markers, we can identify certain classes of interneurons involved in locomotion and their projections such as Chx-10 marker for V2a interneurons that modulate left-right coordination (Kiehn, 2016). With the aid of tissue clearing technique, CLARITY, we can obtain a 3-dimentional view of activated neuron circuitry in TRAP animals during locomotion (Chung and Deisseroth, 2013). This Fos-TRAP model can also be used in comparing neuronal circuit activation prior and post spinal cord injury

to provide a target to epidural stimulation, which has been shown to have effect on restoring some motor function post spinal cord injury in rats and humans (Gerasimenko et al., 2007).

Disclosures: J. Luo: None. **H. Anand:** None. **B.N. Pham:** None. **V. Edgerton:** None.

Poster

152. Neuroethology: Sensory and Motor Systems

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 152.01/PP21

Topic: F.01. Neuroethology

Support: WVCTSI grant U54GM104942
DFG grant BU 857-14
EICCI from the Cluster of Excellence 227 (CITEC), DFG
NSF grant MRI 0959012

Title: Contribution of force dynamics (dF/dt) to activation of muscle synergies in insect legs

Authors: *S. N. ZILL[1], C. HARRIS[1], C. J. DALLMANN[2], J. SCHMITZ[3], S. CHAUDHRY[1], A. BUSCHGES[4]
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Abstract: Feedback from sense organs that monitor forces in the legs of both vertebrates and invertebrates has been shown to fulfill diverse functions, including tuning motor outputs to variations in load and contributing to the activation of groups of muscles as synergists. However, the specific parameters that underlie the transform from force detection to the modification of motor output remain unclear. Previous studies in stick insects showed that forces applied to the legs could activate muscle synergies that generate substrate adhesion and support. However, substantial adaptation of discharges in synergist muscles could occur when forces were applied using linear ramp-and-hold stimuli. Adaptation was significantly reduced when forces were applied using non-linear stimuli whose time profiles and magnitudes were based on joint torques calculated in freely walking animals. Further analysis has suggested that reduction in adaptation to torque stimuli is associated with sustained positive increases in the rate of change of force (dF/dt). In the present experiments, we are examining the response properties and motor effects of campaniform sensilla, receptors that provide feedback by monitoring forces as strains in the exoskeleton, to further characterize the encoding of linear and non-linear forces. Campaniform sensilla show phasicotonic discharges to forces applied using ramp-and-hold stimuli that can be analyzed as discrete static and dynamic components. Receptor sensitivities to force dynamics can be readily fit to power functions (y=ax^b) but different constant and exponent values are

obtained at different force magnitudes. Current experiments are systematically testing the effects of varying force amplitude on rate sensitivities with the goal of generating sets of power functions to delineate the effects of force magnitude on dynamic sensitivities. In addition, these studies have shown that use of torque waveforms with gradual increases in dF/dt significantly decreases adaption in sensory discharges that occurs to stimuli with a static hold phase. Furthermore, these tests indicate that use of torque waveforms also reduces the effects of viscoelasticity in the cuticle, which produces 'creep' during hold stimuli. We plan to extend tests to different groups of campaniform sensilla in cockroaches and stick insects to characterize the relationships between dynamics and magnitude in responses to forces similar to those generated by the animal. Our findings are consistent with the ideas that phasic and static characteristics do not function as separate components and that dynamic sensitivities play a major role in the generation of motor behavior.

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Poster

152. Neuroethology: Sensory and Motor Systems

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 152.02/PP22

Topic: F.01. Neuroethology

Title: Escape responses from looming stimuli in the jumping spider, phidippus regius

Authors: *V. A. GAUDIN, C. L. CLELAND
James Madison Univ., Harrisonburg, VA

Abstract: Animals use withdrawal and escape responses to retreat from threats. Looming stimuli, which represent the approach of a predator, evoke an escape response in jumping spiders that is mediated primarily by visual cues. The majority of studies have focused on spider predation toward prey; only limited studies have explored the escape response. The goal of our research was to determine the strategy used by jumping spiders, *Phidippus regius*, to escape from looming stimuli. In particular, we sought to determine whether jumping spiders employ jumping as part of their escape repertoire. Looming stimuli were created by using the controlled projection of a 3" black polyurethane ball (1 m/s,45 degrees angle, against a white background) toward jumping spiders (*Phidippus regius*, n=9*)*, without actually hitting the spider. The direction of "attack" was varied in 45-degree increments, totaling 8 angles, around the spider. The resulting response was captured from above with high speed video (300 fps) and automated software particle tracking was used to quantify the location and orientation of the spider throughout the escape response. Looming stimuli consistently evoked translation, but not turning. The angle of translation depended significantly on stimulus direction. Typically,

following initial translation the spider executed one or two movements that appeared linked to the stimulus, often ending in a position that faced the looming object. Importantly, three of the spiders sometimes jumped away from the looming stimulus, a movement previously reported only associated with prey capture. These preliminary results suggest jumping spiders may use specific, diverse, multi-stage strategies to escape from looming stimuli. Further, their name-sake behavior, jumping, may be employed for both predation and escape.

Disclosures: V.A. Gaudin: None. **C.L. Cleland:** None.

Poster

152. Neuroethology: Sensory and Motor Systems

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 152.03/QQ1

Topic: F.01. Neuroethology

Support: MEXT KAKENHI 16H06544

Title: Stimulus-intensity impacts on direction encoding and its temporal dynamics in insect mechanosensory projection neurons

Authors: *H. OGAWA[1], K. TANAKA[2], M. SOMEYA[2], H. SHIDARA[1]
[1]Dept. of Biol. Sciences, Fac. of Sci., [2]Grad. Sch. of Life Sci., Hokkaido Univ., Sapporo, Japan

Abstract: Tuning property of stimulus parameters in sensory neurons is crucial for readout of sensory information. Many neurophysiological studies have reported that the tuning property could be modulated depending on the intensity of sensory stimulus. However, it is still unknown how the stimulus-intensity-dependent modulation in the tuning property of individual cells is correlated with the readout accuracy and readout time. We addressed this question by insect mechanosensory system in which several cells are identified and their response properties are unique at the same processing level. In this study, we used cricket cercal sensory system to detect airflow dynamics. The directional information of airflow is processed by local circuit within the terminal abdominal ganglion and conveyed to the brain via several identified projection neurons such as the giant interneurons (GIs). Each GI shows different sensitivity to the direction of airflow in preferred angle and in sharpness of the selectivity. The previous study revealed that the airflow direction is encoded by population activity of two pairs of GIs (Miller and Theunissen, 1991). In the previous studies, the direction selectivity was just measured as total spike counts during stimulus, and it has not been tested whether the directional tuning depends on the stimulus intensity or not. Here, we measured the spike responses of individual GIs to airflow stimulus of various velocities from different angles using intracellular recording method. And, the directional tuning properties were analyzed for different readout time. The modulation of the direction selectivity index (DSI) based on the total spike counts were different from that

based on peak firing-rate in terms of their dependencies on the stimulus velocity. Furthermore, these velocity-dependent modulations were varied between GI types. For example, in GI 10-2, the sharpness of direction selectivity was unchanged according to stimulus velocity. In GI 9-1, DSI value based on total counts was declined depending on the velocity but that based on the peak firing-rate was unchanged. This result suggests that encoding of the stimulus direction in GIs is different in the robustness against the stimulus intensity among the cells which play different roles in sensory processing.

Disclosures: H. Ogawa: None. **K. Tanaka:** None. **M. Someya:** None. **H. Shidara:** None.

Poster

152. Neuroethology: Sensory and Motor Systems

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 152.04/QQ2

Topic: F.01. Neuroethology

Title: Antennae pointing during the escape response in the cricket, acheta domesticus

Authors: *A. M. ZEHER, C. L. CLELAND
James Madison Univ., Harrisonburg, VA

Abstract: In the cricket, the escape response is primarily directed by cercal detection of incoming wind stimuli, though previous experiments showed that vision may play a role. For looming stimuli, the direction of the stimuli affect the direction and magnitude of the escape response. The escape response is often accompanied by the pointing of an antennae toward the incoming object. To date, little research has been done on antennae pointing and its relationship to the properties of the stimulus and escape movement. The goal of this research focuses on determining the characteristics and primary sensory modality responsible for antennae pointing to looming stimuli in *Acheta domesticus*. Looming stimuli were presented by a 3” black polyurethane ball projected (1 m/s, 45 degrees) toward the cricket from eight circumferential directions. The cricket was placed into a rotatable arena of white canvas surrounded by roof flashing. Escape responses and antennae orientations were recorded using high-speed video (650fps) and manually tracked in motion analysis software. Preliminary findings showed that pointing usually (~70 %) accompanied the escape response. Although pointing occurred for all directions of the stimulus, stimuli presented from the posterior end of the cricket often resulted in running or jumping with little movement of the antennae, while anterior stimuli resulted in a turn and run, with more frequent antennae pointing. We noticed that when antennae pointing occurred there was an attempt by the cricket to maintain the pointing throughout escape turning. Pointing appeared to occur in only one antenna at a time, with a preference for the one positioned on the side closest to the incoming looming stimulus. In particular for stimuli presented posteriorly, in which there was no preferred antenna, still only one antenna pointed; there was no evidence that

both antennae pointed. Further studies will identify the relative contributions of vision and wind through the use of vision-only (video) and wind-only (white balls against a white background) stimuli as well as more fully characterize the dependence on stimulus direction and correlation to sensory or movement variables.

Disclosures: A.M. Zeher: None. **C.L. Cleland:** None.

Poster

152. Neuroethology: Sensory and Motor Systems

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 152.05/QQ3

Topic: F.01. Neuroethology

Support: AU Office of Research FY2018 201160

Title: Neural plasticity and behavioral changes as a result of male-exposure in females of an insect model

Authors: *B. A. NAVIA, S. DOSUNMU, C. R. KENT
Dept. of Biol., Andrews Univ., Berrien Springs, MI

Abstract: Syllable-period selective phonotaxis in female cricket (*Acheta domesticus)* as well as the corresponding response of neural elements such as the AN1/L1, AN2/L3 and ON1 neurons (which have been demonstrated to influence phonotaxis) have been the focus of multiple studies. Such studies, have reported individual variability in both responses (behavioral and neuronal). However, differences in their responses based on age are typical for this species. The behavioral and neuronal responses reported in the literature correlated, and ranged from selective to unselective for young and old females respectively. The subjects of all such studies have been virgin females raised in isolation. The current exploratory project investigates the apparent influence in the phonotactic and neuronal responses of male-exposed females of different ages. It had been proposed that the presence of males would significantly reduce selective phonotaxis in females in response to model calls. Preliminary results suggest that in contrast with previous studies, there does not seem to be an age correlation in the phonotactic response exhibited by male-expose females. Regardless of age, male-exposed females do not seem to discriminate between an attractive or unattractive model call and respond to a similar number of syllable periods tested. Additionally, intensity of the call may also affect syllable-period selective phonotaxis in these females. The potential effects of male exposure in the response of prothoracic auditory interneurons such as AN2/L3 are unknown. When presented with attractive calls only, L3s in young virgin females exhibit decrement (reduction in the number of action potentials to consecutive sound pulses within a chirp; and thus is syllable-period selective). AN2/L3s in old virgin females exhibit significantly lower levels of decrement in their responses.

Preliminary results, suggest that regardless of age, AN2/L3s of male-exposed females exhibit similar levels of decrement in response to auditory stimuli, irrespective of syllable period. Exposure to males, seems to affect the females'underlying neuronal connections, which influence recognition and selective phonotaxis, both of which are crucial for reproduction. Additional implications are discussed.

Disclosures: B.A. Navia: None. **S. Dosunmu:** None. **C.R. Kent:** None.

Poster

152. Neuroethology: Sensory and Motor Systems

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 152.06/QQ4

Topic: F.01. Neuroethology

Support: MEXT KAKENHI 16H06544

Title: Trade-off between speed, directional accuracy and behavioral flexibility in action selection of the escape behavior in the cricket

Authors: *N. SATO[1], H. SHIDARA[2], H. OGAWA[2]
[1]Grad. Sch. of Life Sci., [2]Dept. of Biol. Sciences, Fac. of Sci., Hokkaido Univ., Sapporo, Japan

Abstract: In face of danger such as predator's attack, choice of reaction is critical for animals to survive. The escape behavior is effective to avoid approaching predator in various species including vertebrates and invertebrates (Domenici et al., 2011). In some species, the animals use multiple behavioral options as escape strategies and change the choice of escape strategies appropriately according to types of predators or approaches (von Reyn et al., 2014), suggesting that each escape strategy have distinct benefits for successful escape. However, it remains unknown what behavioral trade-off affects the action selection in the escape behavior. A field cricket, *Gryllus bimaculatus*, detects short airflow as predator's approach by the cercal sensory system and displays two different escape reactions, running and jump (Tauber and Camhi, 1995, Sato et al., 2017). In this study, we firstly hypothesized that the crickets chose the escape reactions based on trade-off between the speed and directional accuracy; crickets may escape faster and further away, while their moving direction would be more uncontrollable in jump than running. To test this hypothesis, we quantitatively compared running and jump in several locomotor parameters including moving velocity, distance, reaction time, and angular control of moving direction. This hypothesis, however, was rejected because the moving direction was controlled in jump as accurately as in running. Even in the jump, crickets can move faster, further, and more quickly than running toward the accurately opposite direction to the stimulus. These results seem that jump is more appropriate for escape, but the jump probability was saturated at about 50 %, meaning that the running will be a default choice for the cricket escape

behavior. We assumed that this is because the running is more flexible for escape. For example, the crickets that exhibited jump response to the 'first attack' of the predator might be unable to respond to the 'second attack'. Thus, we applied two successive air puffs in short interval. The response probability to the second puff was lower in the crickets jumping in response to the first puff than the running crickets. These results suggest that crickets choose their escape reaction based on the complex trade-off in motor performance.

Disclosures: N. Sato: None. **H. Shidara:** None. **H. Ogawa:** None.

Poster

152. Neuroethology: Sensory and Motor Systems

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 152.07/QQ5

Topic: F.01. Neuroethology

Support: AU Grant 201160 FY2018

Title: Correlating neural and behavioral responses in an insect model and the effect of neuromodulators in cricket phonotaxis

Authors: *C. R. KENT, B. CHO, C. KIM, B. SHIN, B. A. NAVIA
Biol., Andrews Univ., Berrien Springs, MI

Abstract: Phonotaxis in female cricket *Acheta domesticus* can be selective or unselective in response to model calls with varying syllable periods. Discriminating a model call and walking towards it implies that a certain network(s) of neurons is/are activated when the call is recognized as attractive. Several approaches to demonstrate the influential role of auditory neurons in phonotaxis have been used with different levels of success. One such approach (current study) seeks to evaluate the behavioral and neuronal responses in the same animal, using identical auditory stimuli. This approach allows us to establish a correlation between the neuronal and behavioral responses, as well as to predict the behavior of the animal based on the response of the neuron. The L3 prothoracic auditory neuron has been suggested to influence syllable period selective phonotaxis in this species. In response to calls with attractive syllable periods, the L3 produces a burst of action potentials, which diminish in response to consecutive syllables. Such a decrease in the number of action potentials calculated as percentage is called decrement. Preliminary results indicate that syllable periods that produce positive phonotaxis, also elicit higher decrement values in the neuronal response of the same animal. In the lab, young (5-10 days), virgin females are more likely to respond phonotactically to calls with syllable period ranging between 50 and 70 ms. Older, virgin females (greater than 20 days), exhibit more variability in the range and number of syllable periods they respond to, which may or may not overlap with the range indicated for young ones. Neuromodulators such as juvenile hormone III

(JHIII) have been shown to modify phonotactic selectivity. The effect other neurochemicals may have on cricket phonotaxis remains to be evaluated. Octopamine has been shown to increase aggressive behavior in crickets. Preliminary prothoracic nanoinjections of octopamine show decreased phonotactic responsiveness in young females. A second neuromodulator, chelerythrine chloride (CC), a protein kinase C (PKC) blocker is believed to be part of the signaling mechanism in crickets, particularly regarding JHIII pathway. When older females are nanoinjected with JHIII, selectivity increases. When CC is nanoinjected in young females, selectivity decreases. We hypothesize that older female crickets which are more likely to be unselective, will show little or no change in selectivity following CC injection. This exploratory component of the current study seeks to identify neurochemicals which may be of importance in modulating cricket's phonotactic behavior.

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Poster

152. Neuroethology: Sensory and Motor Systems

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 152.08/QQ6

Topic: F.01. Neuroethology

Support: JSPS KAKENHI Grant Number 16H06544

Title: Phonotactic behaviors in freely-moving female crickets

Authors: *H. SHIDARA[1,2], N. HOMMARU[3], H. OGAWA[3]
[1]Sapporo-Shi, Japan; [2]Hokkaido Univ., Sapporo-shi, Japan; [3]Hokkaido Univ., Sapporo-Shi, Japan

Abstract: Goal-directed behaviors based on sensory cues are crucial for various animals' behaviors such as foraging and homing. Crickets are a good example of understanding the navigation behaviors with sound cues because the female crickets approach males making calling songs for mating. This acoustical navigation called photnotaxis have been well studied in the neuroethological field, using spherical treadmill systems. The experiments with the treadmill systems have especially unraveled detailed behaviors when animals were under various sound situations. In most cases of these experiments, however, the crickets were tethered on the treadmill ball and the sound stimulus was changed in neither sound pressure nor sound location. Thus, the natural process of phonotaxis behavior in the field had not been replicated in the previous research. To clarify the navigation process of approaching sound sources in female crickets, we examined the phonotactic behaviors of freely-moving crickets in an open arena within a sound-proof room. Each female cricket within an open arena (φ=1 m) started from the

center of the arena. The calling song were applied to the cricket from a speaker mounted on the wall of the arena. We extracted the trajectories of crickets from images captured with a video camera above the arena. In response to the song at 75 dB sound pressure level (SPL), most of tested crickets arrived near the speaker. The lower SPL of song, the fewer crickets approached around the speaker. In addition, the crickets moved more rapidly and actively depending on SPLs. To elucidate how crickets showing phonotaxis approach the sound source, we analyzed the trajectories of animals that arrived at the speaker. This analysis revealed that two distinct behavioral states existed in the phonotaxis. Immediately after the start, the crickets moved a short distance locally, and then suddenly started to approach the sound source rapidly. In the later state called 'approach phase', crickets exhibited long runs more frequently. Interestingly, the shift points from initial phase to approach phase was located within about 0.3 m from the start point, regardless of the SPL of the calling song. These results suggested that phonotactic behaviors of crickets are not stereotyped but more sophisticated.

Disclosures: H. Shidara: None. **N. Hommaru:** None. **H. Ogawa:** None.

Poster

152. Neuroethology: Sensory and Motor Systems

Location: SDCC Halls B-H

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Program #/Poster #: 152.09/QQ7

Topic: F.01. Neuroethology

Support: DFG (Deutsche Forschungs Gemeinschaft))

Title: Neurobiological mechanisms of spontaneous behavior and operant feedback in *Drosophila*

Authors: *C. C. ROHRSEN[1], B. BREMBS[2]
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Abstract: Actions are followed by consequences, and each of these consequences has a subjective value. The value assigned to these consequences shape our future actions in what is often called "learning by doing". But how is value conferred in the brain? Biogenic amines have been found to be involved in this process in a variety of different animal preparations as well as in humans. To start addressing this question in the fruit fly *Drosophila*, we have performed experiments where the flies control the on/off state of different subsets of dopaminergic neurons via optogenetics. With these experiments, the animals report to us whether such neuronal activity is experienced as appetitive or aversive. As one major first result, we discovered that the appetitive or aversive role of these neurons varies across operant (feedback) situations and apparently has little relation to their role observed in classical (feedforward) situations. In other words, a dopaminergic population of neurons sufficient to serve as an appetitive unconditioned stimulus in classical conditioning may be sufficient to serve as punishment when brought under

operant control. These results suggest fundamentally different neuronal mechanisms underlying operant and classical learning processes, even at the level of the biologically significant stimulus. By using three different experimental setups and analyzing the differences both within and between them, we found that the reinforcing value of some of the tested neurons is context dependent. We therefore classified the tested neuronal subsets into two groups: context-dependent reinforcers and general reinforcers. To corroborate the results with optogenetically activated neurons, we also performed experiments where flies were allowed to optogenetically inhibit these same neuronal populations. Reinforcement is essential for operant learning, allowing individuals to find more optimized action strategies despite compromising behavioral variability. We are interested in observing how variability in the behavioral repertoire develops over time while an individual learns. Finally, to determine if and to which degree such operant feedback alters the temporal dynamics of otherwise spontaneous actions, we used nonlinear forecasting methods to analyze the temporal structure of spontaneous choices before and after operant learning. The complementary approaches described here are part of a research program designed to converge on a circuit-level understanding of behavioral variability and its modification by reafferent stimuli.

Disclosures: C.C. Rohrsen: None. **B. Brembs:** None.

Poster

152. Neuroethology: Sensory and Motor Systems

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 152.10/DP09/QQ8

Topic: F.01. Neuroethology

Support: Simons Foundation SCGB Fellowship
KIBM innovative grant 2017-954
Mathers Foundation grant

Title: Network models of whole brain activity in behaving adult *Drosophila*

Authors: *S. AIMON[1], T. JIA[3], T. J. SEJNOWSKI[3], R. J. GREENSPAN[2]
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Abstract: We record whole brain calcium and voltage activity at high speed in the brain of head fixed but behaving flies using light field microscopy as described in a previous work (Fast whole brain imaging in adult Drosophila during response to stimuli and behavior bioRxiv 033803; doi: https://doi.org/10.1101/033803). We extract time series by averaging activity in neuropil regions from an anatomical template, or by applying unsupervised machine learning methods such as PCA/ICA and NMF. We use functional connectivity measures to characterize the interaction of

the activity sources at different time scales, and compare the functional networks to anatomical networks. We characterize the flow of information in various situations. For example, when the fly starts walking, we find a flow of activation from motor regions (such as the posterior slope) to central regions (first the lateral accessory lobe, and then the central complex and mushroom body area). Using additional information from anatomical databases, as well as restricted drivers for specific neurotransmitters, we identify simple biophysically-inspired models of the network activity.

Disclosures: S. Aimon: None. **T. Jia:** None. **T.J. Sejnowski:** None. **R.J. Greenspan:** None.

Poster

152. Neuroethology: Sensory and Motor Systems

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 152.11/QQ9

Topic: F.01. Neuroethology

Title: Sensory-motor transformations during *Drosophila* thermotaxis

Authors: *J. M. SIMÕES, J. I. LEVY, M. MANI, W. L. KATH, A. PARA, M. GALLIO
Northwestern Univ., Evanston, IL

Abstract: The ability to navigate the environment is crucial for all motile animals, as it enables them to stay away from dangerous conditions and efficiently plot the best route to find food, shelter or potential mates. We study the decisions that underlie temperature navigation and preference in the fruit fly *Drosophila*. Fruit flies are small, cold-blooded animals and critically depend on fast and reliable information about external temperature to avoid potentially lethal thermal conditions. In order to compute appropriate navigational trajectories, temperature stimuli have to be processed in the brain very quickly (on a time-scale of milliseconds to seconds). This can be directly visualized on the one hand by live calcium imaging of thermal responses in the fly brain and, on the other, during behavior -in artificial arenas in which flies rapidly steer as they encounter abrupt changes in external temperature. Our ultimate goal is to understand what happens in the fly brain as these navigational decisions occur. We recently described second-order projection neurons of the thermosensory system (TPNs) that respond to heating and cooling with characteristic tuning and dynamic properties. We have now adapted unsupervised methods to describe fly navigation, and are using them to study how systematically altering the activity of specific TPNs (by transgenic silencing or activation) affects sensory-motor transformations during thermotaxis.

Disclosures: J.I. Levy: None. **M. Mani:** None. **W.L. Kath:** None. **A. Para:** None. **M. Gallio:** None.

Poster

152. Neuroethology: Sensory and Motor Systems

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Topic: F.01. Neuroethology

Support: Janelia HHMI transition funds
UCSB start up funding
NIH R56NS102416

Title: Mechanosensory inputs contribute to the sequence of *Drosophila* grooming

Authors: *J. H. SIMPSON[1], N. ZHANG[2], L. GUO[2]
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Abstract: Sequential behaviors such as courtship and nest building are observed across animal species. However, little is known about the neural circuits that control these sequences. In this project, we used fruit fly grooming behavior as a model to investigate how animals organize discrete action motifs into a coordinated sequence. Flies perform grooming movements spontaneously, but when covered with dust, they clean their bodies following an anterior-to-posterior sequence. The same sequence can be induced by various dust particles and is observed in different Drosophila species. These phenomena indicate sequential grooming is an evolutionarily conserved solution to variable sensory irritants.
First, we investigated which sensory organs participate in dust-induced grooming behavior. In gain-of-function studies, we used different GAL4 lines to systematically target distinct groups of sensory neurons: we found that grooming can be induced by optogenetic activation of mechanosensory or bitter taste neurons. Interestingly, activating mechanosensory bristle neurons across the whole body first elicited anterior grooming, while increased posterior grooming was observed after the termination of sensory excitation. This sequence resembles the anterior-to-posterior grooming sequence induced by dust. Our loss-of-function experiments were less informative, perhaps because of incomplete blockage of neural activity. But mutant flies with eye bristle defects showed a significant reduction of anterior grooming when covered with dust. Therefore, we propose that mechanosensory bristles play the most essential role in dust-induced grooming behavior.
In dusted flies, sensory organs on different body parts are activated simultaneously. However, only one cleaning module is performed at one time, leading to an action sequence. Next, we investigated how mechanosensory inputs affect grooming sequence. Our computational model based on varied sensory inputs strength from different body parts successfully reproduced the dust-induced grooming sequence. It also suggested grooming hierarchy is determined by ratio of sensory inputs strength from different body parts. This hypothesis was further supported by

grooming progression of mutant flies with eye bristle defects. During grooming, the distribution of sensory inputs changes as flies removing dust. Our model also showed that sensory input dynamics set the speed of anterior-to-posterior cleaning progression. Overall, our results suggest sensory input strength and dynamics play an essential role in sequential behavior. We will further research how specific neural circuits decode these sensory inputs.

Disclosures: J.H. Simpson: None. **N. Zhang:** None. **L. Guo:** None.

Poster

152. Neuroethology: Sensory and Motor Systems

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Topic: F.01. Neuroethology

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Institute for Collaborative Biotechnologies through contract W911NF-09-D-0001 from the U.S. Army Research Office

Title: Modeling *Drosophila* grooming reveals order and duration dependence in behavioral sequence generation

Authors: *P. RAVBAR[1], J. MUELLER[2], J. SIMPSON[1], J. CARLSON[3]
[1]MCDB, [2]Dynamical Neurosci. Program, [3]Physics, UCSB, Santa Barbara, CA

Abstract: Animals assemble complex behaviors from simpler elements. Nervous system dynamics drive action execution and sequencing. Quantification of action sequences suggests that acute sensory information, internal priorities, and movement history contribute to sequence organization, but the respective weights of each are unknown. Grooming, a prominent subset of *Drosophila* behavior, exhibits qualitative properties of complex sequence generation, such as repeated execution of distinct subroutines in variable order. This makes grooming a rich source of data for discovering possible rules implemented by nervous systems to enact protocols which can integrate sensory feedback and internally-driven pattern production. Experimental data demonstrates that some subroutines occur with higher probability and tend to occur early in action sequences that follow a stimulation of flies by an irritant (high priority action).
Here, we develop a progressive series of Markov models that isolate and identify the factors governing grooming action transition dynamics. The simplest is a 1st order discrete-time model which reveals that the *identity of the previous action* influences the selection of the next action. In subsequent models, we include *action duration* as a data dimension, revealing the unexpected role of temporal dynamics in determining behavioral transitions. We verify the primary role of grooming subroutine order and the secondary, though significant, role of action duration by

quantifying how well data shuffled in these dimensions recapitulates observed behavior transition probability matrices. We find that permuting grooming subroutine order significantly degrades the predictive power of the resulting Markov models, as does permuting action duration, though to a lesser extent. In each case, permuting data dimensions reduces the number of free parameters in the ensuing model, but this increased parsimony does not compensate for the simultaneous loss of explanatory power.
The presence of an action duration-dependent component suggests a contribution of patterned internal dynamics in behavioral sequence generation. Thus, the structure of grooming behavioral sequences can be understood as the interplay between discrete decision-making between subroutines and more continuous dynamics organizing the execution of the subroutines. Understanding the control algorithms that govern flexible behavioral sequence generation in *Drosophila* guides identification of the neural circuit implementation by providing model parameters which may correspond to observed neural activity variables, allowing for future model hypothesis testing and refinement.

Disclosures: P. Ravbar: None. **J. Mueller:** None. **J. Simpson:** None. **J. Carlson:** None.

Poster

152. Neuroethology: Sensory and Motor Systems

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Program #/Poster #: 152.14/QQ12

Topic: F.01. Neuroethology

Support: Simons Foundation SCGB #543003
NIH Grant T32HG003284

Title: Temporal processing and chemotactic behavior in *C. elegans* olfactory learning

Authors: *K. S. CHEN[1], A. L. CASTILLO BAHENA[1], M. LUI[2], A. M. LEIFER[3,1]
[1]Neurosci., [2]Lewis-Sigler Inst. for Integrative Genomics, [3]Physics, Princeton Univ., Princeton, NJ

Abstract: Animals flexibly adjust behaviors in response to environmental contexts and learned experiences. In *C. elegans*, associative learning with an olfactory cue modulates behavioral preferences, producing chemotactic behaviors towards the cue if it was paired with food [1]. However, the effects of olfactory learning on sensory-motor processing and chemotactic strategies are not fully understood. Here we investigate butanone-odor associative learning using video tracking microscopy, behavior mapping and high throughput optogenetic stimulation methods [2]. We delivered time varying optogenetic stimulation to populations of *C. elegans* expressing Channelrhodopsin in the AWC chemosensory neuron. By recording over 500 animal-hours of behavior under stimulation, we characterized how temporal properties of signals in the

AWC neuron drive the animal to transition between behavior states like turn and reverse. We identified temporal integration of these sensory signals over seconds. Consistent with previous studies [1], we observe that the animal's reversal behavior becomes more sharply tuned to sensory signals after butanone-odor associative learning. We fit a linear-nonlinear model to predict the animal's behavioral response to optogenetic stimulation and use this framework to quantify changes in the animal's rate of transitions into various behavior states after learning. We draw links between observed changes in sensorimotor processing and the measured statistics of chemotaxis. These findings reveal temporal processing of sensory signals in AWC that evoke behavior, and crucially, show how behavioral response to a stimulus is altered by learned experience.

1. Cho, Christine E., et al. "Parallel encoding of sensory history and behavioral preference during Caenorhabditis elegans olfactory learning." Elife 5 (2016).
2. Liu, Mochi, et al. "Temporal processing and context dependency in C. elegans mechanosensation." arXiv:1803.04085 (2018).

Disclosures: K.S. Chen: None. **A.L. Castillo Bahena:** None. **M. Lui:** None. **A.M. Leifer:** None.

Poster

152. Neuroethology: Sensory and Motor Systems

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Program #/Poster #: 152.15/QQ13

Topic: F.01. Neuroethology

Title: Serotonin modulates neural activity of RID interneuron and the behavior in *Caenorhabditis elegans*

Authors: *H. MORI, K. ASHIDA[1], H. SHIDARA[3], K. HOTTA[4], K. OKA[2]
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Abstract: Various neuromodulators act in neural circuits to modulate behaviors in *C. elegans*. Especially, serotonin (5-HT) modulates locomotion behavior of the worm [Sawin *et al*., 2000]. However, it has not been clarified well how 5-HT induces behavioral changes through the neural circuit modulation. To reveal this, we focused on RID interneuron. RID is involved in locomotion as ablation of RID reduces sustainability of forward locomotion [Lim *et al*., 2016]. RID has 5-HT receptor, MOD-1, and *mod-1* mutant animals are defective in locomotion [Ranganathan *et al*., 2000]. In this study, we investigated the behaviors and neural activities of RID in the worms which were pre-exposed to 5-HT, and found that both of them were modulated

by 5-HT.
To investigate the effect of 5-HT on the behaviors, we performed behavioral assays. We put several worms on the center of an assay plate and recorded their behaviors for 30 minutes. We prepared four groups of pretreatment worms that were exposed to different concentration of 5-HT, and compared them with mock group in various behavioral parameters. We found that the frequency of pirouette, which is a typical behavior to change their locomotive direction was increased in the 5-HT pre-exposed worms.
Next, to investigate neural activity of RID, we used a Ca^{2+} sensor, GCaMP6 and mCherry for the ratiometric analysis [Lim *et al*., 2016]. We measured the Ca^{2+} level in RID and the behaviors of worms simultaneously with a custom-made tracking system. In this experiment, we investigated the worms in 5-HT pre-exposed group and mock group. We analyzed Ca^{2+} activity change when the worm switched from backward to forward locomotion and from forward to backward, respectively. In mock condition, RID neural activity increased during transition from backward to forward and decreased during transition from forward to backward as shown in the previous research [Lim *et al*., 2016]. However, in 5-HT pre-exposed group, these shifts of neural activity were not observed. These results indicate that exogenous 5-HT affected RID activity, and this could change the pirouette frequency.

Disclosures: K. Ashida: None. **H. Shidara:** None. **K. Hotta:** None. **K. Oka:** None.

Poster

152. Neuroethology: Sensory and Motor Systems

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Topic: F.01. Neuroethology

Support: NIH Grant 1R15HL126105
NIH Grant 1SC2GM112570

Title: WormBeat:A strategy to evaluate pharyngeal pumping variability in the nematode *C. elegans*

Authors: *M. B. HARRIS, R. BERLEMONT, B. ORTIZ, B. E. TAYLOR
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Abstract: In C. elegans, the feeding behavior of pharyngeal pumping occurs spontaneously in proportion to metabolism and in response to stimuli. Age- and health-related changes in tissue morphology and function correlate with declines in the frequency of pumping. As such, pumping frequency is an established index of C. elegans “health”, and pumping changes illustrate and quantify functional decline. Timing of pharyngeal pumping is controlled by two pairs of pharyngeal motor neurons (MC and M3). Each pump cycle corresponds to the propagation of a

single pharyngeal muscle action potential, initiated by MC and terminated by M3. We currently model this system with MC acting as an oscillator and synaptically linked to the pharyngeal muscle. Pumping can change by influences on the pace and regularity of the oscillator and/or of the fidelity of pharyngeal muscle response to neuronal inputs. Traditionally, this phenomenon has been reported as an occurrence frequency, describing the number of pumping events observed per unit time over a period of observation. Variation in pumping frequency within and between subjects, and the relative coarseness of this measure may interfere with the resolution of subtle treatment effects and ignores the potential significance of pumping variability. We use the recently developed ScreanChip system (NemaMetrix) to detect individual pump events, and have developed an algorithm (WormBeat) to normalize variation between subjects and distinguish variability associated with MC neuron pace and neuromuscular fidelity of the pharynx. We present the validity of the WormBeat algorithm and demonstrate enhanced resolution in detecting differences in pumping behavior using a training dataset representing ranges of pump frequency and sources of variability. This presentation describes the theory and practice of the WormBeat algorithm. The use of this analysis strategy on an experimental dataset is described in a companion presentation.

Disclosures: M.B. Harris: None. **R. Berlemont:** None. **B. Ortiz:** None. **B.E. Taylor:** None.

Poster

152. Neuroethology: Sensory and Motor Systems

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Topic: F.01. Neuroethology

Support: University of Massachusetts Amherst

Title: Behavioral characterization of Berghia stephanieae: A novel laboratory species for neuroethological research

Authors: *P. D. QUINLAN[1,2], T. N. BUI[1], Y. SAIMIRE[1], M. D. RAMIREZ[1], B. DRESCHER[1], P. S. KATZ[1,2]
[1]Dept. of Biol., [2]Neurosci. and Behavior Program, Univ. of Massachusetts Amherst, Amherst, MA

Abstract: The simple nervous systems and behaviors of sea slugs such as *Aplysia californica* and *Tritonia diomedea* make them useful animals for neuroethological research. However, these species must be caught in the wild or raised in large aquaculture facilities. Here, we introduce the nudibranch, *Berghia stephanieae*, as an experimental system that is easily bred and raised in the lab. The generation time for *Berghia* is approximately two months, allowing developmental studies to be performed. Furthermore, it is inexpensive to generate hundreds of animals, making

it amenable for undergraduate research. We are characterizing behaviors in *Berghia* to develop a foundation for further research on the neural basis of behavior. We describe several behaviors that are easily observed in the lab, including feeding behavior, circadian rhythms, locomotion, and mating. Like *Aplysia*, *Berghia* exhibits rhythmic head-waving when searching for food. However, unlike either *Aplysia* or *Tritonia*, *Berghia* can locate food in the absence of water flow in both an open arena and a T-maze. Food localization requires the rhinophores, the olfactory organs. Additionally, both locomotor and mating behaviors in *Berghia* display circadian rhythms. We are combining this behavioral work with transcriptomic and connectomic approaches to study the neural basis of these behaviors.

Disclosures: P.D. Quinlan: None. **T.N. Bui:** None. **Y. Saimire:** None. **M.D. Ramirez:** None. **B. Drescher:** None. **P.S. Katz:** None.

Poster

152. Neuroethology: Sensory and Motor Systems

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 152.18/QQ16

Topic: F.01. Neuroethology

Title: Neural correlates of adaptive responses to changing load in feeding *Aplysia*

Authors: *J. P. GILL[1], A. P. A. VORSTER[1], D. N. LYTTLE[2], T. A. KELLER[1], S. C. STORK[3], H. J. CHIEL[4]
[1]Dept. of Biol., [2]Departments of Biol. and Mathematics, [3]Departments of Computer Sci. and Cognitive Sci., [4]Departments of Biology, Neuroscience, and Biomed. Engin., Case Western Reserve Univ., Cleveland, OH

Abstract: Animals flexibly and robustly respond to changing environmental conditions. Understanding the neural mechanisms of flexibility could be useful to medicine and robotics. The marine mollusk *Aplysia* shows a complex adaptive response to mechanical loading during feeding (Hurwitz and Susswein 1992). How do *Aplysia* adapt feeding motor programs to changing load? We seek to fully characterize this behavior and its neural control.
Prior work has shown that as intact animals switch from grasping food (biting; no load present) to swallowing (some load present), activity increases in motor neurons that aid in opposing load (Lu et al. 2015), and additional load imposed by the experimenter further increases motor neuronal recruitment (Shaw et al. 2015). Moreover, in a reduced preparation (the suspended buccal mass; McManus et al. 2012), we have observed both increased and decreased motor neuronal recruitment in response to load increases, depending on the magnitude of increase. *Aplysia* may also tear food when load increases to ensure swallowing progress is not lost (Hurwitz and Susswein 1992).
We hypothesized that *Aplysia* deploy at least three adaptive responses to loading during feeding:

(1) when encountering a slow, small load increase, *Aplysia* increase pulling (retraction) and grasper-closing forces proportionally to compensate; (2) when load slowly increases to intermediate levels, *Aplysia* stop swallowing and attempt to tear food to salvage what is already swallowed; (3) when encountering a sudden, large load increase, *Aplysia* rapidly stop feeding and release food to avoid injury.
We also proposed mechanisms for these responses: (1) in load compensation, proprioceptive sensory information biases the central pattern generator to spend more time in the pulling (retraction) phase, resulting in increased retractor and grasper motor neuronal recruitment (e.g., retractor motor neurons B3, B6, B9 [Lu et al. 2015] and grasper motor neurons B8a and B8b [Morton and Chiel 1993b]); (2) in food cutting, motor neuron B38 (which pinches the jaws shut; McManus et al. 2014) increases activity during the pulling (retraction) phase to cause tearing of food; (3) in injury avoidance, fast reflexes inhibit the retractor and grasper motor pools via the B4 and B5 interneurons (Gardner 1993) to prevent pulling and to release food.
Using *in vivo*, *in vitro*, and modeling techniques, we have begun to test these hypotheses in *Aplysia*. By studying the details of how one motor system compensates for load during rhythmic behavior, we may uncover general principles for nervous systems in many other animals.

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Poster

152. Neuroethology: Sensory and Motor Systems

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Program #/Poster #: 152.19/QQ17

Topic: F.01. Neuroethology

Support: R56NS094651

Title: Neural dynamics of a feeding pattern-generating circuit in the marine mollusk *Aplysia* californica

Authors: *J. YANG[1], Y. HUAN[1], N. X. KODAMA[2], R. F. GALÁN[2], H. J. CHIEL[1]
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Abstract: Because of its large, identified neurons, the buccal ganglion of *Aplysia californica* is a model system for understanding the neural basis of feeding behavior, which provides insights into motivated behavior and multi-functionality. We are monitoring in real time the activity of multiple key neurons in the neural circuit that controls feeding behavior. To this end, we are combining nerve recordings of the circuit's motor output with recordings of cell bodies of neurons in the neural circuit using a two-dimensional, high-density (100 um pitch)

microelectrode array (MEA, 120 electrodes). The majority of previous studies have focused on recordings from single neurons, or small groups of neurons. However, many interesting aspects of neural dynamics can only be understood by looking at large populations of neurons and their relationships to each other. We are using the MEA and at the same time recording from buccal nerves 2 and 3, the radular nerve and the I2 muscle to identify key neurons including motor neurons and interneurons on the array during motor patterns. By stimulating the sensory branch of buccal nerve 2, we can reliably induce regular motor patterns. We have successfully recorded from large identified neurons within the ganglion while recording one-to-one extracellular action potentials in the nerves. At the same time, we have observed the reciprocal relationship between key neurons on both MEA recordings and nerve recordings. We have also demonstrated experimental protocols to selectively stimulate individual neurons on the array in isolation or during a motor pattern. By applying different pharmacological treatments, we have been able to inhibit or stimulate large populations of neurons on the MEA. Finally, we have used computational methods to identify the functional connectivity between neurons on the MEA. Our preliminary data suggests this approach will provide deeper insights into a pattern-generating circuit and its behavioral significance.

Disclosures: J. Yang: None. **Y. Huan:** None. **N.X. Kodama:** None. **R.F. Galán:** None. **H.J. Chiel:** None.

Poster

152. Neuroethology: Sensory and Motor Systems

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Program #/Poster #: 152.20/QQ18

Topic: F.01. Neuroethology

Title: A novel serotonergic mechanism for attention in the peripheral nervous system of a predatory mollusk?

Authors: *Y. LIU[1], S. J. HANEY[1], A. C. BORIS[1], T. P. NOREKIAN[3], R. GILLETTE[2]
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Abstract: Soft-bodied animals like mollusks often have an extensive peripheral nervous system (PNS) which can carry out complex computations. The predatory sea slug *Pleurobranchaea californica* uses its cephalic chemotactile oral veil (OV) to locate its prey with computations done in PNS. We previously showed that the oral veil PNS computes a somatosensory map of stimulus location that is usable by a central network as a template for directed turning responses (Yafremava et al., 2011). Interestingly too, a simple working memory for prey tracking via odor plumes in a turbulent environment has also been documented (Yafremava et al., 2007). We have now found that localized punctate stimulation of the OV activates marked feedback from

serotonergic neurons of the central feeding network to the entire oral veil, and that serotonin increases sensory gain at the OV.
We successfully simulated the OV's somatotopic mapping function in a simple two-layered computational model, based on lateral inhibition mechanisms previously implicated in mapping (Yafremava et al., 2011). Moreover, we find that a potential mechanism of attention emerges from extending this model with the findings of serotonergic feedback and sensory gain. In it, serotonin markedly potentiates excitation state in recently excited sensory loci through a hypothetical coincidence mechanism like an eligibility trace, similar to known heterosynaptic plastic mechanisms. With little modification, we demonstrate that the attentional mechanism can account for the working memory shown in prey tracking.

Disclosures: Y. Liu: None. **S.J. Haney:** None. **A.C. Boris:** None. **T.P. Norekian:** None. **R. Gillette:** None.

Poster

152. Neuroethology: Sensory and Motor Systems

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Program #/Poster #: 152.21/QQ19

Topic: F.01. Neuroethology

Title: Regulation of crawling decisions in the sea slug pleurobranchaea californica

Authors: *C. LEE[1], R. GILLETTE[2]
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Abstract: For gastropods that locomote through mucociliary crawling, the neural bases of the decisions to crawl or stop remain unclear. Ciliary locomotion stops rapidly during active feeding and escape avoidance turns, but can also be expressed immediately following swims, avoidance turns, and feeding. How are these behaviors precisely regulated? To address this question, we are studying the neural bases of crawling decisions in the basal nudipleuran *Pleurobranchaea californica*. Our research unfolds in two parts: characterizing the neuronal basis of crawling, and determining relationships between crawling circuitry and those for competing behaviors. We found that stimulating two of the three nerves that innervate the foot - the medial and posterior pedal nerves - induces mucociliary transport. Additionally, we identified a single neuron on the dorsomedial surface of each pedal ganglion that induces mucociliary transport in semi-intact preparations. Inducing fictive escape turns in the isolated CNS by stimulating cephalic sensory nerves inhibits both the crawling neuron and fictive crawling spiking in pedal nerves, and in semi-intact preparations slows mucociliary transport. In the isolated nervous system, inhibition of crawling persists for some seconds after completion of avoidance turns. Induction of fictive escape swimming causes long lasting hyperpolarization the crawling neuron. These preliminary

data confirm expectations that the crawling circuitry is inhibited during the performance of antagonist. However, these data also suggest that the regulation of crawling in *Pleurobranchaea* is under the control of multiple elements that differentially activate and inhibit ciliary movement. More generally, these data provide insight into the transitions between competing behaviors.

Disclosures: R. Gillette: None.

Poster

152. Neuroethology: Sensory and Motor Systems

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Topic: F.01. Neuroethology

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NSF 1250104

Title: How dynamic nervous system influences behavioral stability in hydra vulgaris

Authors: *K. N. BADHIWALA, J. T. ROBINSON
Electrical and Computer Engin., Rice Univ., Houston, TX

Abstract: The cnidarian *Hydra vulgaris* displays remarkable regenerative abilities unlike many invertebrate model organisms like *C. elegans* and *D. melanogaster*. For example, ablation of single neuron in *C. elegans* often leads to permanent behavioral deficits, while *Hydra* can recover complete bisection and resume normal contractile behavior in as little as 48 hours. More remarkably, the cnidarian *Hydra* can regenerate its whole body, reform the entire neural architecture from aggregates of homogenized cells, and regain its full behavioral repertoire. Even in a normal, uninjured *Hydra*, the entire neural population is continually replenished with new cells differentiating from interstitial stem cells as the old cells slough off through the extremities. Thus, *Hydra* has the remarkable ability for both acute regeneration following traumatic injury as well as gradual, homeostatic regeneration that leads to continuous replacement of existing neurons with new ones. Understanding how these two regenerative processes manifest themselves as behavioral phenotypes in a simple model organism can reveal the basic mechanisms of neuroplasticity and neuroregeneration. These regenerative processes lead to highly dynamic nervous system size and architecture in *Hydra*. Despite the constant neuronal differentiation, migration and dynamic connectivity, literature shows that *Hydra* can maintain stable basal behaviors. The apparent behavioral stability despite highly dynamic nervous system offers the opportunity to study how information processing remains robust against changes in the underlying neural architecture. Here, using quantitative behavioral phenotyping techniques, we investigate how these two types of neural reorganization affect animal behavior, and under what

conditions behaviors remain stable despite changes in the number of constituent neurons and their connectivity.

Disclosures: K.N. Badhiwala: None. **J.T. Robinson:** None.

Poster

152. Neuroethology: Sensory and Motor Systems

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 152.23/QQ21

Topic: F.01. Neuroethology

Support: Research as a high impact practice (HIP)

Title: Contributions of taurine to the loser effect and the escape response in the female crayfish, procambarus clarkii and orconectes virilism

Authors: *C. M. MECCA, R. F. WALDECK
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Abstract: Energy drinks are consumed by thousands of Americans daily, despite having many harsh side effects. One of the major ingredients in these beverages is taurine. To improve our overall understanding of energy drinks and their side effects, we must determine taurine's mechanism of action. Taurine is nonessential amino acid that is known agonist for both GABA and glycine receptors (Jia et al., 2008). Invertebrates, such as crayfish, are proposed to have similar GABA-taurine and glutimate-taurine interactions (Picones et al., 1992, and Jia et al., 2008). By investigating the effects of taurine on crayfish aggression, a greater understanding of taurine's role in energy drinks can be achieved. Previous work in our lab conducted conspecific fights to quantify the effects of taurine in losing, adult, female crayfish, *Procambarus clarkii*. Taurine submerged crayfish (25mg/L) showed a decrease in aggression accompanied by a significant increase of submissive behaviors such as tail flips ($p=0.02722$) when compared to baseline. It is still unclear what taurine's mechanism is, but research suggests taurine has an influence on GABA, glutamate, and Ca^{++} (Oja and Saransaari, 2012). Since all three play a role in the tail flip circuit in crayfish (Wine and Krasne, 1982), it is possible that taurine is interacting with one of these molecules, thus producing an increase in tail flip frequency. To further investigate this hypothesis, extracellular electrophysiological recordings of nerve III on ganglion XII of the abdominal nerve chain were taken on both *Procambarus clarkii* and *Orconectes virilism*. The ganglion was administered either taurine (25mg/100mL) or vehicle in 0.5mL drip increments totaling 3mL. A twofold increase of the magnitude of the amplitude range was observed from vehicle to the 2.5mL dose of taurine. Additionally, a twofold increase in activity duration, with a 0.5 increase in peak frequency per stimulation was also found in the 2.5mL dose

when compared to vehicle. These trends support the increase in tail flip frequency observed in the behavioral trials and are critical in determining taurine's mechanism of action on the tail flip.

Disclosures: R.F. Waldeck: None.

Poster

152. Neuroethology: Sensory and Motor Systems

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 152.24/QQ22

Topic: F.01. Neuroethology

Support: 5T32EB009419-08
R35 NS097343

Title: Robustness of a rhythmic motor pattern to varying pH

Authors: *D. HAMPTON[1], J. HALEY[2], E. E. MARDER[3]
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Abstract: Crustaceans must digest food while exposed to a wide range of temperatures, and consequently pHs. Experimental and theoretical work revealed that many different combinations of neuronal conductances and synaptic strengths can lead to the same network output. However, it is not well understood how a small neuronal network retains a robust firing pattern over varying pH. Our study investigates the effect of pH on the conductances of pyloric neurons in the stomatogastric ganglion (STG). This circuit is composed of a pacemaker kernel with three neurons that connects to two types of follower neurons. The pylorus, which this circuit innervates, is responsible for filtering food in the gut.
Recent work in the Marder lab reveals that the activity of the pyloric rhythm of C. *borealis* is largely unaffected by changes in pH in a range from pH = 7 - 9.6. Outside of this range many preparations begin to exhibit 'unhealthy' behavior to various degrees, or activity ceases altogether. To understand how pH affects network activity it is important to study how pH affects critical activity parameters such as conductances known to be responsible for some aspects of activity. By studying these critical activity parameters, the hope is to understand how a highly variable system buffers against environmental insult, and what ways the system breaks down at extreme pH's. Our study uses two-electrode voltage clamp to measure ion channel conductances in the pyloric circuit of the STG over varying pH. We observed a steady decrement in A-type K^+ in LP neurons as pH is decreased. To a lesser extent, this decrement is also observed at high pH's. Additionally, minimum membrane potentials (Vm) are affected at different pHs. Interestingly, Vm is affected differentially according to cell type under conditions when synapses are blocked. Vm depolarizes in PD neurons at low pHs, while Vm hyperpolarizes in LP neurons at high pHs. Further conductance and synaptic measurements are being taken to

understand how robust firing is maintained within a permissible pH range despite differences in network conductance expression.

Disclosures: D. Hampton: None. **J. Haley:** None. **E.E. Marder:** None.

Poster

153. Neuroendocrinology of Social Behavior

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 153.01/QQ23

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH HD089147

Title: Neuropeptidergic regulation of sociality and physiology in juvenile marmoset monkeys

Authors: *J. CAVANAUGH, E. LEICHNER, J. A. FRENCH
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Abstract: Developing and maintaining high-quality social relationships is fundamental to both physiological and psychological well being across the lifespan. The neuropeptides oxytocin (OT) and vasopressin (AVP) have critical and pervasive roles in reproduction and physiology, and have attracted enormous interest as neuromodulators of social and cognitive functioning. While it is clear that these two hormones have important roles in the initiation of social interactions in adulthood, less attention has been given to whether OT and AVP regulate sociality during early development. Thus, the goal of these studies was to determine the extent that OT and AVP modulate social preferences, familial affiliation, and physiological and behavioral responses to stress in prepubertal marmoset monkeys across multiple developmental time-points. The expression of familial affiliation, including the initiation of social approach [$F(2,12)=13.0$, $p=.001$] and total time spent in social proximity [$F(2,12)=4.78$, $p=.03$], decreased as prepubertal marmosets progressed to independence. Neuropeptide treatment also interacted with age to modulate the initiation of social approach [$F(4,24)=2.5$, $p=.07$]; ten-month old marmosets that received AVP experienced the largest decline in social approach behavior. We also show that AVP enhances stress recovery, but not stress reactivity, following social isolation in juvenile marmosets [$F(6,60)=2.14$, $p=.06$]. These findings indicate that neuropeptides differentially modulate affiliation and the physiological stress response in juvenile marmoset monkeys. Ultimately, these results inform the design and application of selective therapeutic treatments for neuropsychiatric disorders that include maladaptive social functioning by providing further clarity on the age- and context-specific roles of OT and AVP in modulating sociality across development.

Disclosures: J. Cavanaugh: None. **E. Leichner:** None. **J.A. French:** None.

Poster

153. Neuroendocrinology of Social Behavior

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 153.02/QQ24

Topic: F.02. Behavioral Neuroendocrinology

Support: CAPES

Title: Behavioral analysis of mice infected with toxoplasma gondii and rosuvastatin treated

Authors: *F. F. EVANGELISTA[1], L. F. BELETINI[1], W. COSTA-FERREIRA[2], F. M. MANTELO[1], A. H. SOUZA[1], P. LAET SANTANA[1], A. A. MARCHIORO[1], A. FALAVIGNA-GUILHERME[1]
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Abstract: The Toxoplasma Gondii (T. Gondii) infection have elevated worldwide prevalenceand may manifest severitily in congenital transmission and in immunocompromised individuals, reaching the retina and the central nervous system, the latter in areas as hypothalamus and amygdala that are involved in emotional behavior responses. Studies in rodents showed that T. Gondii infection may promote the emergence of psychiatric disorders such as anxiety, depression, schizophrenia and locomotor activity. However, there is no study that evaluated Rosuvastatin treatment in the behavior responses of the balb/c mice with T. Gondii infected. Thus, the aim of this work was evaluate the effect Rosuvastatin treatment on behaviors-like anxiety, locomotion, and short-term memory in balb/c mice with T. Gondii infected. For this, were used 88 balb/c mice with 21 days infecteds with 25 cyst of the strain ME-49 per gavage. After 40 days, the animals were Rosuvastatin treated in the dose of 40 mg/kg/day at morning for 21 days. Twenty-four hours after the last treatment, the animals were submitted to the Open Field (OF) test for 5 minutes to anxiety behavior and locomotion evaluated. In the other experiment, we evaluated the Short-Term Memory by object recognition test in the OF. For this, three objects were use: object 1 and 1' object, and object 2. The animals explored objects 1 and 1' for 5 minutes, after the exploration the animals remained in their houses boxes for 10 minutes to then explore objects 1 and 2 for another 5 minutes. The OF arena, consisted in a circular arena with 30 cm of diameter and 30 cm of height. Our results showed that Rosuvastatin treatment increase total locomotion ($F(1,36)= 13.86$, $p<0.05$) within effects of the infection ($F(1,36) = 0.03$, $p>0.05$). In peripheral locomotion, the Rosuvastatin treatment ($F(1,37) = 4.13$, $p>0.05$) and infection ($F(1,37) = 0.02$, $p>0.05$) non affected the number of quadrants explored. In central locomotion, the Rosuvastatin treatment increase the number of quadrants explored ($F(1,37) = 12.83$, $p<0.05$) within effect of the infection ($F(1,37) = 1.82$, $p>0.05$). However, percentage of time in the center, infection decrease time spend in center ($F(1,36) = 6.70$, $p<0.02$) and

Rosuvastatin treatment reversed this effect ($F(1,36) = 5.09$, $p<0.05$). In Short-Term Memory, the infection decrease exploration of the new object ($F(1,44) = 25.70$, $p<0.01$) this effect was reverted with the Rosuvastatin treatment ($F(1,44) = 29.11$, $p<0.01$). Therefore, we conclude that T. Gondii infection leads to the emergence of psychological problems as anxiety and short-term memory injury, and that treatment with rosuvastatin has been able to review such problems.

Disclosures: F.F. Evangelista: None. **L.F. Beletini:** None. **W. Costa-Ferreira:** None. **F.M. Mantelo:** None. **A.H. Souza:** None. **P. Laet Santana:** None. **A.A. Marchioro:** None. **A. Falavigna-Guilherme:** None.

Poster

153. Neuroendocrinology of Social Behavior

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 153.03/QQ25

Topic: F.02. Behavioral Neuroendocrinology

Support: 16H05373

Title: Abnormal membrane protein trafficking in the autistic like the N-ethylmaleimide sensitive factor knockout mice

Authors: *M. XIE[1,2,3], K. IWATA[1], Y. FUKAZAWA[4], H. MATSUZAKI[1]
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Abstract: Autism, characterized by profound impairment in social interactions and communicative skills, is the most common neurodevelopmental disorder. Many studies on the mechanisms of autism have focused on the serotonergic system but its underlying molecular mechanisms remain controversial. In our previous report, we reidentified *N*-ethylmaleimide-sensitive factor (NSF) as new serotonin transporter binding protein, (Iwata et al 2014). In this study, we generated the $NSF^{+/-}$ mice and investigated their behavioral phenotypes. As previous report has already shown that NSF is necessary for AMPA receptors location in the synapse, we examined AMPA receptors location in the synapse of the $NSF^{+/-}$ mice by using freeze-fractured replica-immunolabeling study at first, and revealed the significant decrease in postsynaptic expression of AMPA receptors in CA1 of the hippocampus. Interesting, we also found the membrane expression of SERT half-reduced in the raphe of the $NSF^{+/-}$ mice. Then, we assessed the social interaction behaviors using the three-chambered task and found that the spending time

near the chamber containing the novel mouse were significantly reduced in the *NSF*$^{+/-}$ mice, compared with wild mice. We also analyzed the social communication by ultrasonic vocalizations, and found that the ultrasonic vocalizations significantly reduced in the *NSF*$^{+/-}$ mice, compared with wild mice. It is suggesting that cellular trafficking turbulence of synaptic molecules by lacking NSF gene might be related to the pathophysiology of autistic behavior.

Disclosures: M. Xie: None. **K. Iwata:** None. **Y. Fukazawa:** None. **H. Matsuzaki:** None.

Poster

153. Neuroendocrinology of Social Behavior

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 153.04/QQ26

Topic: F.02. Behavioral Neuroendocrinology

Support: Fond National Suisse
Synapsy NCCR fund

Title: Neuronal signature of social novelty exploration in the VTA: Implication for autism spectrum disorder

Authors: *C. PRÉVOST-SOLIÉ[1], S. BARISELLI[2], H. HÖRNBERG[3], S. MUSARDO[1], L. BURKLE[3], P. SCHEIFFELE[3], C. BELLONE[1]
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Abstract: Novel stimuli attract our attention, promote exploratory behavior, and facilitate learning. Atypical habituation and aberrant novelty exploration have been related with the severity of Autism Spectrum Disorders (ASD) but the underlying neuronal circuits are unknown. Here, we report that the activity of ventral tegmental area (VTA) dopamine (DA) neurons promotes the behavioral responses to novel social stimuli, supports social novelty preference, and mediates the reinforcing properties of novel social stimuli. Social novelty exploration is associated with the insertion of calcium-permeable GluA2-lacking AMPA-type glutamate receptors at excitatory inputs onto VTA DA neurons. These novelty-dependent synaptic adaptations persist upon repeated exposure and sustain social interaction. VTA DA neurons activity is affected in an ASD mouse model and an aberrant expression of GluA2-lacking AMPA-receptors at excitatory inputs onto VTA DA neurons and an occlusion of novelty-induced synaptic plasticity is also involved.

Disclosures: S. Bariselli: None. **H. Hörnberg:** None. **S. Musardo:** None. **L. Burkle:** None. **P. Scheiffele:** None. **C. Bellone:** None.

Poster

153. Neuroendocrinology of Social Behavior

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 153.05/RR1

Topic: F.02. Behavioral Neuroendocrinology

Title: Consumer social interactions and the role of oxytocin in rebuilding trust

Authors: A. A. D. PULGA[1], K. BASSO[1], *K. R. VIACAVA[2]
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Abstract: Trust is present in society and in the life of every human being, playing a crucial role in interpersonal relationships. In the context of the market and relations between customers and companies, trust is related to the customer's expectation that the company will behave predictably in a given situation. However, sometimes companies cannot fulfill the customer's expectation, breaching the trust stablished in the relationship. This can happen through failures in the service outputs or deliveries. To avoid negative consequences, companies must derive attention to rebuild trust after failures or trust violation episodes. One possible path used by managers to regain customer's trust is through a formal apology. However, the use of a formal apology could not consider the interactional nature of the trust. Based on this, some researchers have identified that social interaction between people influence the release of oxytocin, and that this hormone is linked to higher levels of trust behaviors among human beings. So, considering that social interaction is responsible by increase the levels of oxytocin and that this hormone is linked to trust, this study aims to verify the effect of social interaction and the hormone oxytocin on trust regaining after service failures. Three laboratory experiments were conducted. One manipulating the simulated social interaction, with video projections; another manipulating physical social interactions, through hugs; and another applying exogenous oxytocin (or placebo) in the subjects. In all three studies the scenario projected involves a service failure and the dependent measure is the trust after the apology of the company's manager. The results of the first study showed that simulated social interaction causes a positive effect on trust regaining after failure ($p=.02$). The second study demonstrated that physical social interaction (hug) also had a positive effect on customer trust regaining ($p=.02$). The third and last study, a possible confirmation of the underlying mechanism, presents that in the presence of the hormone oxytocin the trust is recovered ($p=.04$), reiterating a neurobiological mechanism involved in the trust, and based on these evidences, on trust regaining. Based on the findings, this research contributes to understanding of the link between social interaction and trust, by elucidating the effect of both simulated and physical interaction in favor of trust regaining between customer and company. Moreover, this research also contributes to the knowledge of the link between oxytocin and trust regaining. As far we know, this is the first study to relate oxytocin and trust regaining.

Disclosures: A.A.D. Pulga: None. **K. Basso:** None. **K.R. Viacava:** None.

Poster

153. Neuroendocrinology of Social Behavior

Location: SDCC Halls B-H

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Program #/Poster #: 153.06/RR2

Topic: F.02. Behavioral Neuroendocrinology

Support: ERA-ANHIWA net WinFish
Swedish Research Council
FACIAS

Title: Boldness and social rank is reflected in the expression of brain dopamine, histamine and opioid receptors in male zebrafish

Authors: *A. MUSTAFA[1,2], G. ANDRÉ[3], P. THÖRNQVIST[1], S. WINBERG[1]
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Abstract: In social animals intraspecific variation in competitive ability gives rise to dominant-subordinate relationships. Dominant and subordinate individuals differ in stress coping styles, dominants being more proactive than subordinates while subordinates being more reactive than dominants. Animals displaying divergent stress coping styles differ in brain dopaminergic functions. Brain dopamine is linked to aggressive behavior and brain dopaminergic activity is associated with social stress. However, not much is known about the involvement of opioid and histamine receptors in dominant-subordinate behavioral phenotypes. In this study 96 male zebrafish of AB strain were selected for stress coping styles (Bold/Shy) and afterwards subjected to staged dyadic fights for social dominance. After 4 days of dyadic contest brain tissue was sampled and analyzed for expression of dopamine, histamine and opioid receptors. The results show that boldness had significant effects on the expression of class 2 type of dopamine receptors, (*drd2b, drd3, drd4rs*), histamine receptor 3 (*hrh3*) and delta opioid receptor 1b (*oprd1b*) in the telencephalon, bold males showing higher expression than shy males. Our study show that boldness and social rank are both associated with specific but different expression profiles of dopamine, histamine and opioid receptors in the brain of the male zebrafish. This is a novel study that adds to our understanding of neuronal mechanisms shaping divergent behavioral phenotypes. The use of animals in this study was approved by the Uppsala Animal Ethical Committee (permit Dnr 55/13) and followed the guidelines of the Swedish Legislation on Animal Experimentation (Animal Welfare Act SFS1998:56), and the European Union Directive on the Protection of Animals Used for Scientific Purposes (Directive 2010/63/EU).

Disclosures: A. Mustafa: None. **G. André:** None. **P. Thörnqvist:** None. **S. Winberg:** None.

Poster

153. Neuroendocrinology of Social Behavior

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Program #/Poster #: 153.07/RR3

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant HL122494
NIH Grant HL096830
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Title: Oxytocin receptor expressing neurons within the prefrontal cortex exert top-down control over social recognition

Authors: *Y. TAN[1], S. SINGHAL[1], S. HARDEN[1], H. HILLER[1], D.-T. NGUYEN[1], L. M. COLON-PEREZ[2], M. FEBO[2], L. WANG[1], K. CAHILL[1], A. D. DE KLOET[3], C. J. FRAZIER[1], E. G. KRAUSE[1]
[1]Dept. of Pharmacodynamics, [2]Psychiatry, Univ. of Florida, Gainesville, FL; [3]Physiol. and Functional Genomics, Univ. of Florida, Col. of Med., Gainesville, FL

Abstract: Autism spectrum disorder is associated with difficulty recognizing novel faces from those previously encountered. Dysregulated oxytocin receptor (OTR) signaling is thought to contribute to impaired social recognition; however, the underlying neural circuits are unknown. The prefrontal cortex (PFC) densely expresses OTR(s) that may affect social recognition. Here, we use the Cre-LoxP system in mice with anatomical, electrophysiological, functional imaging and optogenetic approaches to discern whether excitation of neurons in the PFC that synthesize OTR(s) affects social recognition. Male mice with expression of Cre recombinase directed to the OTR gene (OTR-Cre mice) were delivered Cre-inducible adenoassociated viruses expressing fluorescent protein (control), channelrhodopsin-2 (ChR2) or GCaMP6f into the PFC. *In vitro* application of oxytocin increased GCaMP6f fluorescence intensity in a subset of virally transformed PFC neurons. Neuroanatomical studies indicated that neurons expressing OTR mRNA in the PFC were glutamatergic or GABAergic. These anatomical results were confirmed by morphological characterization and by experiments that used optogenetic approaches to evoke either glutamate or GABA release from OTR neurons *in vitro*. Functional magnetic resonance imaging revealed that *in vivo* optogenetic stimulation of OTR neurons in the PFC evoked BOLD signal increases in the PFC as well as subcortical nuclei including nucleus accumbens, bed nucleus of the stria terminalis and basolateral amygdala (BLA). Viral tract-tracing and ChR2-assisted circuit mapping suggested that the increased BOLD signal in subcortical nuclei resulted from direct glutamatergic projections. To assess the function of OTR neurons in the PFC, OTR-Cre mice were administered ChR2 or control virus in the PFC, and then chronically implanted

with fiber optics bilaterally targeting either the PFC or the BLA. Subsequently, mice were evaluated in paradigms assessing social recognition, novel object recognition, social interaction, anxiety-like behavior and locomotor activity. *In vivo* optogenetic stimulation of OTR neurons in the PFC or their axons in the BLA significantly impaired social recognition but had no effect on novel object recognition, demonstrating that the memory impairment imposed by excitation of this PFC to BLA connection is specific to a social context. Moreover, optogenetic activation of PFC or BLA had no effect on indices of anxiety-like behavior or locomotor activity. Collectively, our results suggest that PFC neurons that express the OTR have connections to the BLA that control the ability to distinguish between familiar and novel conspecifics.

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Poster

153. Neuroendocrinology of Social Behavior

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Program #/Poster #: 153.08/RR4

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH TG 5T32DA018926-9
Hartman Graduate Fellowship Endowment

Title: Social network effects on learning and neural processing of a visual cue discrimination task

Authors: *M. RODRIGUEZ SANTIAGO[1], L. A. JORDAN[2], H. A. HOFMANN[3]
[1]Inst. for Neurosci., Univ. of Texas At Austin, Austin, TX; [2]Dept. of Collective Behavior, Max Planck Inst. for Ornithology, Konstanz, Germany; [3]Dept. of Integrative Biol., Univ. of Texas at Austin, Austin, TX

Abstract: When individuals interact, neural circuits integrate current observations with memories of previous interactions, and predictions about future behavioral outcomes, to respond in a context-appropriate manner. This integration allows individuals to optimize their behavior based on the available information, thereby enhancing their ability to quickly adapt to a changing environment. Social species are tasked with expressing context-appropriate behavior in complex social hierarchies that can undergo dynamic changes depending on group composition and individual experience. These interactions can induce neuronal and physiological responses in individuals that impact their subsequent learning and decision-making. Perturbations of existing network dynamics have been shown to affect the stability and types of interactions within social groups and may therefore influence social decision-making. Here we investigated the

relationship between social network dynamics and learning using detailed behavioral observations of naturalistic social groups of the highly social cichlid fish, *Astatotilapia burtoni*. We monitored groups of males and females over sixteen days and during a visual cue discrimination task to explore the feedback between community level properties and individual learning. To examine the impact network instability may have on individual-level learning and synaptic plasticity, we disturbed the social network during either the acclimation or learning phase. We measured the frequency and type of interactions between individuals during both the acclimation and learning phases to understand how learning occurs and is affected by disturbances in the social network. We also quantified the expression of genes associated with neural activity and synaptic plasticity (*egr-1, c-fos, GRIA1*) across the putative mammalian homologues of the hippocampus, basolateral amygdala, and lateral septum to elucidate how social group disturbances inhibit synaptic plasticity in individuals. We demonstrate that social network properties can have direct and indirect effects on learning, and affects synaptic plasticity at the individual level. Our results show how variably social network dynamics can affect social groups, yet these effects converge to mediate highly conserved neural circuits of synaptic plasticity. By combining behavioral observations of social networks before and during learning with examinations of the underlying neural pathways mediating synaptic plasticity, this research will provide new fundamental insights into the mechanisms regulating learning within dynamic social groups.

Disclosures: M. Rodriguez Santiago: None. **L.A. Jordan:** None. **H.A. Hofmann:** None.

Poster

153. Neuroendocrinology of Social Behavior

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 153.09/RR5

Topic: F.02. Behavioral Neuroendocrinology

Support: The University at Buffalo Research Foundation

Title: Viral-mediated restoration of hypothalamic vasopressin does not ameliorate the hypoaroused behavioral phenotype of vasopressin-deficient adolescent Brattleboro rats

Authors: *K. SCHATZ[1], L. M. BROWN[1], A. BARRETT[1], V. GRINEVICH[2], M. J. PAUL[3]
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Abstract: Arginine vasopressin (AVP) has recently been implicated in adolescent behavioral development, but the mechanisms through which AVP influences adolescent behaviors are not understood. Brattleboro rats have a mutation in the *Avp* gene, and rats homozygous for this mutation (HOM) do not produce functional AVP. Hence, Brattleboro rats can be used to study

the role of AVP and the impact of AVP-deficiency on development. We have previously found that adolescent HOM rats exhibit a hypoaroused phenotype characterized by decreased activity in the open field compared to their heterozygous (HET) littermates, and we proposed that this decreased behavioral arousal impacts other behaviors during adolescence (e.g., social play). Decreased behavioral arousal of Hom rats could be due to the loss of AVP in parvocellular cells of the paraventricular nucleus of the hypothalamus (PVN), which act centrally to facilitate the stress response and autonomic activation. In addition, AVP from magnocellular cells of the PVN could influence arousal via peripheral feedback mechanisms. We asked whether selective restoration of the AVP in AVP cells of the PVN would be sufficient to restore typical levels of arousal in HOM rats. On postnatal day (P)24 (±2 days), male and female HOM rats received bilateral intra-PVN injections of an adeno-associated viral (AAV) vector containing a functional *Avp* gene driven by an AVP promoter (HOM-AVP-AAV) or a control AAV (HOM-CON-AAV); a group of male and female HET rats were also injected with the control AAV (HET-CON-AAV). Behavioral arousal was assessed in adolescence (between P42-47) using the open field test. Water intake measures were recorded at 5-day intervals throughout the experiment to determine whether the viral restoration of PVN AVP ameliorated the diabetes insipidus (excessive drinking and urination) characteristic of HOM rats due to the loss of AVP actions on water reabsorption in the kidney. Pilot studies confirmed that the AVP-AAV restores AVP protein in the PVN, and that AVP restoration does not occur in PVN oxytocin cells. Drinking behavior was markedly decreased in HOM-AVP-AAV rats compared to HOM-CON-AAV rats, but remained elevated above HET-CON-AAV rats. However, injection of the AVP-AAV had no effect on open field activity, and HOM-AVP-AAV rats with the lowest levels of drinking continued to exhibit decreased behavioral arousal. These data demonstrate that restoration of peripheral actions of AVP is not sufficient to restore typical levels of behavioral arousal in HOM rats and suggest that the hypoaroused, low anxiety-like phenotype is due to central actions of AVP.

Disclosures: K. Schatz: None. **L.M. Brown:** None. **A. Barrett:** None. **V. Grinevich:** None. **M.J. Paul:** None.

Poster

153. Neuroendocrinology of Social Behavior

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 153.10/RR6

Topic: F.02. Behavioral Neuroendocrinology

Support: JSPS KAKENHI Grant Number JP 18J14754
JSPS KAKENHI Grant Number JP 16K07507

Title: Comparisons of functions of arginine vasotocin and isotocin and morphology of neurons producing these peptides in gobiid fish with different mating systems

Authors: *K. FUKUDA[1], N. TSUJITA[2], H. KUNIYOSHI[2], T. MUKUDA[3], M. YOSHIDA[2], T. SUNOBE[4], N. YAMAMOTO[1]
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Abstract: Neuropeptides arginine vasopressin, oxytocin and peptides homologous to these nonapeptides are known as key modulators of social behavior across vertebrates. Especially, monogamous and polygamous behaviors are regulated by these peptides in rodents species. Teleost fishes possess various mating system and arginine vasotocin (VT) and isotocin (IT), teleostean homologs of arginine vasopressin and oxytocin respectively, may play an important role in diversification of their mating system. Here, we attempted to detect the functional and neuronal morphological differences between closely related monogamous (*Trimma marinae*) and polygamous (*Trimma caudomaculatum*) gobiid fishes. First, we assessed the role of VT and IT in mating behavior of the two species using intraperitoneal administration of these peptides or peptidergic antagonist against their receptors. In monogamous species, partner preferences and mate guarding behavior, which are two essential female behaviors for forming and maintaining a monogamous mating system, significantly decreased by administration of oxytocin receptor antagonist and V1a vasopressin receptor antagonist to females, respectively. In contrast, administrations of VT or IT to females of polygynous species did not induce these behaviors. These results suggest that VT, IT and associated systems in monogamous species may be different from those in polygaomous species. As a next step, we conducted immunohistochemistry for VT and IT, in search of differences in the morphology and number of cells, projections and density of fibers between monogamous and polygamous species. We will discuss on the comprehensive interspecific difference of VT and IT systems regarding their functions and neuronal morphology from the point of view of mating system in gobiid fish.

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Poster

153. Neuroendocrinology of Social Behavior

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 153.11/RR7

Topic: F.02. Behavioral Neuroendocrinology

Support: Kakenhi Grant 17K08512

Title: Repulsive axon guidance molecule FLRT2 regulates neuronal migration and social behavior

Authors: *S. YAMAGISHI[1], Y. SHINODA[2], S. OGAWA[3], T. MIYAKAWA[4], K. TAKAO[5], K. SATO[1]
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Abstract: During cortical development, migrating neurons and pathfinding axons are guided by molecular cues within the extracellular matrix or on the surface of ambient cells. These cues are interpreted as attractive or repulsive, depending on the set of receptors and signal transducers the cell expresses. We previously identified fibronectin leucine-rich transmembrane protein (FLRT) family as ligand of Unc5 proteins, a well-known Netrin receptors. FLRT2 is expressed in the cortical plate (CP) and inhibited migration of Unc5D+ cells in the subventricular zone (SVZ). The upper layer neurons in FLRT2 mutant mice showed earlier migration to CP, indicating repulsive function of FLRT2. However, the behavioral phenotype of FLRT2 deficient mutant mice was unclear. Here, we comprehensively analyzed behaviors of Emx1 cre induced- FLRT2 conditional knock-out mice using the test battery, including rotor-rod, T-maze, Barns maze, fear conditioning, prepulse inhibition, tail suspension, forced swim, object location test, pattern separation test, open-field, light/dark transition, elevated plus maze, hot plate test and social interaction test. Among these tests, we found that mutant mice showed significant enhancement of anxiety-like behaviors (light/dark transition and elevated maze), pain sensitivity (hot plate) and social interaction. Furthermore, exploratory locomotor activity was decreased (open-field and Y-maze tests). Next, we electrophysiologically assessed synaptic transmission in the mutant mice such as LTP and paired pulse facilitation using hippocampal acute slice. However, we could not find any significant difference. Furthermore, we analyzed the morphological change in primary cultured neurons. Interestingly, neurons from FLRT2 mutant harbored longer axons. These results suggest that mis-migrated/mis-connecting neurons might affect the behaviors of FLRT2 deficient mice such as social interaction and anxiety-like behaviors.

Disclosures: S. Yamagishi: None. **Y. Shinoda:** None. **S. Ogawa:** None. **T. Miyakawa:** None. **K. Takao:** None. **K. Sato:** None.

Poster

153. Neuroendocrinology of Social Behavior

Location: SDCC Halls B-H

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Program #/Poster #: 153.12/RR8

Topic: F.02. Behavioral Neuroendocrinology

Support: NRF-2016R1D1A1B03934263
NRF-2017S1A5A2A01026454

Title: Alteration of adolescent rat brain by pair exposure with conspecific during fear conditioning

Authors: *M. JANG, T. JUNG, J. NOH
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Abstract: We previously demonstrated the association between fear responses and fear memory by social stimuli via pair exposure with conspecific during fear condition. To determine the link of behavioral and brain neural effects by social stimuli in fear-exposed condition, we carried out passive avoidance test and observed expression of c-Fos in the medial prefrontal cortex (prelimbic and infralimbic), amygdala (lateral, basolateral, and central nuclei) and hippocampus (CA1 and CA3) of single- and pair-exposed rats. Whereas single exposed rats showed the significant increase of freezing behaviors and passive avoidance behaviors compared to control rats, pair exposed rats showed the significant reduction of the freezing behaviors compared to single exposed rats. Furthermore, we found the significant increase in expression of c-Fos in the prelimbic prefrontal cortex and basolateral nuclei of the amygdala of pair-exposed rats compared with control and single-exposed rats. Taken together, we suggest that pair exposure during fear situation helps to cope with both freezing response and fear memory systems via differential expression of the prelimbic prefrontal cortex and basolateral amygdala.

Disclosures: M. Jang: None. **T. Jung:** None. **J. Noh:** None.

Poster

153. Neuroendocrinology of Social Behavior

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 153.13/RR9

Topic: F.02. Behavioral Neuroendocrinology

Support: JSPS 16J05070

Title: Distribution of vasopressin receptor 1a and 1b in mouse brain

Authors: *K. HORIE, S. ADACHI, K. NISHIMORI
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Abstract: Arginine vasopressin (AVP) is the neurotransmitter produced in the hypothalamus. The AVP receptors are constituted of three types of G protein coupled receptors, which are arginine vasopressin receptor 1a (*Avpr1a*), 1b (*Avpr1b*), and 2. Avpr1a and Avpr1b are the major receptors to transduce different types of G protein signals in the mice brain. Avpr1a regulates

aggression, olfactory system, social behavior, circadian rhythm and so on. Avpr1b also regulates these behaviors and maternal aggression. Even though the behaviors regulated by Avprs have been reported, there were little research reported the cellular level distribution and co-localization of these two receptors in the mice brain. In this research, we reported that we successfully generated *Avpr1a-P2A-tdTomato* and *Avpr1b-P2A-EGFP* double knocked-in mice by Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) /CRISPR associated protein (Cas) to reveal the distribution and co-localization of these two receptors.We constructed two distinct crispr RNA (crRNA) targeting to each stop codon in Avpr genes. After *in vitro* digestion assay, which is the assay to evaluate the efficiencies of crRNA, we injected each crRNA, trans-activating crRNA, knock in plasmid, and Cas9 protein in to the pronuclear and cytosol of mice embryo. We successfully generated *Avpr1a-P2A-tdTomato*/*Avpr1b-P2A-EGFP* double knocked-in mice highly efficiently. To analyze the distribution of these receptors, double knocked-in mice were fixed and each fluorescence was detected by microscope. We detected Avpr1a signal in previously reported region, such as lateral septum, suprachiasmatic nucleus, and so on, and also, we detected Avpr1b expressed region such as CA2. Additionally, we discovered unreported region expressing *Avpr1b* in the brain.These mutants are invaluable for studying the function of Avprs.

Disclosures: K. Horie: None. **S. Adachi:** None. **K. Nishimori:** None.

Poster

153. Neuroendocrinology of Social Behavior

Location: SDCC Halls B-H

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Program #/Poster #: 153.14/RR10

Topic: F.02. Behavioral Neuroendocrinology

Support: National Institute of Mental Health Intramural Research Program (Z01-MH-002498)

Title: Stimulation of presynaptic fibers projecting from median raphe to CA2 and social behaviors

Authors: *S. LEE[1], S. WILLIAMS AVRAM[1], A. CYMERBLIT-SABBA[1], J. SONG[1], K. COUREY[2], S. YOUNG[1]
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Abstract: The hippocampal cornu ammonis area 2 (CA2) region has been shown to be essential for social memory. Recently, it was shown that targeted CA2 stimulation of vasopressin presynaptic fibers from the paraventricular nuclei (PVN) of hypothalamus strongly enhances social memory in mice. Moreover, the dorsal CA2 area of the mouse hippocampus receives neuronal inputs from other regions including the septal nuclei, vertical and horizontal limbs of the nucleus of diagonal bands of Broca, supramammillary nuclei (SUM), and median raphe

nucleus. However, the functions of these neurocircuits have not been investigated much. Thus, it would be important to pinpoint how these neuronal inputs to CA2 from different regions help orchestrate various behaviors, including social recognition, object recognition, aggression and anxiety-like behaviors. The median, as do other raphe nuclei, contain a population of serotonergic neurons. Serotonergic neurons play a role in anxiety-like and aggressive behaviors. Thus, we investigated the behavioral role of presynaptic fibers from the median raphe nucleus projecting to the CA2. We used a transgenic mouse serotonin transporter promoter (Slc6a4)-driven cre line crossed with B6.129(Cg)-Gt(ROSA)26Sortm4(ACTB-tdTomato,-EGFP)Luo/J or injected with various viruses, to trace and characterize neuronal fibers from the median raphe nucleus to CA2 region. We also injected AAV-EF1a-DIO-hChR2(H134R)-mCherry into the median raphe nucleus and implanted optogenetic fibers into the CA2. The fibers containing channelrhodopsin (ChR2) projecting from the median raphe nucleus to CA2 were optogenetically stimulated during various social behavioral tests, including social exploration and memory, object recognition, marble burying, and open-field. There were no effects on social and object memory, or anxiety-like behavior, yet there was a decrease in social interaction with novel ovariectomized female mice without affecting investigation of physical objects. Thus, serotonergic input may regulate interest in social objects without affecting memory.

Disclosures: S. Lee: None. **S. Williams Avram:** None. **A. Cymerblit-Sabba:** None. **J. Song:** None. **K. Courey:** None. **S. Young:** None.

Poster

153. Neuroendocrinology of Social Behavior

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 153.15/RR11

Topic: F.02. Behavioral Neuroendocrinology

Title: Effects of propranolol on cortisol, alpha-amylase, and circulating interleukin-6 following a social stress task

Authors: *M. M. GAUDIER-DIAZ, J. K. MACCORMACK, E. ARMSTRONG-CARTER, K. A. MUSCATELL
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Abstract: The sympathetic nervous system (SNS) and hypothalamic-pituitary-adrenal (HPA) axis are biological systems activated in response to stress. Clinical studies examining the relationship between stress and disease etiology have identified inflammation as a promising underlying mechanism. Nonetheless, the specific process by which stress induces increases in inflammation remain underspecified in humans. One prominent hypothesis is that the SNS mediates the effect of stress on inflammatory cytokines. To test this, healthy young adults (N=90) were administered placebo (n=47) or propranolol (40 mg; n=43) in a randomized,

double-blind trial. One hour later, they underwent a social stress task and serial collection of biological specimens (e.g., blood and saliva). Salivary cortisol and alpha-amylase are indicative of HPA axis and SNS activation, respectively; furthermore, the area under the curve ratio of alpha-amylase to cortisol (AUC ratio) serves as a measure of dysregulation of the stress response system. When controlling for drug condition there was a significant increase in cortisol ($F(1,85)=18.7$, $p<0.001$) and in the levels of inflammatory cytokine interleukin-6 (IL-6; $F(1, 78)=18.0$, $p<0.001$), but not salivary alpha-amylase ($F(1,81)=0.2$, $p>0.05$), in response to the stressor. The increase in cortisol and IL-6 suggests that the social stress task elicited a stress response in participants. Additionally, propranolol (relative to placebo) was associated with a marginal decrease in alpha-amylase ($t(83)=1.8$, $p=0.076$) and a significant decrease in the AUC ratio ($t(83)=3.6$, $p<0.001$), indicating that propranolol dampened stress-induced SNS activation. In sum, while propranolol effectively blocked SNS responses to stress, it did not attenuate HPA axis or IL-6 responses, suggesting that the inflammatory response to stress may not necessarily depend on SNS activation. An alternative explanation for the absence of a drug effect on inflammation, could be that the inflammatory response extends beyond our study timeline. Thus, to fully understand the mechanisms linking stress to inflammation in humans, further research is necessary.

Disclosures: M.M. Gaudier-Diaz: None. **J.K. MacCormack:** None. **E. Armstrong-Carter:** None. **K.A. Muscatell:** None.

Poster

153. Neuroendocrinology of Social Behavior

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 153.16/RR12

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant R01 MH103322

Title: Oxytocin in the bed nucleus of the stria terminalis promotes avoidance of novel social stimuli

Authors: *N. DUQUE-WILCKENS[1], V. MINIE[3], S. YOKOYAMA[2], A. TRAN[2], V. GRINEVICH[4], B. C. TRAINOR[5]
[1]Animal Behavior Grad. Group, [2]Univ. of California, Davis, Davis, CA; [3]Univesrity of California, Davis, Davis, CA; [4]German Cancer Res. Ctr., Heidelberg, Germany; [5]Univ. of California -Davis, Davis, CA

Abstract: Oxytocin (OT) is usually considered a prosocial hormone, but growing evidence suggests that the behavioral effects of OT are context and sex-specific. Although it has been proposed that OT enhances the salience of positive and negative social stimuli, the underlying

mechanisms are not understood. We previously showed that social defeat induces social avoidance and social vigilance in female but not male California mice. This response is accompanied by hyperactivity in OT neurons in medioventral bed nucleus of the stria terminalis (BNST). In stressed females, one systemic dose of OT receptor antagonist (OTA) reversed the effects of stress on social avoidance and vigilance, and infusion of OTA into the anterior BNST had identical effects. Here, we show that morpholino knockdown of OT in the medioventral BNST prevents stress-induced phenotype in females, and furthermore, infusion of OT into the anterior BNST induces social avoidance and vigilance in females naïve to social defat. Preliminary data using AAV that expresses a fluorescent marker in OT neurons, together with retrobeads injected in anterior BNST, suggest that OT neurons in medioventral BNST send direct projections to anterior BNST. Together, our results suggest that in females, stress-induced hyperactivity of OT neurons in medioventral BNST activate OT receptors in anterior BNST, which results in avoidance of unknown social stimuli and may partly explain how OT can have such diverse effects depending on the context.

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Poster

153. Neuroendocrinology of Social Behavior

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Program #/Poster #: 153.17/RR13

Topic: F.02. Behavioral Neuroendocrinology

Support: JSPS 15H05724
JSPS 17H05549

Title: Distribution of two types of estrogen receptors and co-expression with oxytocin and oxytocin receptors in the neural networks for social and anxiety-related behaviors

Authors: *S. SAGOSHI[1], S. MAEJIMA[3], M. MORISHITA[4], T. SAKAMOTO[5], H. SAKAMOTO[5], S. TSUKAHARA[4], S. OGAWA[2]
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Abstract: Two types of estrogen receptors, ERα and ERβ, are differentially expressed in a number of brain areas involved in neural networks for social and anxiety-related behaviors. In our previous behavioral studies with the use of knockout mouse models, we hypothesized an estrogen-dependent four-gene micronet, composed of ERα, ERβ, oxytocin (OT), and oxytocin receptor (OTR), for the regulation of social recognition (Choleris et al, PNAS, 2003) and anxiety

(Tomihara et al., Physiol Behav, 2009). However, in contrast to ERα dependent action, mechanisms of hormonal action through ERβ have not been well understood and the four-gene micronet hypothesis has not been completely proven, mainly due to difficulties to identify ERβ and OTR expressing cells in the brain. In the present study, we have first generated ERβ-RFP^{tg} mice, in which red fluorescent protein (RFP) was inserted downstream of ERα BAC promotor and verified RFP signal as ERβ. Immunohistochemical assays revealed that RFP expressing cells were mainly localized in the paraventricular neucleus of the hypothalamus (PVN), olfactory bulb, cingulate cortex, island of Calleja, medial preoptic area (MPOA), bed nucleus of stria terminalis, medial amygdala (MeA), granule cell layer of ventral hippocampus, and dorsal raphe nucleus. Double immunohistochemical staining revealed that RFP co-localized with arginine vasopressin, tryptophan hydroxylase-2 and progesterone receptors in a manner consistent with previously reported findings. Furthermore, we could identify neuronal sub-populations those express both types of ERs and those express exclusively ERα or ERβ in the MeA and MPOA. Based on these findings, we further analyzed co-localization of ERβ with OT and OTR and compared with that of ERα. Heterozygous female mice of ERβ-RFP^{tg} were mated with OTR-Venus^{tg} homozygous males (kindly provided by Dr. K. Nishimori) to obtain ERβ-RFP^{tg} /OTR-Venus^{tg} mice. We used the resulting RFP+/Venus+ mice for detection of co-expression of ERα as RFP) with OTR (as GFP) or OT, and RFP-/Venus+ littermates for detection of ERβ with OTR (as GFP) or OT. We found that ERβ and OT were co-expressed in the PVN whereas ERα and OTR were co-localized in the ventromedial nucleus of the hypothalamus. Interestingly, in the postero-dorsal part of the MeA, OTR was expressed in both ERα and ERβ positive cells, which were partially overlapped. These findings collectively provide anatomical evidence for differential roles of ERα and ERβ in estrogenic action on the oxytocinergic system implicated in the regulation of social and anxiety-related behavior.

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Poster

153. Neuroendocrinology of Social Behavior

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Program #/Poster #: 153.18/RR14

Topic: F.02. Behavioral Neuroendocrinology

Support: NSERC

Title: Glucocorticoid receptor activity in the medial prefrontal cortex prevents emotional contagion in mice

Authors: *N. LIDHAR[1], S. SIVASELVACHANDRAN[1], H. N. TURNER[2], S. KHAN[1], M. SIVASELVACHANDRAN[1], S. ABDALLAH[1], J. BANG[1], J. KIM[1], N. M. FOURNIER[2], L. J.

MARTIN[1]
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Abstract: Empirical evidence indicates that rodents are capable of low-level forms of empathic responding, such as emotional contagion. Recent studies have demonstrated that mice have the ability to transmit pain status between paired cagemates resulting in contagious pain hypersensitivity (hyperalgesia). The transmission of pain status only occurs during interactions where both mice of the dyad are in pain and share a social history with each other. We've previously shown that the hypothalamic-pituitary-adrenal (HPA) stress axis is an important modulatory system of pain contagion in mice and people. The objective of the current study was to uncover the stress-related neural circuitry that regulates empathy-like behaviours in mice by using fluorescent immunohistochemistry to detect activated (phosphorylated) glucocorticoid receptors following an emotional contagion of pain paradigm. Our histochemical analysis revealed increased glucocorticoid receptor phosphorylation in unfamiliar mice compared to isolated and familiar mouse conditions in brain areas known to be important for empathy in humans such as the medial prefrontal cortex (mPFC) anterior cingulate cortex (ACC) and anterior insula. Pre-treatment with an intraperitoneal injection of metyrapone, a corticosterone inhibitor, significantly reduced the phosphorylation of glucocorticoid receptors in these regions. To assess the sufficiency of glucocorticoid receptors in specific neural regions, mice were implanted with bilateral cannulae in the prelimbic cortex of the mPFC and the ACC. Microinfusions of RU-486, a glucocorticoid receptor antagonist specifically in the prelimbic cortex, but not the ACC, revealed a significant increase in writhing behaviours among unfamiliar mice. Furthermore, microinfusions of corticosterone in the prelimbic cortex resulted in a significant decrease in writhing behaviours among familiar conspecifics. These experiments suggest a top-down mechanism by which glucocorticoid receptor activity in the prelimbic cortex can suppress empathetic responding in mice.

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Poster

153. Neuroendocrinology of Social Behavior

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 153.19/SS1

Topic: F.02. Behavioral Neuroendocrinology

Support: NSFC Grant 31530032

Title: Massage promotes oxytocin release and activates the orbitofrontal cortex and superior temporal sulcus

Authors: *Q. LI, W. ZHAO, B. BECKER, K. JUAN, K. KENDRICK
Univ. of Electronic Sci. and Technol. of, Sichuan Province, China

Abstract: Massage may be an important method for increasing endogenous levels of oxytocin (OXT) that could be of potential therapeutic benefit in disorders with social dysfunction such as autism. Here we investigated the effects of both hand and machine massage on OXT release and brain activation measured using functional near infrared spectroscopy (fNIRS) in healthy subjects. Additionally, we measured potential modulatory effects of autistic traits. We hypothesized that hand would be more effective than machine massage in releasing OXT and in activating brain regions responsive to social touch (orbitofrontal cortex - OFC and superior temporal sulcus STS). 40 adult male subjects received a light foot massage either by hand (professional masseur), or machine (Panasonic EW-NA84) in a counterbalanced order. Subjects were unable to see the person or machine massaging them. Each subject received 10 min of massage (20s massage/10s rest repeated 20 times on both feet) with 15 min between massage types. Immediately after each massage subjects rated pleasure, intensity and arousal and how much they would pay. Blood samples (6 ml) were taken via a venous catheter immediately before and after each massage condition for OXT measurement (ELISA, Cayman + extraction). Neural activity was measured using fNIRS (34-channels: CW6 Techen) with bilateral placements over the medial and lateral OFC, STS and somatosensory cortex (S1). Skin Conductance Responses (SCR) were also recorded (Biopac). Subjects completed the Autism Spectrum Quotient (AQ) before the experiment. Results showed subjects gave higher ratings of pleasure ($p < 0.001$), but not intensity or arousal, after hand compared with machine massage. There were no differential effects on SCR. Subjects were also willing to pay more for the hand massage ($p<0.001$). Plasma OXT levels increased after both hand ($+ 6.6\pm1.23$ pg/ml, $p<0.0001$) and machine ($+ 2.2\pm0.98$ pg/ml, $p = 0.03$) massage, but more potently after hand massage (+48.5% vs. +17.3%, $p = 0.012$). Basal OXT levels were also negatively associated with AQ scores ($r = -0.53$, $p = 0.001$). For the fNIRS analysis significant differences in the effects of hand vs. machine massage on oxy-Hb were found in the lateral STS and bilateral medial/lateral OFC (all $ps<0.05$ Bonferroni corrected) but not in S1. OFC activation during hand massage was associated with both the amount of money subjects would pay ($r = 0.39$, $p = 0.02$) and AQ score ($r = 0.35$, $p = 0.04$). In summary, our results show that hand massage more potently increases OXT release and activity in brain regions involved in social cognition and reward aspects of affective touch than machine massage, and may therefore have therapeutic potential.

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Poster

153. Neuroendocrinology of Social Behavior

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Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 153.20/SS2

Topic: F.02. Behavioral Neuroendocrinology

Support: Herman Dana Foundation

Title: Prospective follow up study of neuroendocrine and prognostic indices in adolescents with eating disorders

Authors: *T. GOLTSER[1], R. GIESSER[2], A. SHALEV[2], A. MELTZER[2], R. MASARWA[2], D. PEVZNER[3], L. CANNETI[3], E. GALILI-WEISSTUB[2], R. SEGMAN[3]
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Background: Eating Disorders (ED) incidence increases in recent years for unkown reasons, with the most severe form of restrictive Anorexia Nervosa (AN) affecting up to 1% of adolescent girls, of which up to one half recover, another third recover only partially, and the rest suffer a chronic treatment refractory course. Major biological determinants mediating risk for ED remain obscure.
Aims: To prospectively charcterise ED patients based on main clinical course charecteristics, prognostic factors, associated mental and physical complications, and treatment response, and locate biological and psychological markers and predictors and causaly involved contributors, for expressing ED and for its psychiatric and medical course and prognosis.
Methods: ED patients reffered to the ambulatory clinic, day care facility and in patient unit of the Herman Dana Child Psychiatry Center are approached to volunteer to the study. Data collected include comprehensive psychological assesment, horomonal profiles, neurotransmitters and proteins, immune cell protein and RNA levels, in search of correlations with symptoms and prognostic indices.
Results: We will report results of the preliminary neruoendocrine analyses of the prospective cohort thus far collected from this ongoing study.
This work was supported by the Herman Dana Foundation.

Disclosures: T. Goltser: None. **R. Giesser:** None. **A. Shalev:** None. **A. Meltzer:** None. **R. Masarwa:** None. **D. Pevzner:** None. **L. Canneti:** None. **E. Galili-Weisstub:** None. **R. Segman:** None.

Poster

153. Neuroendocrinology of Social Behavior

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 153.21/SS3

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH HL112350
NIU RIPS

Title: Observing a sibling experience a stressor alters behavioral and endocrine stress reactivity in prairie voles

Authors: *O. AKINBO[1], J. J. WARDWELL[1], W. T. WATANASRIYAKUL[1], M. C. NORMANN[1], M. COX[1], S. CIOSEK[1], S. SUJET[1], N. HOLZAPFEL[1], A. J. GRIPPO[2]
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Abstract: Observing another individual experience pain, trauma or stress has been found to induce negative behavioral and endocrine responses in humans. Even without being directly exposed to the stressor, this phenomenon of vicarious stress can influence the observer's emotional states, behaviors, and physiology. The mechanisms underlying vicarious stress are not well defined. The prairie vole is a valuable rodent model for studying responses to social stressors, including mechanisms that mediate vicarious stress. Similar to humans, prairie voles are socially monogamous animals that display pair bonding, communal social groups, and biparental care of offspring. The present experiment studied endocrine and behavioral consequences of an acute exposure to vicarious stress in 35 pairs of male prairie vole siblings. Vicarious stress was modeled by combining the tail suspension test (TST) and open field test (OFT). The TST served as a stimulus to induce stress in one animal (model animal experiencing a direct stressor) while the OFT was used to measure the responses of the sibling (observer animal experiencing potential vicarious stress). Sibling pairs were assigned to one of two conditions for 5 minutes: vicarious stress condition, with both animals tested concurrently in the combined TST-OFT apparatus (n = 18); or control condition, with each animal tested individually in the TST or OFT in separate locations at the same time (n = 17). Behaviors during the TST and OFT were recorded and coded later by experimentally-blind raters, and plasma was assayed for circulating corticosterone reactivity 10 minutes following the test. It was hypothesized that observers in the vicarious stress condition would experience greater behavioral and endocrine reactivity than animals in the control (separated) condition. Animals in the vicarious stress condition displayed increased time spent freezing and decreased grooming and rearing in the OFT, vs. animals in the control condition, suggesting that observing a stressed sibling reduces natural exploratory behavior. Additionally, animals in the vicarious stress condition exhibited elevated levels of corticosterone vs. the control condition, suggesting increased physiological reactivity to observing another animal experience a direct stressor. The data indicate that the observer animal may have vicariously experienced stress in response to its sibling's stress. These results can improve our understanding of mechanisms underlying vicarious stress in humans.

Disclosures: O. Akinbo: None. **J.J. Wardwell:** None. **W.T. Watanasriyakul:** None. **M.C. Normann:** None. **M. Cox:** None. **S. Ciosek:** None. **S. Sujet:** None. **N. Holzapfel:** None. **A.J. Grippo:** None.

Poster

153. Neuroendocrinology of Social Behavior

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 153.22/SS4

Topic: F.02. Behavioral Neuroendocrinology

Support: NSF IOS-1354942
BEACON Center for the Study of Evolution in Action #947
BEACON Center for the Study of Evolution in Action #1081

Title: Developmental origins of social behavior and neuroendocrine function

Authors: *T. SOLOMON-LANE, H. A. HOFMANN
Univ. of Texas at Austin, Austin, TX

Abstract: Early-life social environments shape adult behavior critical for health and evolutionary fitness, yet fundamental questions remain about the behavioral and neuromolecular mechanisms underlying these processes. Social experiences vary across individuals, and accrued experiences can profoundly affect future behaviors, resulting in dramatically different developmental trajectories. Such plasticity is especially likely in brain regions, neuromodulators, and gene networks regulating adult social behavior. We use the highly-social African cichlid fish, *Astatotilapia burtoni*, to investigate juvenile social behavior, how it is shaped by early-life social environments, and the underlying neuroendocrine mechanisms. First, juvenile behaviors appear largely similar to adults, but key differences reveal processes of behavioral development. For example, unlike adults, juvenile agonistic interactions are prosocial, and size-matched juveniles are unable to form stable social status relationships. Second, we show that juvenile brain and behavior are sensitive to multiple early-life social environments, including social and maternal effects. Juvenile behavior forms a syndrome, and individuals reared in social groups are more active in open field and social cue investigation assays, and more interactive in a dominance assay, than juveniles reared in pairs. Importantly, the early-life social environment also significantly affects neural gene expression networks of key neuroendocrine regulators of social behavior. Glucocorticoid and androgen receptor expression drives these differences. The effects of maternal brooding duration on juvenile behavior may be caused by similar neuroendocrine stress axis mechanisms. Together, this research demonstrates the important developmental origins of adult phenotypes and identifies some mechanisms underlying social behavioral variation, which has powerful consequences for health and fitness.

Disclosures: T. Solomon-Lane: None. **H.A. Hofmann:** None.

Poster

153. Neuroendocrinology of Social Behavior

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 153.23/SS5

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant MH110212
NIH Fellowship F31MH113367

Title: Sex differences in social reward and sex-specific effects of oxytocin in the ventral tegmental area on social motivation

Authors: *J. M. BORLAND[1,2], K. O'LAUGHLIN[1,2], K. GRANTHAM[1,2], L. AIANI[1,2], K. J. FRANTZ[1,2], H. E. ALBERS[1,2]
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Abstract: The rewarding and motivating properties of social interaction are a fundamental element in the expression of adaptive social behaviors and the development and maintenance of social relationships. Furthermore, dysfunctions in social reward likely contribute to the etiology of many psychiatric disorders. Because social behavior evolved in response to different selective pressures in males and females, the neurobiological mechanisms mediating social reward are likely sex-dependent, and it seems likely that these sex differences may contribute to sex differences in the prevalence of psychiatric disorders. Using the traditional Conditioned Place Preference (CPP) test and a recently validated Operant Social Preference (OSP) test, we report that both male and female Syrian hamsters found same-sex social interactions rewarding. However, females displayed about a two-fold greater preference for same-sex social interactions compared to males. Next we investigated potential sex differences in the role of oxytocin (OT) in regulating social reward. We have previously shown that activation of OT receptors (OTR) in the ventral tegmental area (VTA) is essential for social reward in male hamsters. However, because the mesolimbic dopamine system and the OT system are sexually differentiated, we hypothesized that OT in the VTA has sex specific effects. OT neurons in both the paraventricular nucleus and supraoptic nucleus are activated during same-sex social interactions in males and females. Furthermore, inhibition of OTRs in the VTA during social interaction conditioning decreased social reward in both males and females. However, exogenous stimulation of OTRs or treatment with OT in the VTA had sex-specific effects on social reward. In females, injections of OT or a selective OTR agonist decreased social reward. However, very interestingly for males, OT or the OTR agonist increased social reward. In conclusion, females found same-sex social interactions more rewarding than males, and although the OT system is necessary for social reward in both males and females, exogenous stimulation of the OT system can increase social

reward in males, but decreases social reward in females. Thus, similar to well-established sex differences in drug reward, females may be more sensitive to the rewarding effects of social interactions and there may be an inverted-U shaped dose response curve for social reward value with females shifted more to the left compared to males.

Disclosures: J.M. Borland: None. **K. O'Laughlin:** None. **K. Grantham:** None. **L. Aiani:** None. **K.J. Frantz:** None. **H.E. Albers:** None.

Poster

153. Neuroendocrinology of Social Behavior

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 153.24/SS6

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant RO1 AG043467

Title: Analysis of neuronal activation in response to novel social interaction in forebrain oxytocin target neurons of young and old F344 female rats

Authors: *J. R. RAVENEL[1], A. E. PERKINS[2], A. DEFENDINI[1], T. DEAK[2], R. L. SPENCER[1]
[1]Dept. of Psychology and Neurosci., Univ. of Colorado, Boulder, CO; [2]Behavioral Neurosci. Program, Binghamton Univ., Binghamton, NY

Abstract: We previously found that young adult female rats, but not age-matched male rats, show enhanced neural activation (*c-fos* mRNA) after conspecific social interaction during the final 10 min of a 30 min test chamber (context) exposure when compared to context exposure alone. These differences were especially pronounced in several forebrain regions important for social behavior and stress processing—bed nucleus of the stria terminalis (BNST), medial amygdala (MeA), prelimbic cortex, hypothalamic paraventricular nucleus (PVN), and hippocampal CA3 region (HC) (Perkins et al, Brain Res 1672:113-121, 2017). In a follow-up experiment, we compared young (3 mos) and old (18 mos) female rats under the same test conditions, and mapped c-fos mRNA expression patterns throughout much of the forebrain (radiolabeled *in situ* hybridization). We observed overall blunting of the *c-Fos* mRNA response to social interaction of the aged female rats that was most pronounced in some of the same brain regions that were selectively responsive to social interaction (BNST, MeA, HC and neocortex). The age-dependent blunting of *c-Fos* expression was associated in the same rats with decreased social investigative behavior (Deak et al. Soc Neursci Abs 2016:596.01/L7). Because central oxytocin neurotransmission is an important modulator of social behavior, we hypothesized that changes in the central oxytocinergic system with aging may contribute to age-related declines in social behavior and the underlying neural control of that behavior. To test this hypothesis, we examined whether the age difference in neural response to novel social interaction extends to

differences in neural activation of oxytocin target neurons, i.e. neurons that express oxytocin receptors (*Otr* mRNA). We used an *Otr* and *c-Fos* mRNA fluorescent *in situ* hybridization double-label strategy. We performed this analysis on tissue from the same young and old female brains described above in which we had mapped the overall *c-Fos* mRNA profile. We observed enriched oxytocin receptor expression especially in the female ventromedial hypothalamus (VMH), and olfactory nucleus, but also lower level expression in the PVN, central nucleus of the amygdala and the BNST. We found that very few cells in the VMH of both young and old female rat brains were activated (*c-Fos* positive) in response to the social interaction. Interestingly, however, there was a significant decrease in Otr expression (~50% reduction) in the VMH of aged rats. We are now examining *c-Fos* and *Otr* expression in other brain regions that showed the strongest response to the social interaction experience.

Disclosures: J.R. Ravenel: None. **A.E. Perkins:** None. **A. Defendini:** None. **T. Deak:** None. **R.L. Spencer:** None.

Poster

153. Neuroendocrinology of Social Behavior

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Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 153.25/SS7

Topic: F.02. Behavioral Neuroendocrinology

Support: Vetenskapsrådet 348-2014-4396
Conacyt CB-2013/22173
PAPIT IN205217

Title: Role of the vasopressin V1b receptor in the amygdaloid modulation of social behaviors

Authors: *O. R. HERNANDEZ PEREZ[1], M. CRESPO-RAMIREZ[1], K. FUXE[2], M. PEREZ DE LA MORA[1]
[1]Cognitive Neurosci., Inst. de Fisiologia Celular, UNAM, México City, Mexico; [2]Dept. of Neurosci., Karolinska Institutet, Stockholm, Sweden

Abstract: The amygdala plays a paramount role in the modulation of anxiety and numerous studies have shown that arginine vasopressin (AVP) elicits anxiogenic effects following either its systemic or septal administration. The aim of this paper was to study the involvement of vasopressinergic neurotransmission in the amygdaloid modulation of unconditioned anxiety and to ascertain whether or not AVP receptor subtypes may have a differential role in this modulation. Anxiety behavior was evaluated both in Shock-Probe Burying Test and Light-Dark Box following the bilateral microinfusion of AVP alone or AVP together with either AVP 1a or AVP V1b receptor antagonists into the central amygdala (CeA). AVP microinfusion elicited at low (1 ng/side) but not at high doses (10 ng/side) anxiogenic-like responses in the Shock-Probe

Burying Test but not in the Light-Dark Box. SSR149415, an AVP V1b antagonist unlike Manning compound, an AVP V1a antagonist, fully prevented AVP effects in the Shock-Probe Burying Test when it was administered simultaneously with AVP. In addition, oxytocin receptor blockade also failed to affect AVP effects. No effects of any AVP antagonist by itself were observed in both anxiety paradigms. Our results indicate that AVP V1b receptor contribute to the amygdaloid modulation of anxiety at least in the context of the Shock-Probe Burying Test since no effects were noticed in the Light- Dark Box. It remains to the future to ascertain whether AVP receptor subtypes have indeed differential actions either in the modulation of global or specific features of unconditioned anxiety.

Disclosures: O.R. Hernandez Perez: None. **M. Crespo-Ramirez:** None. **K. Fuxe:** None. **M. Perez de la Mora:** None.

Poster

153. Neuroendocrinology of Social Behavior

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 153.26/SS8

Topic: F.02. Behavioral Neuroendocrinology

Support: Brinkman Family Foundation

Title: The effects of acute intranasal oxytocin on anxiety and social behaviors using the valproic acid model of autism spectrum disorder

Authors: *S. M. HARDING[1], A. C. AGUDELO RIVERA[2], N. S. LOCURTO[2]
[1]Psychology Dept, [2]Fairfield Univ., Fairfield, CT

Abstract: Autism Spectrum Disorder (ASD) affects 1 in 68 children and is characterized by deficits in social behaviors and communication. In humans, in utero exposure to the antiepileptic compound valproic acid (VPA) is associated with an increased risk of developing ASD. Previous research has also shown that VPA exposure during development in rodents produces a reliable animal model for studying ASD. The present study examined the effects of acute intranasal oxytocin (OT) on anxiety and a variety of social behaviors in the ASD animal model. Pregnant Long Evans rats were administered saline or VPA (600mg/kg body weight) on gestational day 12.5. After weaning, males and females were assigned to the following groups: Saline-saline, VPA-saline, or VPA-OT (n=7 males and n=6 females per group). Saline or OT (0.8IU/kg) was administered intranasally 30-60 minutes before behavioral tests for anxiety (elevated plus maze), sociability, and reproductive behaviors (copulation tests and partner preference tests) in adolescence and adulthood. On the elevated plus maze, male VPA rats showed significantly increased open arm latency compared to Saline-saline controls ($p < .024$), unless OT was administered. Surprisingly, no differences between groups were observed on sociability tests. In

tests for reproductive behaviors, OT reduced mount latency in VPA-exposed males, and impaired partner preference in VPA-exposed females. These findings have important implications for ASD, and suggest that sex is an important variable to consider when administering treatment.

Disclosures: S.M. Harding: None. **A.C. Agudelo Rivera:** None. **N.S. LoCurto:** None.

Poster

153. Neuroendocrinology of Social Behavior

Location: SDCC Halls B-H

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Program #/Poster #: 153.27/SS9

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH NS034950
NIH MH101373
NIH MH 096220
Arnold O. Beckman Postdoctoral Fellowship

Title: Differential effects of androgen signaling on social behavior in a cichlid fish

Authors: *B. A. ALWARD, A. AGRAWAL, P. H. CATHERS, S. A. JUNTTI, R. D. FERNALD
Biol., Stanford Univ., Stanford, CA

Abstract: Animals exhibit different patterns of social behavior throughout life. During early development members of many species perform different types of affiliative behavior, but after reaching sexual maturity begin performing territorial and reproductive behaviors. The underlying molecular mechanisms of these
behaviors, however, remains to be established. We investigated the role of steroid hormones in the regulation of social behavior and physiology in the African cichlid fish *Astatotilapia burtoni.* In *A. burtoni,* juveniles can be observed shoaling (or, a less organized form of schooling), but once sexual maturity is
reached males begin showing brighter coloration than females and, through agonistic interactions between males, establish a clear social dominance hierarchy. Dominant (DOM) male fish maintain territories through aggressive interactions and court females, while non-dominant males perform simple
behaviors such as fleeing from DOM males. DOM males are brightly colored, have higher levels of testosterone (T) and 11-ketotestosterone (11-KT; a fish specific androgen), and possess larger testes compared to ND males. To investigate how androgens affect shoaling behavior, we combined steroid
immersion treatment with high-throughput tracking procedures. *A. burtoni* fry (~7 days post

fertilization) were immersed in vehicle (DMSO), ethinyl estradiol (EE), or 17α-methyltestosterone (MT) for four days. We measured shoaling behavior at 43 and 52 dpf. DMSO and EE fish shoaled more than MT fish on both days, but all groups shoaled less over time. *A. burtoni* possess two androgen receptors (ARs), *ar1* and *ar2*. Using CRISPR-Cas9 gene editing, we generated *A. burtoni* homozygous for frame-shift alleles encoding *ar1* (*ar1-/-*), *ar2* (*ar2-/-*), or both *ar1* and *ar2* (*ar1-/-;ar2-/-*). In adult *A. burtoni*, both *ar1-/-* and *ar2-/-* males and females are fertile. *ar2-/-* males are drably colored like females and possess substantially smaller testes compared to sibling wild-type males. *ar1-/-* males show bright coloration typical of their wild-type counterparts yet possess much larger testes. Preliminary studies suggest *ar1-/-* males court females significantly less than wild-type males. Therefore, androgens play an important role in the regulation of social behavior and physiology in *A. burtoni*. Investigating the distinct roles played by ARs in *A. burtoni* will provide insight into the fundamental molecular mechanisms of social behavior and its evolution.

Disclosures: B.A. Alward: None. **A. Agrawal:** None. **P.H. Cathers:** None. **S.A. Juntti:** None. **R.D. Fernald:** None.

Poster

153. Neuroendocrinology of Social Behavior

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 153.28/SS10

Topic: F.02. Behavioral Neuroendocrinology

Title: Impact of life style, physical and mental training on brain structure and functioning

Authors: *R. COURA[1], S. GRANON[2]
[1]Neurotélos, Paris, France; [2]Inst. des Neurosciences Paris-Saclay (CNRS), Orsay, France

Abstract: In the last few years, studies have been showing the effects of physical exercise, breathing and meditation on brain structure and functioning. At the same time, advances in Neurosciences have been pinpointing the necessary conditions for keeping the brain healthy and providing an optimal performance. Moreover, mental processes and mental states are known to have an impact on everything we do, from body and brain physiology to behavior, social interaction, and consciousness. Here, for the first time, we propose to evaluate the impact of different parameters at the same time on brain structure, wiring and functioning. These parameters include life style (including nutrition, quality of human relationships, sleeping, and others parameters), specific physical exercises, breathing techniques, relaxation, as well as concentration, selective attention, and meditation training. All the behavior and techniques belong to the DeROSE Method, an international methodology specialized in improving performance and quality of life. We will assess qualitative and quantitative data. Qualitative data will be computed through a validated questionnaire that we developed in order to analyze

welfare and performance from the point of view of the subjects and their subjective experience and perceptions in the physical, emotional and mental levels. Quantitative will consist mainly of EEG data, cerebral imaging (scan), blood count and biochemical analyses. We will use a compact electroencephalography system (Muse), a validated tool allowing rapid EEG data collection from many participants. Measures will be done during different moments of physical and mental training, as well as during decision-making tasks.The study is being carried out in Paris, New York and Brazil. The population is of 30-50 controls, 30-50 beginners (from 1-3 months practicing), 30-50 junior experienced (one to five years practicing) and 30 senior experienced (more than five years practicing) subjects in each location, which makes a total population of 90-150 individuals for each group, with equal representation of both genders. Preliminary results from qualitative data showed an improvement in healthy state and welfare perception, as well as in performance on sports and at work. Subjects also reported better interpersonal relationships, as well as improved decision making, and also stress management ability.

Disclosures: R. Coura: None. **S. Granon:** None.

Poster

153. Neuroendocrinology of Social Behavior

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 153.29/SS11

Topic: F.02. Behavioral Neuroendocrinology

Support: University of Nebraska Omaha Graduate Research and Creative Activity Grant

Title: Heterogeneity of social rejection experiences: A comparison of affective, behavioral, and physiological responses

Authors: *S. KISTER[1], E. BASS[1], I. FRENCH[1], K. MOSER[2], R. BRODSKY[1], A. NELSON[1], K. REIDELBERGER[1], E. OBAID[1], D. SCHNEIDER[1]
[1]Univ. of Nebraska At Omaha, Omaha, NE; [2]Univ. of Nebraska Omaha, Omaha, NE

Abstract: Due to the fundamental need for belonging (Baumeister & Leary, 1995), social rejection threatens wellbeing. Yet, there has been inconsistency in physiological (stress hormone; Blackhart et al., 2007 vs. Bass et al., 2014), affective (Baumeister et al., 2017 vs. Gerber & Wheeler, 2009), and behavioral responses (Maner et al., 2007 vs. Twenge et al., 2007). Due to shared neural substrates of physical and social pain, research suggests differences may be accounted for by severity of rejection experiences (Cyberball vs. Future Life laboratory manipulations; Bernstein & Claypool, 2012a, 2012b). This current study is the first to compare physiological, affective, and behavioral responses (and interrelations) to two different rejection experiences within a single study. Ongoing experimental research involves 95 participants to

date (75% female; Mage = 21.40, SD = 5.44) randomly assigned to either Cyberball (less severe) or Future Life manipulation (more severe) and either acceptance or rejection. Participants self-reported fear of negative evaluation before the manipulation, self-reported positive and negative affect and basic needs satisfaction (belonging, self-esteem, meaningfulness, control) before and after the manipulation; provided saliva for stress hormone analyses (cortisol, alpha amylase); and engaged in a simulated social experience with the opportunity to be prosocial or antisocial. Those rejected in either paradigm exhibited decreases in all basic needs; effects were greater for those excluded in Cyberball. Those predicted to have a future alone also had reduced positive and negative affect, whereas those excluded in Cyberball had increased negative affect. Reduced self-esteem and sense of belonging following rejection in either paradigm were greater for women. All responses were greatest for women high in fear of negative evaluation. A predicted future alone lead to more antisocial responses for those who experienced a decrease in positive and negative affect, self-esteem, belonging, meaningfulness, or control; the effect was stronger for those high in fear of negative evaluation. Stress hormones will be analyzed at the conclusion of data collection (08/2018).Results support aversive responses to rejection (varying by type of rejection, gender, and fear of negative evaluation) and potential for antisocial responses. Evidence is consistent with the neurophysiology of social pain: emotional analgesia for predicted future alone (major social injury) versus negative emotion for Cyberball exclusion (minor social injury), and antisocial responses for predicted future alone only.

Disclosures: E. Bass: None. **I. French:** None. **K. Moser:** None. **R. Brodsky:** None. **A. Nelson:** None. **K. Reidelberger:** None. **E. Obaid:** None. **D. Schneider:** None.

Poster

154. Neuroendocrine Processes: Sexual Differentiation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 154.01/SS12

Topic: F.03. Neuroendocrine Processes

Support: NIH R01 GM108885
NSF IOS 1353075

Title: A conserved genetic timer regulates the sexual maturation of neural circuits and behavior

Authors: *D. S. PORTMAN[1], H. LAWSON[2]
[1]Ctr. for Neural Develop. and Dis., [2]Univ. of Rochester, Rochester, NY

Abstract: As juvenile animals mature through adolescence into adulthood, extensive changes take place in the nervous system, many of which generate sex differences in circuit function and behavior. In humans, the mechanisms underlying the temporal control of the onset of these changes are poorly understood. In the nematode *C. elegans*, adult circuit function and behavior

exhibit numerous sex differences, many of which are controlled cell-autonomously by the sex-determination hierarchy. For example, adult hermaphrodites and males differ in their sensory responses to food and sex pheromones, and genetic sex-reversal of specific sensory neurons is sufficient to recapitulate the sex-typical physiological and behavioral features of one sex in the other. However, while these neurons are fully differentiated at birth and their genetic sex remains constant, the sex-specific features controlled by genetic sex emerge only at the larval-to-adult transition. To explore mechanisms that might temporally gate the effects of genetic sex on post-mitotic neurons, we considered a role for the conserved developmental regulator LIN-28 and its target miRNA *let-7*. We find that mutations in these "heterochronic genes" delay or advance the timing of sexually dimorphic changes in gene expression and behavior that normally occur at the juvenile-to-adult transition. *lep-2*/Makorin, a recently described *C. elegans* heterochronic gene, also functions in this mechanism; moreover, these factors act cell-autonomously to control nervous system maturation. Remarkably, LIN28 and the Makorin MKRN3 are also associated with the timing of puberty in mammals, including humans. Our results indicate that a conserved, miRNA-dependent regulatory module may act cell-autonomously in the brain to mediate the timing of the functional maturation of the mammalian nervous system.

Disclosures: D.S. Portman: None. **H. Lawson:** None.

Poster

154. Neuroendocrine Processes: Sexual Differentiation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 154.02/SS13

Topic: F.03. Neuroendocrine Processes

Support: F31NS0933947
R01MH52716

Title: Phagoptosis by microglia determines the size of the sexually dimorphic nucleus (SDN) of the POA

Authors: *L. A. PICKETT, M. M. MCCARTHY
Pharmacol., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: The largest neuroanatomical sex difference in the mammalian brain was reported in 1971 and later named the sexually dimorphic nucleus (SDN) due to its larger size in males compared to females. Many sex differences in the brain are determined by elevated androgens and estrogens in males during the perinatal period. In the SDN, males and females generate the same number of neurons but the lack of estradiol in females causes neurons in the SDN to selectively die off early in life, whereas the production of estradiol from precursor androgens in males exerts a neuroprotective effect. How estradiol prevents cell death in the SDN is unknown

but appears to be unique from mechanisms established for the AVPV and BNST, two nuclei that also display sex differences in volume. Recent discoveries on the critical role of non-neuronal cells, such as microglia, in sculpting the developing brain led us to explore the role of these cells in development of sex differences in neuronal architecture. Our lab has demonstrated that microglia are essential for the masculinization of dendritic spine density on POA neurons, as well as male sexual behavior. We further discovered that the female central MPN of the POA contains more "surveying" phagocytic microglia than males during the first postnatal week, a phenomenon that peaked on postnatal day 8 (PN8). Inhibition of microglial phagocytosis by intracerebral injections of minocycline or antibodies against CD11b, a microglial receptor important for phagocytic activity, on PN5-7 increased the volume of the SDN in both males and females when examined on PN8, a finding consistent with the interpretation that microglia are engaging in phagoptosis (engulfment of stressed, but viable cells) in order to shape the size of the SDN. This discovery, coupled with the known role of estradiol in this system, predicts that estradiol suppresses microglial phagoptosis in the male SDN, rather than preventing conventional cell-autonomous apoptosis. We anticipate that microglia initiate phagoptotic events in females, in the absence of estradiol. Further studies are underway to determine the impact of estradiol on microglial phagoptosis and the specific cellular targets of phagoptosis in the developing SDN. Completion of the experiments proposed will challenge the dogma that estradiol prevents neuronal apoptosis in the male SDN; and reveal novel hormone and neuroimmune mechanisms that regulate phagoptotic and neuroprotective cascades during normal brain development. Understanding these mechanisms will not only facilitate studies of sex differences in other brain regions, but may also lead to novel insights into windows of vulnerability during development.

Disclosures: L.A. Pickett: None. **M.M. McCarthy:** None.

Poster

154. Neuroendocrine Processes: Sexual Differentiation

Location: SDCC Halls B-H

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Topic: F.03. Neuroendocrine Processes

Support: Seed grant from the Brains & Behavior Program at Georgia State University

Title: 5-hydroxymethylcytosine as a stable epigenetic mark possibly involved in sexual differentiation of the brain

Authors: *C. D. CISTERNAS[1], L. CORTES[1], E. C. BRUGGEMAN[2], B. YAO[2], N. G. FORGER[1]
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Abstract: A transient exposure to gonadal testosterone around the time of birth has lasting effects on the brain and behavior of male rodents. The mechanisms underlying this cellular memory are not completely understood, but the role of epigenetic regulation including DNA methylation have recently been implicated. DNA methylation at the 5-carbon position of cytosine (5mC) can be oxidized to 5-hydroxymethylcytosine (5hmC) and downstream derivatives by the Ten-eleven Translocation (Tet) enzymes. While 5hmC was viewed as an intermediate step, recent evidence indicates that 5hmC accumulates in neurons during postnatal life, suggesting 5hmC as a stable epigenetic mark regulating gene expression. Although 5hmC is highly enriched in mammalian central nervous system, whether 5hmC has a role in sexual differentiation of the brain has not been examined. As a first step, we evaluated sex differences and the effect of neonatal testosterone on the expression of the Tet enzymes in the mouse brain across development. Newborn mice received testosterone propionate or peanut oil on the day of birth (P0) and P1. Mice were sacrificed at P1, P25 or P60 and the mRNA expression of Tet1, Tet2 and Tet3 was examined by quantitative RT-PCR in the ventromedial hypothalamic region, anterior preoptic region and hippocampus/cortex. Region-specific sex differences were found for all Tet enzymes during development (P1 or P25), but not in adulthood. Female mice expressed lower levels of Tet1, Tet2 and Tet3 in the ventromedial hypothalamic region while expressing higher levels than males in the hippocampus/cortex. The sex difference in Tet expression was not affected by testosterone treatment of females. Expression of all three Tet enzymes was also much higher at P1 than at P25 or P60. This was somewhat surprising, because a previous study reported higher Tet expression in adults than in development. Using 5hmC-specific dot blots, we found that global 5hmC levels were higher at P25 compared to P1, confirming that this epigenetic mark accumulates in the brain over time. Our finding of peak expression of Tet enzymes at birth suggests an important developmental role for DNA demethylation, or a neonatal placement of stable 5hmC marks. Region-specific sex differences during the critical period for sexual differentiation suggest that active DNA demethylation machinery plays a sex-specific role in the development of these brain regions.

Disclosures: C.D. Cisternas: None. **L. Cortes:** None. **E.C. Bruggeman:** None. **B. Yao:** None. **N.G. Forger:** None.

Poster

154. Neuroendocrine Processes: Sexual Differentiation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 154.04/TT1

Topic: F.03. Neuroendocrine Processes

Support: NSF Graduate Research Fellowship
GSU Brains & Behavior Seed Grant

Title: Effects of a neonatal testosterone and a DNA methyltransferase inhibitor on the sexual differentiation of cell phenotype in the mouse brain

Authors: *L. R. CORTES, C. D. CISTERNAS, I. GOLYNKER, N. G. FORGER
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Abstract: Some sex differences in the brain relate to the number of cells expressing a specific phenotype. For example, females have more cells than males expressing estrogen receptor (ER) alpha in the ventrolateral region of the ventromedial nucleus of the hypothalamus (VMHvl), while males have more cells expressing calbindin in the sexually dimorphic nucleus of the preoptic area (CALB-SDN). We recently found that neonatal treatment with zebularine, an inhibitor of DNA methylation, increased the number of ER alpha cells in the VMHvl of males and CALB-SDN cells in females, without altering apoptosis or total cell number. This suggests that DNA methylation is important for sexual differentiation of neuronal cell phenotype. The sex difference in CALB-SDN cell number is due to organizational effects of neonatal testosterone and is present prior to puberty. Here, we tested whether this is also true for ER alpha expression in the VMHvl and for kisspeptin in the preoptic periventricular nucleus (PeN), and whether an inhibition of DNA methylation at birth would interfere with the masculinizing effects of testosterone. Newborn C57BL/6 female mice received testosterone propionate (T) or peanut oil, sc, as well as intracerebroventricular injections of zebularine or saline on the day of birth (P0) and P1. Mice were sacrificed at weaning (P25) and their brains were collected for immunohistochemical detection of ER alpha, calbindin, and kisspeptin. Males had more CALB-SDN than females, while females had increased ER cell number in the VMHvl and kisspeptin cell number in PeN. Neonatal testosterone treatment of females completely masculinized all three markers. Neonatal zebularine abolished differences between females and T-treated females in the CALB-SDN. Zebularine also increased ER alpha cell number in the VHMvl of males and T-treated females, but not to the level seen in control females. In contrast, neonatal zebularine had no effect on kisspeptin cell number in any group. This suggests that effects of neonatal testosterone on sexual differentiation of CALB-SDN and ER alpha are at least partly mediated by DNA methylation, while masculinization of kisspeptin may be independent of DNA methylation. Additionally, our results suggest that inhibiting DNA methylation masculinizes or feminizes depending on the cell phenotype analyzed.

Disclosures: L.R. Cortes: None. **C.D. Cisternas:** None. **I. Golynker:** None. **N.G. Forger:** None.

Poster

154. Neuroendocrine Processes: Sexual Differentiation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 154.05/TT2

Topic: F.03. Neuroendocrine Processes

Support: CMU College of Medicine Internal Funds

Title: miRNA regulation of androgen receptor mediated sexual differentiation in the spinal nucleus of the bulbocavernosus (SNB) system

Authors: *J. A. JOHANSEN[1], M. ALTEMUS[2], M. N. FLORENDO[3]
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Abstract: The spinal nucleus of the bulbocavernosus (SNB) is a sexually dimorphic neuromuscular system highly dependent on androgens, and is a powerful model for understanding androgen mediated sexual differentiation of the central nervous system. The mechanisms that control SNB motor neuron number and size however are unclear. The androgen responsive bulbocavernosus and levator ani (BCLA) target muscles have similar levels of androgen receptor (AR) mRNA when compared to other non-responsive skeletal muscles; however, the BCLA muscles have much more AR protein than other skeletal muscles. This suggests that the amount of AR protein is regulated by translational or post-translational mechanisms in the muscle. A microRNA (miRNA) can regulate mRNA targets by degrading target mRNA molecules or inhibiting their translation. Using NanoString technology we assessed the gene expression of 603 miRNAs in gonadally intact wildtype male mice BCLA and extensor digitorum longus (EDL) skeletal muscles. We found 89 miRNAs differentially expressed between the two muscle types, 9 predicted to bind ARs. Because AR is a transcription factor itself, we next determined if the upregulated miRNAs were dependent on androgens. We castrated male mice and implanted either blank or testosterone filled silastic capsules, and assessed miRNA gene expression on BCLA and EDL muscles using Nanostring technology. There was no significant effect of hormone treatment on miRNAs previously upregulated in the EDL, suggesting that they are not regulated by androgens. Therefore, these miRNAs could be potential mediators of translational or post-translational modifications of AR in skeletal muscle. Overall our results suggest that miRNAs may provide a potential mechanism for the differential androgen receptor protein expression among skeletal muscles and demonstrate a role for miRNAs in the sexual differentiation of the SNB system.

Disclosures: J.A. Johansen: None. **M. Altemus:** None. **M.N. Florendo:** None.

Poster

154. Neuroendocrine Processes: Sexual Differentiation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 154.06/TT3

Topic: F.03. Neuroendocrine Processes

Support: HHMI

Title: Estrogen's role in suppression and refinement of the sexually dimorphic song learning system in zebra finches

Authors: *H. N. CHOE[1], H. MATSUNAMI[2], E. D. JARVIS[3]
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Abstract: Hormones can alter the organization of the brain during early development and can also coordinate various behaviors throughout life. This includes the vocal learning system in songbirds. In zebra finches, song learning is limited to males, and the associated song learning brain pathway also only matures in males. This brain pathway atrophies in females, and this atrophy can be partially reversed by giving females exogenous estrogen during early post-hatch development. Due to prior experiments being limited in their estrogen manipulations, we were concerned about whether male song pathway development was conclusively shown to develop independently of estrogen. Here, we used for the first time in songbirds a potent third generation estrogen synthesis inhibitor, exemestane, from day 1 of hatching throughout the entirety of juvenile development. We find that males treated long-term with exemestane had complete removal of circulating estrogen, but still developed song nuclei in the brain. However, these males had dramatically delayed male plumage development, and stunted song learning abilities. Conversely, providing females with estrogen long-term supported prior findings of prevention of atrophy in the song learning system, and further, we found, resulted in specialized gene expression in their song nuclei close to the levels seen in normal males. These findings support the hypothesis that song learning may be an ancestral trait in both sexes, that was subsequently suppressed in females throughout the evolution of some species, and that estrogen has come to play a role in this suppression as well as in refinement of the song learning system.

Disclosures: H.N. Choe: None. **H. Matsunami:** None. **E.D. Jarvis:** None.

Poster

154. Neuroendocrine Processes: Sexual Differentiation

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Program #/Poster #: 154.07/TT4

Topic: F.03. Neuroendocrine Processes

Support: F.R.S.-FNRS grant CDR J.0142.17
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Title: Key role of estrogen receptors beta (ERβ) in the sexual differentiation of Japanese quail

Authors: L. COURT, L. FAGOT, J. BALTHAZART, *C. A. CORNIL
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Abstract: Estrogens play a key role in the sexual differentiation of brain and behavior in both birds and rodents. Although estrogen actions on early brain programming appear to rely on diametrically opposite mechanisms in birds and mammals (estrogens masculinize male rats but demasculinize female quail) early estrogen exposure has the same ultimate effects in the two vertebrate classes: males mount while females show a receptive posture. Similar brain differences have also been detected in the brain (e.g. the male preoptic area presents more fibers immunoreactive for arginine-vasopressin than females). However, studies using estrogen receptor knock-out models or early pharmacological treatment with specific estrogen agonists or antagonists reported conflicting data regarding the identity of the receptors involved in the organization of sex differences in brain and behavior. This study aimed at determining which receptor(s) plays a key role in the sexual differentiation of copulatory behavior in Japanese quail using agonists specific for the two nuclear estrogen receptor (ERα and ERβ). Eggs were injected with the ERα agonist PPT (300µg), the ERβ agonist DPN (300µg), estradiol benzoate (EB 25µg, as positive control) and their vehicle, propylene glycol (PG), as negative control at embryonic day 7 that marks the beginning of the sensitive period to estrogens ending at embryonic day 12. Males were gonadectomized 3 weeks post-hatch and then treated with exogenous testosterone to ascertain that they would be in a similar endocrine adult condition. They were then tested for male sexual behavior. Most males treated with PG (10 out of 14) or PPT (12 out of 16) expressed active male sexual behavior. By contrast, all females (n=12) and males treated with EB (n=17) showed a complete absence of male sexual behavior while only one male treated with DPN out of 15 expressed this behavior. The statistical analysis of behavioral frequencies confirmed these observations with control males and males treated with PPT showing normal behavioral frequencies while the three other groups showed frequencies close to zero. Together these data clearly demonstrate a role for ERβ in the demasculinization of male sexual behavior in Japanese quail, which fits in well with the high expression of this receptor in the medial preoptic nucleus during the critical period. The brain of these subjects are currently analyzed to determine the impact of the treatment on the sex difference in the relative area covered by fibers immunoreactive for vasotocin, the avian homolog of arginine-vasopressin, a clear marker of sexual differentiation in quail.

Disclosures: L. Court: None. **L. Fagot:** None. **J. Balthazart:** None. **C.A. Cornil:** None.

Poster

154. Neuroendocrine Processes: Sexual Differentiation

Location: SDCC Halls B-H

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Program #/Poster #: 154.08/TT5

Topic: F.03. Neuroendocrine Processes

Support: NIH Grant DK090823
American Heart Association 16GRNT31110008

Title: Sex differences in gene expression profiles of various brain regions in adolescent rodents with implications for behavioral vulnerabilities

Authors: *K. KROLICK[1], J. CAO[1], D. TAPP[2], M. MCMURRAY[2], A. J. KISS[3], H. SHI[1]
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Abstract: Prominent sex-different genetic susceptibilities exist in male and female brains during adolescence leading to neurodevelopmental and neurochemical differences, thus foreshadowing sex differences witnessed in later-life neurodegenerative, neuropsychiatric, and related diseases. The molecular basis of these sex differences could be due to alterations in transcriptome profiles between male and female brains existing during the early life stage in animals including humans. Furthermore, sex dimorhphisms in the brain may develop due to genetic factors either prior to or due to gonadal hormone effects. Important adolescent differences in the male and female brains in limbic (e.g. basal lateral and central medial amygdala and hippocampal regions CA1, CA3 and dentate gyrus), hypothalamic (e.g. paraventricular, ventromedial, and arcuate nuclei), and cortical regions (e.g. orbitofrontal cortex) are involved in neurodegenerative and neuropsychiatric diseases. Progress in studying the molecular basis of neuronal vulnerabilities has been hampered by the lack of genome-wide information on sex differences in gene expression in various brain regions during adolescence. Recent approaches have focused on specific subregions of brain structures with homologous cell types yielding unambiguous results. In this study, brains were collected and flash frozen along with trunk blood from postnatal day 44 male and normal-cycling female Sprague Dawley rats. ELISAs were performed and circulating estradiol and testosterone levels were measured from trunk blood. Total RNA was isolated to study gene expression in the aforementioned nine brain sub-regions using RNA-Seq employing Unique Molecular Indices, to reduce PCR bias. Differential Gene Expression analysis was performed using a bootstrapped receiver operating characteristic (bROC) approach with a confidence detection of ≥0.95 cut-off for DGE between males and females. Here we show that sex differences in gene expression was dependent on sub-region examined and were widespread in the adolescent brain, being detectable in all major brain structures, including the cortex, limbic system, and hypothalamus. Furthermore, in a different cohort involving a behavioral experiment, we analyzed sex hormone measures of maladaptive cognitive functioning. We give examples of genes where sex-distinct expression is disease-relevant and likely to have functional consequences, and provide evidence suggesting that sex biases in expression during adolescence may lead to sex-biased gene regulatory structures and function, contributing to sex differences in neurological disorders in later life stages.

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Poster

154. Neuroendocrine Processes: Sexual Differentiation

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Program #/Poster #: 154.09/TT6

Topic: F.03. Neuroendocrine Processes

Support: NIH R01 MH109471

Title: Sex, age, and regional differences of estrogen receptors and aromatase in the rat striatum

Authors: *A. A. KRENTZEL, A. JOHNSON, J. WILLETT, J. E. MEITZEN
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Abstract: The striatum comprises multiple subregions instrumental for behaviors and neurological disorders that show sex differences in incidence and function. It has been suggested that estrogens are primarily driving these sex differences; however, little is understood of the mechanism by which sex differences occur. Estrogen receptor or aromatase expression may facilitate these sex differences. In adult female rodents, striatal regions express non-nuclear estrogen receptors. However, little is known whether estrogen receptor expression varies across the life span and how this changes by sex. Aromatase mRNA expression has been described in both the striatum and nucleus accumbens; however, to our knowledge, a study testing the differences of aromatase expression across the lifespan and in both sexes has not been done in the striatal brain regions. Both receptor and enzymatic ends of estrogenic signaling present a critical gap in knowledge because sex-specific estrogen action varies according to developmental stage. We determined how protein expression of two estrogen receptors, GPER1 and ERα, as well as aromatase changes between males and females at several developmental time points. We collected brains from males and females at ages P3, P20, and adulthood and stained for GPER1, ERα, and aromatase using immunofluorescence. We used a confocal microscope to take multiple scans throughout the tissue slices in three major regions of the striatum: dorsal striatum, nucleus accumbens core, and nucleus accumbens shell. We also imaged the cingulate cortex, arcuate nucleus of the hypothalamus, and the amygdala as positive controls for GPER1, ERα, and aromatase expression, respectively. We found that GPER1 expression decreased in the dorsal striatum and increased in accumbens core and shell from P3 to P20, indicating that GPER1 may be regulated differently between the dorsal and ventral subregions before puberty begins. All subregions decreased expression in adulthood. We did not detect robust sex differences in GPER1 expression. For ERα, although expression was low, we did observe nuclear staining patterns, especially in P3 females. This expression disappeared as animals aged and was pronounced in females compared to males. Quantification of aromatase staining patterns are ongoing. This study has provided developmental time windows in which to explore changes in estradiol sensitivity across the striatum. Future directions will determine what is driving the

differential regulation between region and sex, testing how mRNA of receptors and aromatase changes with age.

Disclosures: A.A. Krentzel: None. **A. Johnson:** None. **J. Willett:** None. **J.E. Meitzen:** None.

Poster

154. Neuroendocrine Processes: Sexual Differentiation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 154.10/TT7

Topic: F.03. Neuroendocrine Processes

Title: Dynamic hormone regulation of corticotropin releasing factor receptor 1-expressing cells in the anteroventral periventricular nucleus

Authors: *R. M. DE GUZMAN[1], Z. J. ROSINGER[1], J. S. JACOBSKIND[1], M. MALONE[1], N. BULANCHUK[1], N. J. JUSTICE[2], D. G. ZULOAGA[1]
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Abstract: Corticotropin releasing factor (CRF) signaling through CRF receptor 1 (CRFR1) regulates autonomic, endocrine, and behavioral responses to stress and CRF/CRFR1 activity has been implicated in the pathophysiology of several disorders, including anxiety, depression, and addiction. Using a validated CRFR1 reporter mouse line (bacterial artificial chromosome identified green fluorescence protein (BAC GFP-CRFR1)), we report a novel sex difference in CRFR1-expressing cells within the anteroventral/rostral periventricular nucleus (AVPV/PeN), which is exclusively expressed in female mice. The AVPV/PeN is interconnected with many forebrain structures involved with endocrine activity, stress, and reproductive behaviors (e.g., preoptic area, bed nucleus of the stria terminalis, arcuate nucleus, and paraventricular nucleus of the hypothalamus). The AVPV/PeN CRFR1 sex difference is apparent in the early neonatal period and is regulated by perinatal, and not adult, gonadal hormones. A single injection of testosterone on the day of birth reverses the AVPV/PeN CRFR1 sex difference, while adult gonadectomy has no effect. Dual-label fluorescent immunohistochemistry shows nearly all CRFR1 cells co-express estrogen receptor alpha (ERα); therefore, ERα is a likely receptor through which perinatal hormones sexually differentiate this nucleus. AVPV/PeN CRFR1 is also increased in female, but not male, mice following chronic variable stress. Most of these cells also co-localize glucocorticoid receptors, indicating stress effects on CRFR1 may be mediated by glucocorticoid expression. Finally, AVPV/PeN CRFR1-GFP cell number is also increased during the postpartum period, although the specific maternal hormones that produce these effects have not yet been elucidated. Together, these results indicate that CRFR1 cells in the AVPV/PeN are dynamically regulated by hormones throughout the lifespan and may contribute to an array of functions, including stress-associated (anxiety/depression), maternal, and reproductive behaviors.

Disclosures: R.M. De Guzman: None. **Z.J. Rosinger:** None. **J.S. Jacobskind:** None. **M. Malone:** None. **N. Bulanchuk:** None. **N.J. Justice:** None. **D.G. Zuloaga:** None.

Poster

154. Neuroendocrine Processes: Sexual Differentiation

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Program #/Poster #: 154.11/TT8

Topic: F.03. Neuroendocrine Processes

Support: NIH Grant 5R21HD076430-02

Title: Calbindin-D28K cell distribution in neonatal progesterone receptor knockout (PRKO) mice

Authors: *D. LALITSASIVIMOL, C. K. WAGNER
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Abstract: Steroid hormones regulate neural development and direct the formation of almost all sex differences in brain structure and function. As a potent transcription factor, progesterone receptor (PR) may influence sexual differentiation. There is a dramatic sex difference in PR expression in the medial preoptic nucleus (MPN) from E19 to P10, in which the male MPN has fifty times greater number of PR immunoreactive (PRir) cells than the female (Wagner at al, 1998), thus creating a developmental window during which the male MPN is more sensitive to progesterone than the female MPN. Inhibition of PR activity in males demasculinizes MPN subnuclei (Quadros et al., 2001), suggesting that PR plays a crucial role in the development of sexually dimorphic MPN morphology. The CALB-SDN is a sexually dimorphic cluster of Calbindin-D28K immunoreactive (CALBir) cells within this region in adult mice (Gilmore et al., 2012), making the CALB-SDN an excellent model to determine the role of PR expression in sexual differentiation of the mouse MPN. We quantified CALBir cells during early neonatal (P4, P7, P10) development in male and female PR knockout (PRKO) and wildtype (WT) mice. We analyzed the total number of and the anatomical distribution (using a grid overlay) of CALBir cells within the MPN. There were no significant main effects in the total number of CALBir within the preoptic area in PRKO and WT mice. However, there was a main effect of sex on the distribution of CALBir cells. Within the center grid box, females had fewer CALBir cells compared to males, and there was a significant interaction between genotype and sex. These results indicate a diffuse pattern of CALBir cell growth in females compared to males, and that PR expression is required for a male-like distribution pattern. These findings extend our previous adult studies, where there was a significant genotype by grid box interaction in CALBir distribution. Overall, these findings suggest that PR expression is critical for the masculinization of MPN morphology.

Disclosures: D. Lalitsasivimol: None. **C.K. Wagner:** None.

Poster

154. Neuroendocrine Processes: Sexual Differentiation

Location: SDCC Halls B-H

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Program #/Poster #: 154.12/TT9

Topic: F.03. Neuroendocrine Processes

Title: Progesterone receptor expression in the SF-1knockoutmouse brain during postnatal development

Authors: *Y. IKEDA, A. TAGAMI, M. MAEKAWA
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Abstract: Expression of progesterone receptor (PR) is sexually dimorphic in several brain regions, including the preoptic area (POA), the arcuate nucleus (ARC) and the ventromedial hypothalamus (VMH) during postnatal development. To study the role of gonadal hormones in development of sexually dimorphic PR expression, we examined PR expression in the POA, ARC, and VMH during postnatal period of 4 weeks, in agonadal steroidogenic factor-1 (SF-1) knockout (KO) mice, which have never been exposed to endogenous gonadal steroid hormones at any stage of development. Furthermore, we treated SF-1 KO mice with a synthetic estrogen, diethylstilbestrol (DES), during perinatal development and examined PR expression at postnatal day (P) 7, P14 or P21. At P0 and P7, abundant PR immunoreactive ($^{+}$) cells were detected in the POA in WT males, whereas few PR^{+}cells were detected in WT females and SF-1 KO males and females. However, abundant PR^{+}cells were detected in both sexes in both WT and SF-1 KO mice at P14 and P21. Quantification of PR IHC showed that PR-immunoreactivity (IR) levels in the POA were significantly higher in WT males than in WT females or either sex of SF-1 KO mice at P0 and P7, were similar between sexes and between the two genotypes at P14, and were reduced to be significantly lower in both sexes of SF-1 KO mice compared with WT males at P21. SF-1 KO mice treated neonatally with DESshowed elevated POAPR-IR compared with the vehicle-treated group at P7, and the levels in the neonatal DES group were equivalent to those in WT males. No apparent changes in the PR-IR levels in any DES-treatment group were detected at P14 or P21. In previous rodent studies, POA PR expression in females was shown to increase around P10, which roughly coincides with the onset of ovarian estrogen production, while that in neonatally ovariectomized female rats significantly decreased but was restored by estrogen treatment. Those results have suggested that ovarian estrogens are thought to be responsible for inducing female PR expression. However, based on our results, it is likely that estrogens of non-gonadal origin may contribute to induction of POA PR expression around P14.

Disclosures: Y. Ikeda: None. **A. Tagami:** None. **M. Maekawa:** None.

Poster

154. Neuroendocrine Processes: Sexual Differentiation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 154.13/TT10

Topic: F.04. Stress and the Brain

Support: The Collaborative Health Initiative Research Program

Title: Pituitary genetic signature common to short-term sleep deprivation and stress in male and female mice

Authors: *M. G. OYOLA[1], E. A. SHUPE[5], A. SOLTIS[2], G. SUKUMAR[2], C. L. DALGARD[6], M. WILKERSON[2], S. ROTHWELL[3], O. LARCO[4], T.-Y. J. WU[4]
[1]Obstetrics and Gynecology, [2]Colaborative Hlth. Initiative Res. Program, [3]Anatomy, Physiol. and Genet., [4]Uniformed Services Univ., Bethesda, MD; [5]Obstetrics and Gynecology, Uniformed Services Univ. of the Hlth. Scienc, Bethesda, MD; [6]Anat. Physiol. Genet., Uniformed Services Univ. of the Hlth. Sci., Bethesda, MD

Abstract: Background: The pituitary gland plays a crucial role in the regulation of sleep and the stress system, and it dysfunction leads to sleep-related disorders such as anxiety, post-traumatic stress disorder, and depression. Despite the importance of the pituitary gland in the regulation of these two critical biological functions, information pertaining to the interaction of these functions remains unclear. In this study, we performed a comparative whole transcriptome analysis to identify candidate genes and relevant pathways common to sleep deprivation and the stress system. **Method**: We used a modified Paradoxical Sleep Deprivation (PSD) method to deprive animals of sleep for 12hrs. One day later, animals were restraint for 20mins to study the pituitary stress response. Gene expression changes in the pituitary were assessed via deep sequencing RNA-Seq and pathway analysis in male and female mice using the DESeq2 software and a generalized linear model design. **Results**: PSD in male and female mice elicited an upregulation (FDR q-value ≤ 0.05) of 533 pituitary genes of which 53 overlap between sexes and are involved in the regulation of homeostasis, circadian rhythm, and rhythmic process including Bdnf, Cartpt, Per2, Roc, and Vgf. We also observed a downregulation of 228 genes after PSD, four of which are common between male and females and are involved in the immune response. There was a marked sex difference response to PSD and restraint stress in pituitary genes. PSD elicited upregulation of neurogenesis, axonogenesis and brain development genes in females, while it increased metabolism and oxidation-reduction in males. Restraint stress caused upregulation of 266 genes and downregulation of 65 genes. Pathway analysis of the upregulated 266 genes revealed that these genes are involved in the regulation of the glucocorticoid, lipid, and immune system responses, phosphoregulation, kinase activity, and fatty acid transport. The downregulated genes are linked to retinoic acid signaling, reproduction, cellular differentiation,

and morphogenesis. **Conclusion**: We show potential regulatory targets of the circadian clock and its involvement in the stress response. Genes that are differentially expressed in the pituitary gland after short-term sleep deprivation and restraint may serve as targets to be considered when studying the importance of sleep and the response to stress. This study aimed to simulate the potential effects on the pituitary function after skipping a night of sleep, especially in the stress response.

Disclosures: M.G. Oyola: None. **E.A. Shupe:** None. **A. Soltis:** None. **G. Sukumar:** None. **C.L. Dalgard:** None. **M. Wilkerson:** None. **S. Rothwell:** None. **O. Larco:** None. **T.J. Wu:** None.

Poster

154. Neuroendocrine Processes: Sexual Differentiation

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Program #/Poster #: 154.14/TT11

Topic: G.03. Emotion

Support: NIH Grant R01MH100536

Title: Sex differences in rat basolateral amygdala projections to the bed nucleus of the stria terminalis and prolonged cued fear conditioning responses

Authors: *J. E. VANTREASE[1], B. AVONTS[1], J. H. URBAN[2], J. A. ROSENKRANZ[1]
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Abstract: Women are twice as likely as men to develop anxiety disorders, yet studies examining sex differences in the neurobiology related to these disorders are limited. The basolateral amygdala (BLA) is a critical component of the neurocircuitry involved in anxiety and is more active in patients with anxiety disorders and in females performing specific affective tasks. This suggests that BLA activity may contribute to sex differences in the pathophysiology of anxiety disorders. Prior studies from our laboratory demonstrated that BLA neuronal activity is sexually dimorphic; however, it is not known whether specific subsets of BLA neurons exhibit this disparity. BLA outputs to the lateral bed nucleus stria terminalis (BSTL) play a critical role in the expression of prolonged cued fear and general anxiety-like behavior with female rats expressing less general anxiety-like behaviors compared to males. The purpose of this study was to determine if BLA-BSTL neurons in female rats are less active than in males. We utilized in vivo single-unit extracellular electrophysiological recordings to record anti-dromically identified BLA-BSTL neurons in anesthetized male and cycling female rats. We found that BLA-BSTL neurons in females have lower firing frequencies compared to males, despite females having greater spontaneous BLA neuronal activity overall. Fewer BLA-BNST neurons were observed in females, which suggested that while female BLA neurons are more active overall, there is a

reduction in the specific populations of BSTL- projecting neurons and that these neurons are less active compared to those of males. We also examined whether prolonged cued fear conditioning, which is dependent, in part, on BLA-BSTL connections, is less robust in female rats compared to males. Rats were conditioned to an 8 min tone, during which footshocks (0.3mA) were delivered at random. Four days later (when females were in the same estrous stage as training), rats were tested in a novel context and time spent freezing during the presentation of the tone was measured. No sex differences were observed during training, but proestrous females froze less during training compared to males and metestrous females. However, during extinction, females extinguished freezing behavior more quickly than males, and proestrous females froze less than metestrous females. These results coincide with our electrophysiological data suggesting that decreased BLA-BSTL activity in females leads to a decreased freezing behavior during prolonged cued fear. These results may help explain the apparent disconnect between overall sex differences in BLA neuronal firing and its relation to particular anxiety behaviors.

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Poster

154. Neuroendocrine Processes: Sexual Differentiation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 154.15/TT12

Topic: F.04. Stress and the Brain

Support: R01MH109975

Title: Changes in morphology and spine densities in orexin neurons after repeated stress in male and female rats

Authors: *L. GRAFE[1], K. R. URBAN[2], E. GENG[3], S. BHATNAGAR[4]
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Abstract: We recently found that female rats have increased orexin expression and activation compared with males, leading to impaired HPA habituation to repeated restraint stress and subsequent cognitive deficits. In the studies described here, we extended our study of sex differences in the role of orexins to examining morphology and spine densities in orexin neurons and whether these differ between male and female rats both in non-stress conditions as well as after 5 consecutive days of 30-min restraint stress. To assess orexin neuronal morphology, adult male and female Sprague Dawley rats were sacrificed either in basal conditions or after repeated restraint stress, and brains were stained using a Golgi-cox procedure. Preliminary data are

presented here with n = 8 animals per group and 4 cells analyzed per animal. While total dendritic length, nodes, and branching did not differ between non-stressed males and females, there was a main effect for repeated restraint stress in decreasing all three measures. Further analyses using post hoc tests revealed that this main effect was driven by differences in males with stress significantly decreasing total dendritic length and number of nodes in male rats but not in female rats. However, stress significantly decreased dendritic branching in female rats but this was only a trend in male rats. Sholl analysis revealed that control male and female rats differed in number of intersections per sholl radii, depending on the distance away from the orexin neuron. Additionally, stressed male rats had fewer intersections per sholl radii than control male rats, but this was not observed in stressed females. Analysis of total number of spines indicated that control females have significantly more dendritic spines than control males. Once again, stressed males show a decreased number of spines compared to control males, but this was not observed in females. In summary, our data indicate that orexin dendritic complexity decreases after repeated restraint stress in males but not females. This might be reflective of decreased orexin system function after repeated restraint in males, which allows males to habituate more fully to repeated restraint than females, as we have previously shown. In addition, the higher number of spines observed in control female rats compared to male rats might indicate more basal orexinergic synaptic connections, supporting our previous findings of higher orexin expression and activation in control female rats. These findings extend our understanding of sex differences in orexins by suggesting that morphological characteristics of orexin neurons may be less adaptive to repeated stress in females.

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Poster

154. Neuroendocrine Processes: Sexual Differentiation

Location: SDCC Halls B-H

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Program #/Poster #: 154.16/TT13

Topic: F.03. Neuroendocrine Processes

Support: NIH Grant 5R21HD076430-02

Title: Exposure to a synthetic progestin in clinical use to prevent preterm birth alters innervation of the postnatal rat medial prefrontal cortex: Differential effects in males and females

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Abstract: The synthetic progestin 17-alpha-hydroxyprogesterone caproate (17-OHPC) is a commonly prescribed treatment to delay parturition in women at risk for preterm birth. 17-OHPC is administered between 16 and 20 weeks of gestation, and continues until week 36. 17-OHPC is

observed in the fetal compartment of the human placenta and can be detected in fetal plasma up to 44 days after the last injection. Despite potential exposure, the effects of 17-OHPC on the developing fetal brain are virtually unstudied. The period of gestation in which 17-OHPC is administered corresponds to a critical window of development for the mesocortical dopamine pathway, a behaviorally critical neural circuit that regulates complex cognitive function. In rodent models, nuclear progesterone receptor (PR) is expressed in dopaminergic neurons of the ventral tegmental area (VTA) that project to the medial prefrontal cortex (mPFC). In addition, PR is expressed in the subplate and in layer 5 of the mPFC. This suggests that the development of the mesocortical dopamine pathway is sensitive to the actions of progestins. Indeed, exposure to 17-OHPC during neonatal development in rats significantly decreased the amount of tyrosine hydroxylase-immunoreactive (THir) fibers in females, and significantly narrowed the distribution pattern of THir fibers in layer 5 of prelimbic (PL) mPFC compared to control females. Such treatment differences are not observed in males, suggesting that 17-OHPC may exert differential effects in males and females. Additionally, a sex difference in THir fiber distribution observed in control animals was abolished in 17-OHPC treated pups. Furthermore, pups treated with17-OHPC during development exhibit deficits in cognitive flexibility in adulthood. Overall, these findings suggest that exposure to 17-OHPC during development may alter innervation of the mPFC. Ongoing studies compare the number of synaptophysin-ir terminal boutons in the PL mPFC in 17-OHPC exposed neonatal rats and controls. Preliminary results suggest that 17-OHPC may differentially impact the number of synaptophysin-ir puncta in PL mPFC in males and females. Taken together, these findings suggest that consideration should be given to the potential effects of 17-OHPC exposure on neural and cognitive development in children.

Disclosures: C.K. Wagner: None.

Poster

154. Neuroendocrine Processes: Sexual Differentiation

Location: SDCC Halls B-H

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Program #/Poster #: 154.17/TT14

Topic: G.03. Emotion

Support: R37HD083217 (RMS)
F32MH112232 (MO)
F31 MH112372 (AS)
R21MH105846 (CA)

Title: Early life abuse alters GABAergic synaptic contacts in the basolateral amygdala of juvenile rats in a sexually dimorphic manner

Authors: *K. Y. LIM[1], A. N. SANTIAGO[1], M. OPENDAK[2,3], R. M. SULLIVAN[2,3], C. J. AOKI[1]
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Abstract: Early life abuse from a caregiver is a major risk factor for the development of psychiatric disorders involving abnormal threat responses. Weaning-aged rats (PN23) with a history of maternal abuse show an elevated innate threat response and hyperactivity of the basolateral amygdala (BLA), a region known for its critical role in regulating such responses. However, the neural substrates and circuitry mediating this BLA hyperactivity remain to be explored. Using a naturalistic model of early life trauma, the Scarcity Adversity Maltreatment Model (SM) of low resources (i.e., low amount of cage bedding (PN8-12)), we investigated the hypothesis that this early life abuse directly alters the inhibitory neurocircuitry of the BLA. Fast-spiking parvalbumin positive (PV) inhibitory interneurons, in particular, are known to play a critical role in BLA fear response by dampening BLA excitability. These PV interneurons are encapsulated by extracellular matrix structures called perineuronal nets (PNNs), which have recently been shown to enhance the activity of PV cells. Last year, we reported a reduction of perineuronal nets needed to optimize parvalbumin-containing (PV) interneurons' inhibition of BLA's excitatory outflow, and showed that reduced PV inhibitory synapses onto Pyr cells may contribute to hyper-responsiveness of weaning age rats following early life abuse. In view that sex-specific differences are abundant in the development of many stress-related psychiatric disorders, we hypothesized that BLA hyperactivity could be mediated additionally by other synaptic-level changes in GABAergic circuitry differently for females and males. This year, we characterise presynaptic non-PV axo-somatic inputs onto postsynaptic PV, nonPV, and pyramidal cells in BLA at weaning age and show additional synaptic mechanisms that may mediate BLA hyperactivity following early life trauma. Specifically, non-PV axo-somatic inputs onto PV were greater for females than for males, suggesting sex-specific effects of early life maternal abuse. Dual immunohistochemistry and electron microscopy revealed that a majority of these non-PV inputs are GABAergic, suggesting that this population are cholecystokinin-expressing basket cells or PV cells that have lost immunoreactivity at the terminal. These findings inform us about treatments for individuals exhibiting fear regulation problems following a history of early life abuse that are optimized for each of the sexes.

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Poster

154. Neuroendocrine Processes: Sexual Differentiation

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Program #/Poster #: 154.18/TT15

Topic: G.03. Emotion

Support: K08 MH014743-01A1

Title: Differential response in male and female infant rats to maternal presence during fear conditioning

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Abstract: Beginning in infancy, social cues play a crucial role in the regulation of emotional state and behavior. In addition to providing their child with nutrition, warmth, and safety, mothers can suppress amygdala activity, cortisol release, emotional distress, and fear learning in their child, an effect termed "maternal buffering." When infant rats undergo fear conditioning in the presence of a calm mother, they do not acquire the association between the neutral cue and mild foot shock. There is an emerging literature on sex differences in expression of fear and in the social regulation of fear in adults; however, sex differences in maternal buffering of fear have not received much attention. Here, we examined whether maternal presence differentially modulates fear in female and male rat pups. On postnatal day 13 (P13), pups received 11 peppermint odor-tail shock pairings in the presence or absence of an anesthetized dam. On P18, pups were exposed to the conditioned odor 3 times and freezing in response to the cue was measured. In females, we found a significant main effect of maternal presence ($p = 0.008$); females that were conditioned in the presence of an anesthetized dam ($n = 6$) froze significantly less at test than females that were conditioned in the absence of an anesthetized dam ($n = 7$). However, we did not observe significant differences between males that were conditioned in the presence of an anesthetized dam ($n = 7$) and males that were conditioned in the absence of an anesthetized dam ($n = 5$) ($p = 0.75$). These data demonstrate that female pups are more susceptible to maternal buffering of fear, and suggest that sex differences in social regulation of emotion emerge very early in life.

Disclosures: A.M. White: None. **J. Debiec:** None.

Poster

154. Neuroendocrine Processes: Sexual Differentiation

Location: SDCC Halls B-H

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Program #/Poster #: 154.19/TT16

Topic: F.03. Neuroendocrine Processes

Support: IC 094843
IC 103169

Title: The role of progesterone in stress and binge-like eating behaviors and its influence on the central nervous system in male and female rats

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[1]CRIUCPQ, Quebec, QC, Canada; [2]Dep Psychiatry and Neurosci., Laval Univ., Quebec, QC, Canada; [3]CRIUSMQ, Univ. Laval, Quebec, QC, Canada

Abstract: Women are more likely than men to develop binge eating disorder, which is triggered by stress and involve the intake of palatable food. This prevalence may be explained by the difference in sex hormones between genders. Progesterone is found in high concentration in women and is mainly synthesized by the ovaries. In intact male and female rats, and in ovariectomized (OVX) / adrenalectomized rats, progesterone injection increased food intake. However, no effect was reported on females OVX only. Interestingly, progesterone injection in OVX rats increased motivation to experience foot shock stress to get more palatable food. However, the precise role of progesterone on stress and palatable food consumption is not well understood. The aim of this study is to understand the role of progesterone in male and female rats on stress and anxiety; on palatable food consumption in normal conditions and in a context of stress. Subcutaneous injections (n=6/group) of progesterone (1mg/kg) or oil were done on castrated male and OVX female Wistar rats 4 hours prior any tested condition. Behavioral tests were performed to measure anxiety level, compulsion to palatable food and consumption of sucrose solution in home cage. During euthanasia, blood was collected for plasma corticosterone assay and brains were removed to evaluate the expression of corticotropin-releasing factor (CRF) mRNA levels with *in situ* hybridization. In castrated males, CRF mRNA expression in the parvocellular part of the paraventricular hypothalamic nucleus (PVNp) and plasma corticosterone levels decreased after progesterone injection. Progesterone injection had no effect on 10 minutes-sucrose intake in home cage and during the modified light/dark box test (LD). The latency to eat palatable food during the novelty suppressed feeding (NSF) test was not different between groups. The elevated-plus maze (EPM) test shows that progesterone reduced anxiety. In OVX females, plasma corticosterone levels and CRF expression in the PVNp were not changed after progesterone injection. Progesterone injection had no effect on 10 minutes-sucrose intake in home cage, but increased it in LD. Progesterone decreased latency to consume palatable food in NSF and did not change anxiety level in EPM. We conclude that in castrated males, progesterone decreased the activity of the hypothalamic-pituitary-adrenal (HPA) axis and thus reduced stress without effects on food consumption. In OVX females, progesterone did not act on HPA axis or on palatable food intake in a safe context. However, progesterone injection increases palatable food consumption in a context of stress, a feature of binge eating disorder.

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Poster

155. Stress and the Hypothalamus, Amygdala, and Bed Nucleus

Location: SDCC Halls B-H

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Program #/Poster #: 155.01/TT17

Topic: F.04. Stress and the Brain

Support: National Natural Science Foundation of China (31371115, 81527901)
Tsinghua IDG/McGovern Institute for Brain Research and Center for Brain-Inspired Computing Research.

Title: A potential pathway mediating sound-induced anxiety-like behavior

Authors: *W. YIWEI[1], M. LIU[2], D. CAI[3], F. XIE[5], L. YOU[2], Y. YUE[2], K. YUAN[4]
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Abstract: Anxiety behavior is essential for animal to fight against potential threats in their environment. However, the neural circuits responsible for sound-induced anxiety-like behavior remain largely unclear. As an important division of medial hypothalamic defensive system, the ventromedial hypothalamus (VMH) is closely related to animal innate defensive behavior. Since it was reported that VMH receives direct inputs from non-lemniscal auditory thalamus, we propose that the non-leminscal auditory thalamus→VMH pathway might play an important role in sound-induced anxiety-like behavior. Optogenetic activation of the non-leminscal auditory thalamus→VMH fibers was sufficient to initiate anxiety-like behavior, such as a dramatic increase in thigmotaxis, rearing and mice respiration rate. Open field test showed that mice spent less time in the center of the chamber, and their average velocity as well as center entries decreased after laser stimulation. Elevated plus maze test exhibited that mice spent significantly longer time in closed arm during laser stimulation. In anesthetic mice, selective photo-activation of non-lemniscus auditory thalamus→VMH pathway increased respiration rate. Moreover, fiberphotometry recording showed considerably stronger responses of VMH neurons receiving direct non-lemniscal auditory inputs to threatening sounds compared with responses to white noise. This is the first study demonstrating sound-driven responses in mammalian VMH, and suggested that, besides non-lemniscal auditory thalamus→amygdala pathway, the non-lemniscal auditory thalamus→VMH pathway might play a role in sound-induced defensive behavior as well. Further loss-of-function studies would be necessary to consolidate this conclusion.

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Poster

155. Stress and the Hypothalamus, Amygdala, and Bed Nucleus

Location: SDCC Halls B-H

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Program #/Poster #: 155.02/TT18

Topic: F.04. Stress and the Brain

Support: NIMH R01 MH105675
NIMH K08 MH109735

Title: Bed nucleus of stria terminalis (BNST) CRF microcircuits for chronic stress-induced anxiety-like behaviors

Authors: *A. GARCIA[1], S. E. CANETTA[2], T. KASH[4], A. HARRIS[3], E. D. LEONARDO[5]
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Abstract: While exposure to acute stress can produce short-term adaptive changes in the brain, chronic stress can lead to long-term maladaptive changes that can precipitate neuropsychiatric disorders. The bed nucleus of stria terminalis (BNST), a critical node that regulates behavioral and physiological responses to potential threats, is dysfunctional in many stress-related illnesses. Yet, the effects of chronic stress on BNST-mediated responses are not well understood. Specifically, the BNST is a heterogeneous structure in which different sub-regions and cell-types can modulate avoidance behaviors bi-directionally, suggesting that opposing circuits exist within the BNST, the balance of which determines the behavioral outcome. For example, among the different sub-regions of the BNST, its oval division (BNSTov) integrates information about mood and negative valence and is sensitive to chronic stress. However, its circuitry and role in chronic stress-induced anxiety remains unexplored. Within the BNSTov, we find two populations of neurons; one that expresses CRF and a non-overlapping population that expresses PKC-δ in mice. We show that acute exposure to a novel environment results in increased activation of BNSTov PKC-δ cells. In contrast, chronic stress potentiates the activation of BNSTov CRF cells while decreasing activation of PKC-δ cells. In addition, acute photoinhibition of BNSTov CRF cells during testing ameliorates chronic stress-induced anxiety. Further, we find that acute optogenetically mediated activation of BNSTov CRF cells is sufficient to mimic chronic stress-induced anxiety-like behaviors. Together, this data suggests that these two separate populations within the BNST are differentially recruited in response to chronic stress and that BNSTov CRF cells play a key role in chronic stress-induced anxiety. We are currently working on determining the role of BNSTov PKC-δ cells in chronic-stress induced changes on behavioral and physiological responses to potential threats.

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Poster

155. Stress and the Hypothalamus, Amygdala, and Bed Nucleus

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Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 155.03/TT19

Topic: F.04. Stress and the Brain

Support: NSF Career Award IOS 0845550

Title: Preimmunization with a heat-killed preparation of Mycobacterium vaccae reduces corticotropin-releasing hormone mRNA expression in the central nucleus of the amygdala and the bed nucleus of the stria terminalis in a fear-potentiated startle paradigm

Authors: *K. M. LOUPY[1], M. R. ARNOLD[1], J. E. HASSELL, Jr.[1], M. LIEB[1], L. N. MILTON[1], K. E. CLER[1], J. H. FOX[1], P. H. SIEBLER[1], D. SCHMIDT[2], S. I. S. R. NORONHA[3], H. E. W. DAY[1], C. LOWRY[1]
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Abstract: Posttraumatic stress disorder (PTSD) is a trauma and stressor-related disorder that is characterized by dysregulation of glucocorticoid signaling, chronic low-grade inflammation, and impairment in the ability to extinguish learned fear. Corticotropin-releasing hormone (Crh) is a stress- and immune-responsive neuropeptide secreted from the paraventricular nucleus of the hypothalamus (PVN) to stimulate the hypothalamic-pituitary-adrenal (HPA) axis; however, extra-hypothalamic sources of Crh from the central nucleus of the amygdala (CeA) and bed nucleus of the stria terminalis (BNST) govern specific fear- and anxiety-related behaviors. We previously reported that preimmunization with a heat-killed preparation of the immunoregulatory environmental bacterium *Mycobacterium vaccae* NCTC 11659 enhances fear extinction in a fear-potentiated startle paradigm. In this follow-up study, we utilized an *in situ* hybridization histochemistry technique to investigate *Crh*, *Crhr1*, and *Crhr2* mRNA expression in the CeA, BNST, and PVN of the same rats from the original study [Fox et al., *Brain, Behavior, and Immunity*, 66: 70-84]. Here we demonstrate that preimmunization with *M. vaccae* NCTC 11659 decreases *Crh* mRNA expression in the CeA and BNST of rats exposed to the fear-potentiated startle paradigm, and further, that *Crh* mRNA expression in these regions is correlated with fear behavior during extinction training. These data are consistent with the hypothesis that *M. vaccae* promotes stress-resilience by attenuating Crh production in anxiogenic circuits. These data suggest that immunization with *M. vaccae* may be an effective strategy for prevention of fear- and anxiety-related disorders.

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Poster

155. Stress and the Hypothalamus, Amygdala, and Bed Nucleus

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Program #/Poster #: 155.04/TT20

Topic: F.04. Stress and the Brain

Support: F32 AA022832
R01 MH087592
R01 MH102638

Title: Parallel bed nuclei of stria terminalis --> lateral hypothalamus circuits for opposing emotional states

Authors: *W. J. GIARDINO[1], A. EBAN-ROTHSCHILD[2], D. J. CHRISTOFFEL[1], S.-B. LI[1], R. C. MALENKA[1], L. DE LECEA[1]
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Abstract: The mechanisms underlying aversion and reward are beginning to be uncovered, with substantial efforts focused on the intricate connectivity of the lateral hypothalamus (LH). LH neurons containing the neuropeptide hypocretin (Hcrt; orexin) modulate affective components of arousal, yet the precise neuronal inputs linking Hcrt-LH activity and emotional behavior remain to be fully defined. We set out to uncover how Hcrt-LH neurons respond to ethologically salient stimuli, generate hedonically-valenced behavioral states, and integrate diverse synaptic inputs from specific upstream cell types. Here, we used modified rabies monosynaptic input mapping to identify major sources of long-range input onto *Hcrt*-LH neurons originating from neuronal populations in the bed nuclei of stria terminalis (BNST; a heterogeneous structure of the extended amygdala). To functionally interrogate BNST-->LH circuitry, we used viral, optical, chemogenetic, and slice electrophysiological methods for monitoring and manipulating neural activity with genetically-defined and pathway-specific resolution in mice. Specifically, we characterized two non-overlapping and spatially-segregated GABAergic BNST cell groups that both exhibit axonal projections to the LH, but express distinct neuropeptide markers (corticotropin-releasing factor, *Crf*; and cholecystokinin, *Cck*). We found that *Crf*-BNST and *Cck*-BNST neurons provide differentially abundant synaptic inputs onto *Hcrt*-LH neurons, display discrete physiological responses to salient stimuli, drive opposite emotionally-valenced behaviors, are differentially required for appetitive behavioral approach, and receive synaptic

inputs from unique upstream neural networks. By performing additional *Crf* and *Cck* BNST optogenetic manipulations in Hcrt-Ataxin3 mice in which *Hcrt*-LH cells are ablated, we found that *Hcrt*-LH neurons are essential for *Crf*-BNST-induced behavioral avoidance, but are dispensable for *Cck*-BNST-induced preference/approach. Together, the data provide an advanced model for how parallel genetically-defined BNST-->LH pathways promote divergent emotional states via distinct connectivity patterns of circuit-specific neuronal subpopulations. Our novel findings suggest a mechanistic framework for BNST-->LH circuit dysregulations in psychiatric disorders, and will therefore be broadly relevant to the fields of neuroscience and mental health, as future studies will inform development of improved therapeutic approaches.

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Poster

155. Stress and the Hypothalamus, Amygdala, and Bed Nucleus

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Topic: F.04. Stress and the Brain

Support: NIH Grant R01 MH-095972
NARSAD Independent Investigator Grants

Title: Evidence for involvement of the bed nuclei of the stria terminalis in memory consolidation via an HPA-modulatory circuit

Authors: *R. LINGG[1], S. B. JOHNSON[2], E. B. EMMONS[2], R. M. ANDERSON[2], S. A. ROMIG-MARTIN[2], N. S. NARAYANAN[3], R. T. LALUMIERE[1], J. J. RADLEY[1]
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Abstract: Memory consolidation following stressful experiences is a key means of adaptation, allowing an individual to create a long-term representation of the event, and thereby evaluate the severity of future threats of the same or similar kind. It is well documented that the consolidation process is enhanced by glucocorticoids, the end-products of the HPA cascade, via activation of cognate receptors in the basolateral amygdala. For example, both systemic and intra-amygdaloid administration of corticosterone or glucocorticoid receptor agonists following training enhance later retention. Nevertheless, there remain lingering questions concerning what changes in endogenous glucocorticoid activity are necessary to enhance memory, which neural systems are involved in this modulation, and whether they overlap with the neural systems more directly implicated in memory encoding. Previous work from our laboratory has established an important role for the anteroventral subdivision of the bed nuclei of the stria terminalis (avBST) in the

modulation of the HPA axis. Moreover, avBST maintains extensive connectivity with a network of limbic cortical circuitry canonically associated with consolidation processes. As such, avBST is well positioned to act within the traditional framework of the consolidation network and as a regulatory locus for the hormonal modulation of memory strength. Here we used an optogenetic approach to manipulate activity within avBST and downstream pathways to examine its role during consolidation using a single trial inhibitory avoidance task. First, we showed that 10-minute post-training optogenetic manipulations of avBST somata bi-directionally modulated retention when rats were tested two days later. Specifically, photoinhibition (Arch) and photoexcitation (ChR2) led to a two-fold enhancement and decreased retention (by 45%, $p < 0.05$ for each), respectively. Follow-up experiments revealed that GABAergic projections from avBST to the paraventricular hypothalamus, but not to the midbrain central gray, increased retention latencies (by 200%, $p < 0.05$) following post-training photoinhibition (Halo) of avBST axonal fibers in PVH. As our results suggested that this effect was contingent upon an elevated pituitary-adrenal output, in a final study rats were pre-treated with metyrapone (50mg/kg s.c.) prior to inhibitory avoidance training and post-training photoinhibition of avBST. Blockade of training-induced HPA activation precluded the memory enhancing effects of avBST inhibition. Together, these data support a role for avBST activity as an upstream initiator of memory modulation via its influence over the HPA axis.

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Poster

155. Stress and the Hypothalamus, Amygdala, and Bed Nucleus

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 155.06/TT22

Topic: F.04. Stress and the Brain

Support: NIMH MH-072908
NIH P51OD011132

Title: Cell type specific expression of muscarinic receptors in the oval nucleus of BNST: Modulation by chronic stress

Authors: *J. GUO, A. MENIGOZ, L. J. YOUNG, D. G. RAINNIE
Dept of Psychiatry, Emory Univ., Atlanta, GA

Abstract: The bed nucleus of stria terminalis (BNST) is an important relay in the circuits regulating emotional behavior. The BNST is not a homogenous nucleus; instead it contains many subnuclei and different cell types. We have previously shown that the oval nucleus of the BNST

(BNSTov) contains three physiologically and neurochemically-distinct cell types (Type I-III). The CRF-expressing Type III neurons and non CRF-expressing Type I or II neurons may play opposite roles in stress-induced anxiety behavior. Acetylcholine is a neurotransmitter critically involved in advanced brain function and emotional disorders. The BNST is heavily innervated by cholinergic afferents and expresses multiple muscarinic receptors. Five muscarinic receptor subtypes have been identified, including Gq-coupled M1,M3,M5 and Gi-coupled M2, M4 receptors. Our previous study showed that the BNSTov expresses mRNAs for all five muscarinic receptor subtypes. However, the expression of muscarinic receptor subtypes in BNSTov cell types remains unknown, as does the expression of muscarinic receptors after chronic stress. Using a combination of *in vitro* patch clamp recording and single cell RT-PCR, we examined the expression of M1-M5 receptors in control rats and rats subject to 7-day unpredictable shock stress. We found that muscarinic receptor expression is distinct among cell types in the BNSTov. Type I neurons express medium to high levels of M1,M2,M4 receptor mRNA. Type II neurons express high levels of M1 mRNA, and low levels of M2,M4 receptor mRNA. Lastly, type III neurons express high levels of mRNA for M3 and M5 receptors, but not mRNAs for M1,M2,M4 receptor. Significantly, after chronic USS, muscarinic receptor expression changed in a cell type dependent manner. In type I neurons, no significant changes was observed for any receptor subtypes. However in type II neurons, the expression of M1, M4 receptors decreased, whereas M5 expression increased significantly. In type III neurons, expression of M1, M4 receptors increased, whereas M3 and M5 receptor expression decreased. Together these data suggest that muscarinic receptors may play important roles in modulating BNST function, and contribute to chronic stress induced anxiety disorders.

Disclosures: J. Guo: None. **A. Menigoz:** None. **L.J. Young:** None. **D.G. Rainnie:** None.

Poster

155. Stress and the Hypothalamus, Amygdala, and Bed Nucleus

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 155.07/TT23

Topic: F.04. Stress and the Brain

Support: KHIDI HI15C2887
NRF 2017M3C7A1043845
NRF 2016R1A4A1010796
KHIDI HI17C2665

Title: Functional dissection of the bed nucleus of the stria terminalis in stress-related states

Authors: *S.-R. KIM, G. HEO, H.-E. PARK, S.-Y. KIM
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Abstract: Stress—a condition that seriously disturbs the physiological and psychological balance of an animal—represents a critical etiology for a wide range of psychiatric disorders such as anxiety disorders and depression. The bed nucleus of the stria terminalis (BNST) is a critical structure mediating diverse stress-related states and is linked to relevant mental diseases, yet their cell type-specific function remains incompletely understood. Here we identify a novel genetically defined neural population in the BNST as a key node for stress-related behaviors. By monitoring deep-brain calcium dynamics, we show that the BNST subpopulation is activated by diverse anxiogenic and stressful stimuli, such as open space, social attack, and tail suspension. Optogenetic stimulation of the BNST neurons induce anxiety- and depression-like behaviors and alter physiological responses including heart rate. These neurons project to many other brain areas implicated in stress responses, including the hypothalamus, amygdala, and nucleus accumbens. Our preliminary data suggest that the BNST subpopulation represents a critical node in the stress system. We are currently characterizing its inputs and outputs, and their projection-specific functions to further dissect the BNST circuitry in stress-related states.

Disclosures: S. Kim: None. **G. Heo:** None. **H. Park:** None. **S. Kim:** None.

Poster

155. Stress and the Hypothalamus, Amygdala, and Bed Nucleus

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 155.08/TT24

Topic: F.04. Stress and the Brain

Support: NIH BP-Endure

Title: Downregulation of bdnf expression within mesolimbic brain structures following intranasal oxytocin treatment on cocaine conditioning in male rats

Authors: *S. D. FONSECA[1], D. O. OJEDA[2], G. C. MOLINA[2], A. DEFENDINI[2], C. S. MALDONADO[2]
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Abstract: Cocaine-paired environments have shown to prompt cocaine seeking behavior due to their cue-induced memory triggering effects, which is one of the main factors contributing to cocaine's high rate of relapse. The neuropeptide oxytocin (OT) have shown to attenuate both cue-induced reinstatement and cue-induced anxiety on a cocaine conditioning paradigm. A possible mechanism of action is by reducing hypothalamic-pituitary-adrenal axis (HPA) stress responses triggered by cocaine-associated cues which leads to changes in synaptic transmission in the ventral tegmental area (VTA). Furthermore, elevated brain-derived neurotrophic factor (BDNF) levels during withdrawal have also shown to prolong long-term potentiation (LTP) in the VTA, which results in drug-seeking behavior. BDNF transmission in the VTA may trigger

neural adaptations that enable the persistence of enhanced cocaine craving as a result of long-term neuroplasticity. This results in alterations of BDNF levels within mesocorticolimbical regions such as: nucleus accumbens (NAc), pre-frontal cortex (PFC) and amygdala. This study aims to characterize the role of OT in mediating changes in BDNF expression within the mesolimbic system on cocaine-conditioned rats after the withdrawal period. Separate groups of rats were administered intraperitoneal cocaine (10 mg/kg) for five consecutive days. After a day of withdrawal, rats were administered intranasal infusions of OT (1ug/uL) or vehicle (saline), 30 minutes prior the exposure to the cocaine-associated environment. Anxiety was tested with elevated plus maze (EPM) and biochemical analyses were performed using western blot. Our preliminary data confirms the anxiolytic properties of OT and suggests that OT downregulates BDNF expression in the NAc, PFC and amygdala, brain regions enervated by the VTA. Further studies assessing BDNF levels after longer periods of withdrawal are needed to fully understand the role of OT in regulating BDNF expression that might be, in part, responsible for attenuating cocaine seeking behavior and anxiety-like behaviors.
Keywords: Cocaine, anxiety, oxytocin, BDNF, nucleus accumbens, prefrontal cortex, amygdalaFunding source: 5R25NS080687 NeuroID grant

Disclosures: S.D. Fonseca: None. **D.O. Ojeda:** None. **G.C. Molina:** None. **A. Defendini:** None. **C.S. Maldonado:** None.

Poster

155. Stress and the Hypothalamus, Amygdala, and Bed Nucleus

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 155.09/UU1

Topic: F.04. Stress and the Brain

Support: Innovative Seed Grant Program, Univ. Colorado Boulder

Title: Determination of steady-state transcriptome modifications associated with repeated homotypic stress in the rat posterior hypothalamic region

Authors: *S. CAMPEAU[1], R. DOWELL[2], J. STANLEY[2], S. K. SASSE[4], P. R. DURGEMPUDI[3], A. KEEFER[3]
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Abstract: Recent results (Nyhuis et al., 2016, J. Neurosci., 36(3):795) indicate that the rat rostral posterior hypothalamic region significantly contributes to the brain circuits responsible for robust response reduction (habituation) associated with repeated homotypic stress. In the present study, 24 adult male Sprague-Dawley rats (~320 g) were handled for one week prior to placement in

acoustic-attenuation boxes. Rats were randomly grouped into 4 different conditions (n=6), consisting of a no stress control, 1, 3, or 7 exposures to 30 min of loud noise (white noise, 100 decibels sound pressure level, A scale), 24-hrs apart. All groups received their last noise or control exposure on the same day, at the end of which a tail blood sample was collected. 24-hrs later, and without further manipulations, all rats were euthanized, and the brains rapidly removed and sectioned (~2 min) to obtain 2x2x1-mm tissue blocks in the posterior hypothalamic region (RNA later, Qiagen). Total RNA was extracted (PureLink RNA Mini Kit #12183018A with TRIzol #15596026, Life Technologies), paired-end libraries were constructed (2x76, dual 8 bp index - KAPA HyperPrep mRNA kit) from polyA enriched samples, and run on a NextSeq 500 sequencer. Approximately $18x10^6$ reads per library were obtained, visualized (IGV) by first mapping back (HISAT2) onto the annotated rat genome (Assembly v6.0, build 91), and analyzed for differential gene expression with DESeq2 (adjusted p values < 0.05). An enzyme-linked assay (Arbor Assays, #K014-H1) on the plasma samples indicated reliable corticosterone increases in all stressed groups as compared to the control group, with the smallest increase in the 7-stress group, indicating significant glucocorticoid habituation compared to the other stressed groups. While no steady state differentially expressed genes were reported between the control and 1-stress group, 55 and 352 genes were differentially expressed between the control, 3- and 7-stress groups, respectively. Of the 352 genes differentially expressed after 7 stress exposures, 121 were downregulated, 231 were upregulated, with log2 fold changes ranging from -0.45 to 0.50. An initial gene ontology analysis (LAGO) of the differentially expressed genes between the control and 7-stress groups indicated neuron part, nervous system development, neuron and cell projections, plasma membrane bounded cell projection, synapse, and neurogenesis as the most significant classifications. Transcripts with the highest log2 fold changes ($> \pm 0.4$), representing different classifications, and identified through pathway analysis, are currently being validated using in situ hybridization histochemistry.

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Poster

155. Stress and the Hypothalamus, Amygdala, and Bed Nucleus

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 155.10/UU2

Topic: F.04. Stress and the Brain

Support: NSERC Discovery Grant

Title: The impact of stress on synaptic function and appetite regulation in the dorsomedial nucleus of the hypothalamus

Authors: *K. M. CROSBY, S. A. WILSON, E. J. STEEVES
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Abstract: Stress hormones, namely cortisol in humans and corticosterone in rats, affect numerous homeostatic functions including appetite regulation. Evidence suggests that acute stress decreases, while chronic stress increases food intake, but the underlying mechanisms are poorly understood. In rats, corticosterone binds to glucocorticoid receptors that are expressed in brain regions including the dorsomedial hypothalamic nucleus (DMH). Neurons in the DMH are important in appetite regulation; therefore, it is possible that corticosterone influences neuronal excitability or synaptic transmission in this region, to modulate food intake. We therefore pursued the following objectives (1) to determine if stress affects the excitability of DMH neurons, through changes in intrinsic properties or alterations in glutamate and GABA signaling onto these neurons, and (2) to determine whether corticosterone modulates food intake when administered directly into the DMH. To pursue these objectives, young male Sprague-Dawley rats (postnatal day 21-30) were used. To determine whether stress affects neuronal excitability and synaptic transmission, whole cell electrophysiological recordings were obtained from DMH neurons from control animals or animals subjected to a single restraint or repeated restraint episodes (to mimic acute and chronic stress). Stress had no effect on intrinsic neuronal excitability, as assessed by several variables including resting membrane potential, threshold to fire action potentials, and action potential frequency, amplitude, and duration. Glutamate and GABA-mediated postsynaptic currents were also measured and our results suggest that stress increased GABA release onto DMH neurons but had no effect on glutamate transmission. To determine if corticosterone alters food intake when administered directly into the DMH, we surgically implanted bilateral guide cannulas into the DMH to allow for administration of corticosterone (or vehicle) into the DMH. Following a one-week recovery period, animals received either a single injection of corticosterone or vehicle, or five consecutive daily injections to mimic a prolonged stressor. Food intake and body weight were measured over a four hour period post-injection and brains were subsequently removed and sliced to confirm accurate placement of cannulas. Our preliminary results suggest that neither acute nor chronic administration of corticosterone into the DMH altered food intake or body weight. Overall, we conclude that stress increases GABA release onto DMH neurons, but that stress hormones may not be acting in the DMH to modulate appetite.

Disclosures: K.M. Crosby: None. **S.A. Wilson:** None. **E.J. Steeves:** None.

Poster

155. Stress and the Hypothalamus, Amygdala, and Bed Nucleus

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 155.11/UU3

Topic: F.04. Stress and the Brain

Title: Chronic unpredictable stress modulates neuronal activity of AgRP and POMC neurons in hypothalamic arcuate nucleus

Authors: *X. FANG[1], S. JIANG[2], J. WANG[2], Z. ZHANG[2], Y. LEI[1], X.-Y. LU[1]
[1]Med. Col. of Georgia At Augusta Univ., Augusta, GA; [2]Binzhou Med. Univ., Binzhou, China

Abstract: Chronic unpredictable stress modulates neuronal activity of AgRP and POMC neurons The arcuate nucleus (ARC) of the hypothalamus contains two distinct subpopulations of neurons, expressing orexigenic agouti-related peptide (AgRP) and anorexigenic pro-opiomelanocortin (POMC). POMC and AgRP neurons respond differentially to metabolic stress and acute emotional/psychological stress. The goal of this study was to investigate the effects of chronic stress on intrinsic and synaptic properties of AgRP and POMC neurons. We demonstrated that chronic unpredictable stress (CUS) induces depressive-like behavioral phenotypes in mice, including anhedonia and behavioral despair. Electrophysiological recordings were made from identified AgRP and POMC neurons in brain slices of Agrp-ires-cre;tdTomato and Pomc-cre;tdTomato reporter mice exposed to 10 days of CUS. We found that CUS decreased the firing rate of AgRP neurons but increased the firing rate of POMC neurons. Furthermore, synaptic input onto AgRP and POMC neurons were also differentially altered by CUS. Chemogenetic stimulation of these neurons cause opposite effects on CUS-induced behavioral changes. Our results suggest that AgRP and POMC neurons play an important role in chronic stress adaptation and associated behavioral deficits.

Disclosures: X. Fang: None. **S. Jiang:** None. **J. Wang:** None. **Z. Zhang:** None. **Y. Lei:** None. **X. Lu:** None.

Poster

155. Stress and the Hypothalamus, Amygdala, and Bed Nucleus

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 155.12/UU4

Topic: F.04. Stress and the Brain

Support: NKFIH 109622
NKFIH 124424

Title: Acute-, repeated- and chronic stress differentially affects central metabolic homeostasis

Authors: *K. J. KOVÁCS[1], D. KUTI[2], D. ZELENA[3], S. FERENCZI[2], Z. WINKLER[2]
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Abstract: Exposure to physiological or psychological stressors result in significant changes in metabolic regulation. Acute stress challenges mobilize and redirect fuels to prepare the organism

for fight or flight. By contrast, long lasting stress exposure protects the energy stores and promotes adaptation. The aim of the present studies was to compare body composition, energy expenditure (EE), respiratory exchange ratio (RER) and locomotor activity of male rats during acute and repeated restraint stress. Furthermore, metabolic recovery after chronic psychogenic stress was also followed in Phenomaster metabolic phenotyping units. Hypothalamic expression of stress- and metabolic related genes were assessed by RT-qPCR and in situ hybridization histochemistry. To reveal if hypothalamic corticotropin-releasing hormone (CRH) drives stress-induced metabolic changes we used a chemogenetic approach via injection of rAAV8/hsyn-DIO-hM3D(Gq)-mCherry into the paraventricular nucleus (PVH) of CRH-IRES-Cre animals. CRH expressing PVH neurons were stimulated by systemic CNO injections. Following acute restraint stress, there is a rebound increase of food intake, which remains elevated for 24 h. VO2, EE and fatty acid oxidation decrease shortly after stress, while RER increases 3-4 hrs after restraint and remains elevated, suggesting that carbohydrates are the major fuel. We found these metabolic changes to be accompanied by rapid hypothalamic upregulation of CRH, NPY and AgRP genes. Selective chemogenetic stimulation of paraventricular CRH neurons does not fully recapitulate acute stress-induced metabolic changes. Repeated restraint and chronic variable stress result in changes of metabolic variables similar to those observed after acute exposure, however there is a significant habituation. Three days recovery from three weeks chronic stress, restored locomotor activity, RER and fatty acid oxidation to the control levels. These data suggest differential mechanisms driving metabolic responses to acute and chronic stress, which might be implicated in stress-induced metabolic pathologies.

Disclosures: K.J. Kovács: None. **D. Kuti:** None. **D. Zelena:** None. **S. Ferenczi:** None. **Z. Winkler:** None.

Poster

155. Stress and the Hypothalamus, Amygdala, and Bed Nucleus

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 155.13/UU5

Topic: F.04. Stress and the Brain

Support: R00 AA023559

Title: Sex dependent effects of binge alcohol exposure on a thalamolimbic stress circuit

Authors: *O. LEVINE[1], K. E. PLEIL[2], M. SKELLY[2], J. MILLER[1,3]
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Abstract: Alcohol use disorder (AUD) is highly comorbid with anxiety disorders, both of which are debilitating diseases that impose a tremendous social and economic burden on our society.

Comorbid AUD and anxiety disorders are more prevalent in women compared to men, but the precise neural underpinnings by which this occurs remain unknown. Females across mammalian species binge drink more than males and the human and rodent literature indicates a role for endogenous estrogen (E2) in the regulation of alcohol intake. Binge drinking is a primary risk factor for these diseases, and neurons expressing the stress neuropeptide corticotropin releasing factor (CRF) in the bed nucleus of the stria terminalis (BNST) regulate binge drinking and anxiety in mice. Our data show marked differences in the synaptic and neuronal function of BNST CRF neurons that indicate this population is more active and sensitive to synaptic inputs in females than males and enhanced after binge alcohol drinking in both sexes. For example, females receive a more robust monosynaptic excitatory input from the paraventricular nucleus of the thalamus (PVT), a region that also regulates anxiety and we found drives binge drinking, and alcohol exposure enhances neurotransmission at the PVT-BNST CRF synapse in males. Here, I test the hypothesis that E2 plays a positive modulatory role in glutamatergic transmission at PVT-BNST CRF synapses to increase the activity of BNST CRF neurons and drive binge drinking behavior. The experiments presented here aim to enhance our understanding of the mechanisms underlying alcohol addiction and comorbid anxiety disorders to elucidate potential novel pharmacotherapeutic targets for both diseases.

Disclosures: O. Levine: None. **K.E. Pleil:** None. **M. Skelly:** None. **J. Miller:** None.

Poster

155. Stress and the Hypothalamus, Amygdala, and Bed Nucleus

Location: SDCC Halls B-H

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Topic: F.04. Stress and the Brain

Support: NIH Grant R01AA013983
NIH Grant F31AA025827

Title: Persistent female social defeat stress and escalated alcohol drinking in female C57BL/6J mice

Authors: *E. L. NEWMAN[1], K. C. BURK[1], M. B. BICAKCI[1], J. F. DEBOLD[1], K. A. MICZEK[1,2]
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Abstract: Exposure to stress can increase the risk of developing an alcohol use disorder. We have shown that male C57BL/6J (B6) mice exposed to repeated attacks from a dominant, male resident will later consume significantly more alcohol than non-defeated controls, and that CRF-R1 antagonism can selectively reduce stress-escalated drinking. We have developed a model of social stress that relies on interfemale aggression to evaluate the effects of social defeat stress on

alcohol consumption, and to test the effectiveness of CRF-R1 antagonism in reducing stress-escalated drinking in females. Five days before 7- or 14-day chronic social defeat stress (CSDS), resident CFW females were pair-housed with CFW males in large cages with pine shavings. These cages were divided in half with clear, perforated partitions and breeding pairs were housed on one side of the divider. During daily 5-minute confrontations, each CFW male was replaced with a B6 female. Similar to intermale aggression, 96% of resident CFW females attacked the B6 intruder within three seconds, and female aggression occurred in the absence of neuroendocrine manipulations. B6 females were then housed opposite the CFW female that defeated them and CFW males were rehoused with their CFW females. B6 females were defeated and housed with a new CFW female daily. Control B6 females were housed opposite one another in partitioned cages and engaged in daily non-aggressive encounters. Ten days after the final defeat or social encounter, females were tested for social interaction with a novel CFW female. Then, B6 females received continuous access to 20% EtOH and water for ten weeks; beginning in week six, they were treated with weekly intraperitoneal injections of the CRF-R1 antagonist, CP376395 (0-17 mg/kg). Defeated females consistently drank more EtOH than controls, but CSDS did not permanently disrupt estrous cycling or induce social avoidance-like behavior. CP376395 significantly reduced alcohol consumption but also suppressed water drinking. The present protocol relies on interfemale aggression in a model of CSDS. As in males, a social stress history increased alcohol consumption in defeated females. While evidence points to CRF/CRF-R1 signaling in male stress-escalated drinking, CP376395 does not selectively diminish female stress-escalated alcohol intake. This female CSDS protocol may serve as a productive tool for examining novel pharmacotherapeutic targets for sex-specific effects in models of stress-related psychopathologies. Our ongoing work uses male and female CRF-Cre mice to evaluate mechanistic differences in stress-escalated drug-taking, focusing on VTA-projecting BNST neurons.

Disclosures: E.L. Newman: None. **K.C. Burk:** None. **M.B. Bicakci:** None. **J.F. DeBold:** None. **K.A. Miczek:** None.

Poster

155. Stress and the Hypothalamus, Amygdala, and Bed Nucleus

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 155.15/UU7

Topic: F.04. Stress and the Brain

Support: NIAAA R00AA023559
Kellen Foundation

Title: Estrus stage modulates stress reactivity and alcohol drinking in female mice

Authors: *J. RIVERA[1], H. TAKASHIMA[2], M. J. SKELLY[2], J. D. MILLER[2], K. E. PLEIL[2]
[1]Neurosci., [2]Pharmacol., Weill Cornell Med., New York, NY

Abstract: Repeated binge drinking to intoxication and withdrawal is an important risk factor for the morbidity and mortality of alcohol use disorder (AUD) and is correlated with numerous comorbid psychiatric disorders. In addition, stress-related neuropsychiatric diseases such as anxiety and depression are more common in women than men, and the occurrence of binge drinking and AUD among women is increasing at an alarming rate, narrowing the gender gap in this disease. Females experience periods of altered mood and sensitivity to the rewarding effects of alcohol and other drugs across the estrus/menstrual cycle, implicating cyclical fluctuations of ovarian hormones such as estrogen in the dynamic regulation of alcohol consumption, anxiety, and depressive like behaviors. Here, we tested whether fluctuations in estrogen levels across the estrus cycle in female mice dynamically regulated their anxiety and stress coping behavior. We found that individual females' behavior changes across assays in accordance with their current estrogen status, such that high estrogen (during proestrus) was anxiolytic and promoted an active coping strategy compared to females with low estrogen (during met/diestrus); ongoing experiments are examining estrus cycle modulation of binge drinking behavior. In addition, the underlying neural mechanisms by which these hormonal effects occur remain unknown. The bed nucleus of the stria terminalis (BNST) is a sexually dimorphic brain region that participates in the regulation of these behaviors and may be a site for the effects of ovarian hormones. Current experiments are testing the hypothesis that cycling levels of estrogen modulate the excitability of CRF neurons in the BNST, whose activity we have previously shown regulates binge alcohol drinking and anxiety behavior. We hypothesize that this effect is driven by estrogen modulation of the direct excitatory projections from the paraventricular nucleus of the thalamus to BNST CRF neurons. This study aims to enhance our understanding of the overlapping circuits driving binge alcohol drinking and comorbid neuropsychiatric disorders and characterize the functional mechanisms of estrogen action, enabling the development of pharmacotherapeutic treatments to help the millions afflicted by these debilitating diseases.

Disclosures: J. Rivera: None. **H. Takashima:** None. **M.J. Skelly:** None. **J.D. Miller:** None. **K.E. Pleil:** None.

Poster

155. Stress and the Hypothalamus, Amygdala, and Bed Nucleus

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 155.16/UU8

Topic: F.04. Stress and the Brain

Support: PAPIIT IN306216

Title: The effect of stress on the object recognition memory in female rats during the diestrus and proestrus

Authors: *M. R. GONZALEZ LOPEZ, N. L. GARCÍA-SALDÍVAR, J. C. ROMERO-GUADIANA, J. P. C. ARRIAGA-RAMÍREZ, S. E. CRÚZ-MORALES
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Abstract: Neuroendocrine changes during the estrous cycle, diestrus (D2) and proestrus (P) have been related to variations in the memory of female rats. With object recognition memory (OR), differences have been found in different days of the cycle with short inter-trial intervals, less exploration has been reported in D2 with OR. In this study the effect of stress produced by: restriction (R), shock (SH), or the administration of 5 mg / kg of corticosterone pre-training in OR during the phases D2 and P, and the release of corticosterone in plasma were studied. Sixteen groups (n = 8) of rats cycled, eight in D2 and eight in P were assigned to the following treatments: two without treatment (I), four subjected to R of which two were immediately sacrificed (R15-0) and two sacrificed 2 hr later (R15-2). Two more groups were trained in OR (T-0), two that were trained and tested in OR, two were restricted 15 min before OR (R+OR), two received a shock of 2.5 mA (SH+OR) pre-training and two received corticosterone 5 mg / kg ip before OR. The subjects who performed the task were habituated 10 min in the recognition box without objects. 24 hr after, at the training session, the subjects were allowed to explore for 5 min two familiar objects (FO) and two hr later they performed the test where they were exposed for 5 min to a novel object (NO) and a FO. To evaluate memory in OR, the discrimination index (DI) was obtained (DI = 100 * 100 (TN- TF) / (TN + TF), where TN = time spend in the NO, and TF = time spend in the FO; the time of exploration to the NO was measured. To measure anxiety the permanence time on the periphery of the box was recorded (thigmotaxis). At the conclusion of the treatments, all subjects were sacrificed and blood samples were obtained to measure corticosterone with ELISA. The performance on OR without stress was similar in both phases. R and SH impaired memory in D2 but had no effect on P. Corticosterone improved memory and produced anxiety in D2 but had no effect on P. Plasma corticosterone was lower in groups stressed before OR in both phases, this effect may be related to inhibition of corticosterone release. R or OR training produced stress in both phases, similar to other studies. It was concluded that the effect of stress due to restriction or shock on OR was dependent on estrous cycle. D2 was more affected, exogenous corticosterone improved memory in this phase. Memory formation of OR in P is more resistant to the effects of stress.

Disclosures: M.R. Gonzalez Lopez: None. **N.L. García-Saldívar:** None. **J.C. Romero-Guadiana:** None. **J.P.C. Arriaga-Ramírez:** None. **S.E. Crúz-Morales:** None.

Poster

155. Stress and the Hypothalamus, Amygdala, and Bed Nucleus

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 155.17/UU9

Topic: F.04. Stress and the Brain

Support: Office of Naval Research Grant

Title: A profile of hypothalamic-pituitary-adrenal axis function following photoperiod alteration and the influence of dietary isoflavones

Authors: *K. BUBAN[1], B. M. BAUMAN[1], A. L. RUSSELL[2,3], R. J. HANDA[4], T.-Y. J. WU[1,3]
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Abstract: The hypothalamic-pituitary adrenal (HPA) axis mediates the physiological response to stressors, both physical and psychogenic, to optimize an organism for survival in their environment. This system relies on a number of redundant feedback mechanisms at multiple levels, including the hypothalamus, pituitary gland, adrenal glands and the limbic system. HPA feedback occurs via glucocorticoid (MR/GR) and CRF (1/2) receptors to ensure proper homeostatic functioning. Therefore, changes in the environment and diet can greatly impact neuroendocrine function and behavioral outcome. Previous research has found that circadian disruption in the form of a light cycle shift can cause a dysregulation in the HPA axis, whereby rats show increases in basal corticosterone (CORT) secretion and anxiety like behavior (Dulcis et. al., 2013). However, little research has been done on identifying at what level the dysregulation occurs, and how diet may further influence this dysregulation, as the isoflavones in standard rodent chow can also impact HPA axis function. In this study, male C57BL/ 6J mice were maintained on either a standard 12:12 light-dark (LD) cycle or moved to a shortened activity period of a 16:8 LD cycle and given either a diet of standard rodent chow or an isoflavone free (IF) chow. Animals exposed to the shortened activity period showed increased basal CORT secretion ($p<.05$), however this increase was attenuated in animals fed the IF chow ($p<.05$). At the level of pituitary, animals maintained on the 16:8 LD cycle and fed IF chow showed increases in MR and GR expression ($P<.05$). Differential expression of MR and GR receptors were seen in the anterior and posterior divisions of the bed nucleus of the stria terminalis (aBNST/pBNST respectively) following light alteration. Mice fed the standard chow had increases in basal MR expression following light alteration ($p=.05$). Furthermore, in the aBNST animals in the 16:8 LD cycle fed the standard chow showed decreases in basal GR expression ($p<.05$). Our data suggests that the dysregulation in CORT secretion may be mediated through the relay nuclei of the BNST. Furthermore, the attenuation seen in the IF chow groups

could be modulated via compensatory mechanisms at the level of the pituitary. These findings provide insight into how circadian disruption via a light cycle shift can alter HPA axis function, and further illuminates the importance of the BNST in the regulation of the HPA axis.

Disclosures: K. Buban: None. **B.M. Bauman:** None. **A.L. Russell:** None. **R.J. Handa:** None. **T.J. Wu:** None.

Poster

155. Stress and the Hypothalamus, Amygdala, and Bed Nucleus

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 155.18/UU10

Topic: F.04. Stress and the Brain

Support: KIOM Grant K18181

Title: A mechanical acupuncture instrument mitigates the endoplasmic reticulum stress and oxidative stress of ovariectomized rats

Authors: *S. SEO
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Abstract: Acupuncture has become a popular complementary and alternative treatment approach for anxiety and depression. However, there is little research on the detailed mechanism of acupuncture therapy relieving depression. Previously, 17β-estradiol was shown to prevent oxidative stress and endoplasmic reticulum (ER) stress in ovariectomized (OVX) rats. This study investigated whether stimulation of Sanyinjiao (SP6) using a mechanical acupuncture instrument (MAI) can mitigate depression-like behavior caused by estrogen deficiency in OVX rats. Furthermore, we found that acupuncture reduced ER stress and oxidative stress-related protein expression. The OVX operation was performed on female SD rats that were divided into four groups: the E2 (2.5 μg/kg, i.p.) injection control group (OVX + E2), the OVX group (OVX), and the OVX with acupuncture stimulation group (OVX + SP6). Non-acupoint stimulation rats served as controls (OVX + NonAcu). The acupuncture point stimulation began three weeks after surgery. The depressive behavior was analyzed by the forced swim test (FST) and open field test (OFT). The 8-OHDG, BiP, Sigma receptor 1, pJNK, PDI, Ero1-Ia and Calnexin protein levels were evaluated by immunoreactivity in the amygdala. Acupuncture stimulation reduced depressive behavior and altered depression-related proteins. Stimulation of SP6 decreased the immobility time of the FST and altered the ER stress and oxidative stress marker proteins, such as 8-OHDG, BiP, pJNK, PDI, Ero1-Ia and Calnexin. Our results indicated that stimulation of SP6 had a significant antidepressant-like effect on an OVX-induced depression rat model, and mitigation of ER stress and oxidative stress was implicated in this effect.

Disclosures: S. Seo: None.

Poster

155. Stress and the Hypothalamus, Amygdala, and Bed Nucleus

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 155.19/UU11

Topic: F.04. Stress and the Brain

Support: NIDA Grant R01DA035680

Title: Hypothalamic CRF neurons integrate multimodal stimuli to program a functional circuitry for avoidance behavior

Authors: *M. WAGLE[1], J. SCHULKIN[2], S. GUO[1]
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Abstract: Corticotropin releasing factor (CRF) is an ancient neuropeptide present from nematodes to vertebrates. It serves as an information molecule to signal potential danger and elicit physiological and behavioral response. While considerable progress has been made in linking CRF to cautious avoidance behaviors, it remains poorly understood the precise contribution of CRF to behaviors at a circuit level. Here we address this question employing larval zebrafish, which has a CRF system analogous to mammals and is amenable to neural circuit dissection at cellular resolution. hypothalamic CRF neurons (CRF^{Hy}) extended axonal terminals and varicosities proximal to pituitary and multiple visual-motor neuronal types in the brain. Chemogenetic ablation of CRF^{Hy} neurons or a block of CRF receptor activity disrupts physiology measured by cortisol level and camouflage response, and moreover abolished dark aversion. Conversely, heat stimulus elevates cortisol and enhances dark aversion. Finally, natural stimuli including dark and heat both activate CRF^{Hy} neurons. This further modulates the activity in the visual-motor neuronal types receiving CRF innervation. These findings provide evidence to support that CRF neurons integrate sensory stimuli of distinct modalities and in turn regulate both sensory and motor targets in a neuronal circuit for avoidance behavior.

Disclosures: M. Wagle: None. **J. Schulkin:** None. **S. Guo:** None.

Poster

155. Stress and the Hypothalamus, Amygdala, and Bed Nucleus

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 155.20/UU12

Topic: G.03. Emotion

Support: NIH Grant MH113026

Title: Differential cortical and limbic brain inputs to dorsal lateral and medial subregions of the anterior bed nucleus of the stria terminalis revealed by new viral genetic mapping

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Abstract: The anterior portion of the bed nucleus of the stria terminalis (BNST) modulates fear and stress responses. The dorsal component of the anterior BNST (adBNST) can be anatomically subdivided into the lateral and medial divisions. Although output projections of BNST subregions have been studied, the local and global input connections to these subregions remain poorly understood. To further understand BNST-centered circuit operations, we have applied new genetic-directed viral tracing and functional circuit mapping to determine detailed synaptic circuit inputs to lateral and medial subregions of adBNST in the mouse. Monosynaptic canine adenovirus type 2 (CAV2) and rabies-based retrograde tracing from the lateral and medial adBNST subregions shows surprisingly sparse local connections between these two subregions. Rather, lateral versus medial BNST subregions have distinct patterns of long-range cortical and limbic brain inputs which implies different functional specialization for each subregion. Overall, the amygdalar complex, hypothalamus and hippocampal formation account for the majority of inputs to adBNST. Parsing these inputs by subregion, the lateral adBNST subregion has more extensive and stronger connections from prelimbic, cingulate, insular and motor cortex, anterior thalamus and perirhinal cortex. In contrast, the medial adBNST subregion receives proportionally more inputs from medial amygdala, lateral septum, preoptic nucleus and subiculum. Further, anatomical and functional circuit mapping reveal a previously undescribed significant input from the amydalohippocampal transition area to the adBNST subregions. These results provide important information of the differential afferent inputs to adBNST subregions, and provides insight into the functional operations of BNST circuitry in anxiety-related behaviors.

Disclosures: X. Xu: None. **T.C. Holmes:** None. **Y. Sun:** None.

Poster

155. Stress and the Hypothalamus, Amygdala, and Bed Nucleus

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 155.21/UU13

Topic: F.04. Stress and the Brain

Support: Simons Foundation 322839
NIH MH100556

Title: Gut microbiota regulate social behavior via stress response pathways in the brain

Authors: *W.-L. WU, M. D. ADAME, W. TANG, C. E. SCHRETTER, M. I. WANG, R. ABDEL-HAQ, K. BEADLE, B. E. DEVERMAN, V. GRADINARU, S. K. MAZMANIAN
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Abstract: Social impairment is a major symptom of neuropsychiatric conditions, such as autism spectrum disorder (ASD), schizophrenia, anxiety and depression. While the microbiome has been linked to social interaction in animals, gut-brain connections that regulate this complex behavior remain entirely undescribed. Herein, we demonstrate that depletion of microbiota in mice not only impairs social behavior, but also activates specific brain regions related to canonical stress responses. Social deviation in germ-free and antibiotic-treated mice is associated with elevated levels of the stress hormone corticosterone, which is primarily produced via activation of hypothalamus-pituitary-adrenal (HPA) axis. Accordingly, removal of the adrenal gland, antagonism of the glucocorticoid receptor, and pharmacological inhibition of corticosterone synthesis effectively correct social deficits. Genetic ablation of the glucocorticoid receptor in specific brain regions and chemogenetic inactivation of hypothalamic neurons dramatically increase social behavior. Further, we identify specific bacterial metabolites that suppress activation of the HPA axis and improve social impairment. These findings reveal that the gut microbiome regulates social behavior by co-opting neuronal circuits that control stress responses in mice.

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Poster

156. Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 156.01/UU14

Topic: F.04. Stress and the Brain

Title: Homeostatic synaptic scaling maintains stability in stress circuits

Authors: *N. RASIAH[1], D. G. ROSENEGGER[2], N. DAVIU[3], T. FUZESI[4], T.-L. STERLEY[2], J. S. BAINS[3]
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Abstract: Survival for any organism requires that stress circuits respond appropriately to intrinsic and extrinsic challenges. Inappropriate activation of these circuits can be a harbinger for a number of psychopathologies including depression, anxiety and mood disorders. How these circuits maintain stability to ensure appropriate responses to challenge is not known. Corticotropin releasing hormone (CRH) neurons in the paraventricular nucleus of the hypothalamus (PVN) control the endocrine and behavioral response to stress. Their activity drives, and is curtailed by corticosteroids. Here we used a mouse model of chronic corticosteroid administration to examine cellular and synaptic mechanisms that contribute to stability in PVN:CRH neuron output.
Crh-IRES-Cre;Ai14 mice had access to 25µg/ml CORT in the drinking water for up to 7 days. We then used fibre photometry to assess CRH neuron activity *in vivo* or prepared brain slices and used on-cell and whole-cell electrophysiology to study CRH activity and synaptic properties *in vitro*. Seven-day CORT treatment had no effect on the *in vivo* activity of CRH neurons ($p=0.97$ vs control), but did decrease the firing of these neurons *in vitro* ($p=0.0022$ vs control). This was accompanied by a homeostatic and multiplicative increase in the amplitude of miniature excitatory postsynaptic currents (mEPSCs) ($p = 0.0026$ vs control) with no effect on mEPSC frequency, AMPA:NMDA, or PPR. Brain-derived neurotrophic factor (BDNF) has been implicated in synaptic scaling, and adding 160µg/ml DHF, an orally available BDNF analog, to the drinking water solution prevented synaptic scaling. Finally, to directly test whether loss of BDNF signaling may be required to maintain CRH output *in vivo*, we used photometry to examine the effects of CORT+DHF. In animals given CORT+DHF, there was a significant decrease in CRH neuron activity ($p=0.0014$ vs control) demonstrating that synaptic scaling is required for maintaining circuit output during a CORT challenge. This is one of the first accounts of homeostatic synaptic scaling in adult vertebrates, and is the first to establish a role for homeostatic synaptic scaling in reengaging set-point activity in an intact neural circuit

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Poster

156. Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 156.02/UU15

Topic: F.04. Stress and the Brain

Support: CIHR Grant 86501

Title: Vasopressin decreases synaptic metaplasticity after stress

Authors: *S. P. LOEWEN, J. S. BAINS
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Abstract: Acute stress primes glutamate synapses onto corticotropin-releasing hormone (CRH) neurons in the hypothalamic paraventricular nucleus (PVN), resulting in a stress-induced metaplasticity. We have recently shown that this priming of synapses by a single stress is buffered in females, but not males, by the presence of a partner. However, the cellular substrates underlying this sex-specific buffering of stress have not been elucidated. Here we examined the effects of vasopressin (AVP) on short-term potentiation (STP) elicited by high-frequency electrical stimulation (HFS), which induces metaplasticity at glutamatergic synapses in the PVN. We used female Crh-IRES-Cre; Ai14 mice in which CRH neurons express a tdTomato fluorophore. Stress was induced by subjecting mice to a footshock protocol (0.5 mA for 2 seconds delivered every 30 seconds over 5 minutes). Immediately following this footshock protocol, mice were anaesthetized and coronal brain slices were prepared for electrophysiological experiments. PVN slices were incubated in artificial cerebrospinal fluid containing 100 nM AVP for 30 minutes. Evoked excitatory postsynaptic currents were recorded from CRH neurons before and after HFS. STP was still evident in cells from AVP-incubated slices (115.2 ± 4.1%, n=14, p=0.002 vs. baseline), but was significantly reduced compared to cells from non-incubated control slices (154.0 ± 8.5%, n=16, p=0.0005). Additionally, the paired-pulse ratio of evoked synaptic currents was decreased following HFS in cells from non-incubated slices (83.5 ± 3.9%, n=16, p=0.001 vs. baseline), but remained unchanged in cells from AVP-incubated slices (94.7 ± 3.0%, n=14, p=0.5 vs. baseline). These results demonstrate that AVP erases the synaptic effects of acute stress in female mice.

Disclosures: S.P. Loewen: None. **J.S. Bains:** None.

Poster

156. Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 156.03/UU16

Topic: F.04. Stress and the Brain

Title: A novel method to study chloride homeostasis in brain slices

Authors: *A. J. LANZ, G. R. GORDON, J. S. BAINS
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Abstract: The potassium-chloride co-transporter (KCC2) is a electrogenically neutral pump that establishes and maintains chloride gradients in adult neurons. Physiological or pathological regulation of KCC2 activity can alter the chloride Nernst potential to modulate the direction and strength of synaptic inhibition. Both acute and chronic stress reduce KCC2 activity in corticotropin-releasing hormone (CRH) neurons of the paraventricular nucleus (PVN) of the hypothalamus. Despite the potential importance of KCC2 in regulating neuronal excitability, there are few methods to directly and accurately measure KCC2-mediated chloride extrusion. Here we combined whole cell voltage-clamp electrophysiology with optogenetics to measure KCC2 function in CRH neurons in hypothalamic brain slices of naïve and acutely-stressed mice. By using GABA-puff evoked currents as a proxy for chloride driving force, we track chloride gradients during and after halorhodopsin-mediated chloride loading. Photostimulation of halorhodpsin robustly decreases outward chloride currents (tau = 7.172 s); cessation of light delivery results in a monoexponential recovery of currents (tau = 11.13 s). After an acute stress, chloride currents collapse in response to photostimulation, but show a complex, non-exponential partial recovery. These preliminary data provide new approaches to study chloride homeostasis in healthy and pathological brain circuits.

Disclosures: A.J. Lanz: None. **G.R. Gordon:** None. **J.S. Bains:** None.

Poster

156. Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 156.04/UU17

Topic: F.04. Stress and the Brain

Support: CIHR

Title: Fear and anxiety in the hypothalamus

Authors: *T. FUZESI[1], D. G. ROSENEGGER[2], N. DAVIU[2], N. RASIAH[2], G. PERINGOD[2], G. R. GORDON[2], J. S. BAINS[2]
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Abstract: It is well established that corticotropin-releasing hormone (CRH) neurons in the paraventricular nucleus of the hypothalamus (PVN) control the endocrine response to stress. This concept is built on hormone assays and proxy, but static measures of activity. Recent work indicates that PVN CRH neurons also control specific stress-related behaviors. This does not require hormone release. Precisely how these neurons encode both rapid behavior and slow hormone release is not known. Here, we used in vivo single fibre photometry to assess real-time calcium changes in PVN CRH neurons in freely behaving mice. We injected an adeno-associated virus containing a Cre-dependent GCaMP6s construct into the PVN of a CRH-Cre transgenic mouse. Two weeks later, an optical fiber was implanted directly above the PVN. Experiments began after one week of recovery and handling.We examined PVN CRH activity in a number of scenarios. Introduction of the animal to a novel environment elicited a sustained elevation in the GCaMP signal. Repeated exposure (4 days) to the same environment did not alter the amplitude of this persistent increase. Consistent with this observation, there was no habituation in the corticosterone response during repeated exposure to novel environment. In response to footshock, CRH neurons showed a rapid, but transient increase in Ca^{2+}. Re-introduction of the animal to this context on subsequent days induced a sustained increase of PVN CRH Ca^{2+} that was not altered over the course of many days. Finally, handling itself also induced an increase in Ca^{2+} that was similar in magnitude to that observed during footshock. The Ca^{2+} response remained unchanged by repeated handling sessions. These observations indicate that PVN CRH neurons show distinct activity profiles that reflect the duration and intensity of the stressor.

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Poster

156. Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 156.05/UU18

Topic: F.04. Stress and the Brain

Support: NIH Grant R01MH102595

Title: A mouse model of stress-enhanced fear and anxiety-like behavior

Authors: *A. M. HASSIEN, F. SHUE, M. LEE, B. E. BERNIER, M. R. DREW
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Abstract: Stressful experiences can cause long-lasting sensitization of fear and anxiety that extends beyond the circumstances of the initial trauma. The mechanisms underlying stress-induced fear and anxiety are not well understood. In particular, it is debated whether sensitization is a nonassociative phenomenon or reflects the generalization of a learned Pavlovian association. Here, we established a mouse model of stress-enhanced fear and anxiety-like behavior. C57bl6/j or 129svev/Jax mice were given four 1-mA foot shocks in an 8-minute session or received an equivalent amount of context exposure without shock. Shock exposure suppressed open field exploration and potentiated freezing in response to a loud noise presented in a neutral context. The suppression of exploratory behavior in the open field persisted for at least 56 days after shock exposure. Chemogenetic inhibition of the basolateral amygdala during foot shock prevented acquisition of contextual fear conditioning and blocked the effects of foot shock on open field exploration and noise-induced fear, demonstrating that both associative fear conditioning and sensitization require basolateral amygdala (BLA). To determine whether foot shock-induced sensitization is associative or nonassociative, additional groups of mice received footshock (or shock-free context exposure) followed by context extinction, which reduced associative conditioned fear. Extinction training caused open field exploration to return to normal levels but failed to attenuate sensitized fear of a loud noise. Stress-Enhanced Fear Learning (SEFL) was assessed in extinguished and non-extinguished mice by administering a single, unsignaled shock in a novel context. 129svev/Jax displayed the SEFL effect but C57bl6/j mice did not. The SEFL effect in 129svev/Jax was not prevented by prior context extinction training. Together, these findings indicate that stress-induced fear sensitization involves both associative and nonassociative components. Stress-enhanced anxiety-like behavior likely reflects generalization of the conditioned response, whereas stress-induced fear and fear learning are non-associative.

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Poster

156. Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 156.06/UU19

Topic: F.04. Stress and the Brain

Support: NARSAD Young Investigator Award (DD) R01MH111918 (DD)

Title: Identification of whole-brain structural connectivity patterns of stress-activated basolateral amygdala neurons in resilient versus susceptible mice

Authors: *C. FILLINGER[1], Y. S. GROSSMAN[2], A. MANGANARO[3], J. ZHANG[1], H. BITO[4], C. A. DENNY[5], D. DUMITRIU[6]
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Abstract: Depression is the most common mood disorder, considered to be the foremost contributor to the worldwide burden of disease in 2030. Mechanisms underlying depression are not well understood, but stress is a well-known trigger. Interestingly, not all individuals respond to stress equally: some are resilient to it, while others are stress-susceptible. In prior work, we showed differences in mesoscale functional connectivity of resilient versus susceptible mice using c-Fos co-activation mapping immediately after acute social defeat stress (ASDS). These differences in co-activation were driven in large part by the basolateral amygdala (BLA) but were not a consequence of difference in absolute level of activation. To understand the structural connectome underlying such individual variability in stress-sensitivity and co-activation patterns, we analyzed social defeat stress-activated neurons inputs in the basolateral amygdala (BLA) using an innovative combination of activity dependent labeling and transsynaptic viral-mediated tracing. To label activated neurons, we are using two different methods: Arc-CreER^{T2} transgenic mice (Denny et al Neuron 2014) and viral-mediated E-SARE-CreER-PEST expression (Kawashima et al Nat Methods 2013), both leading to functional Cre expression in activated neurons in the presence of 4-hydroxytamoxifen (4-OHT). We combine this activity-dependent Cre expression with a prior injection of Cre-dependent viral-mediated expression of avian receptor TVA and glycoprotein G in the BLA. This allows us to label presynaptic partners of stress activated neurons by injecting a transsynaptic pseudotyped G-deleted rabies virus into the BLA two weeks after 4-OHT-mediated recombination. Because the pseudotyped G-deleted rabies virus can only infect and translocate from TVA- and G-expressing neurons, this technique allows us to highlight features of the presynaptic whole-brain structural connectome of stress-activated BLA neurons of resilient versus susceptible mice. We are currently using iDISCO brain clearing in combination with light-sheet microscopy to dissect connectomic differences. Our results will be an important first step in uncovering the individual variability of functional and structural circuits that mediate divergent stress-responses.

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Poster

156. Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 156.07/UU20

Topic: F.04. Stress and the Brain

Title: Circuit mapping of medial amygdala (MeA) to the paraventricular nucleus (PVN)

Authors: *L. AKBARI[1], J. YEOH[1], C. D. ADAMS[1], C. S. MITCHELL[1], J. S. BAINS[2], Z. B. ANDREWS[3], B. A. GRAHAM[1], C. V. DAYAS[1]
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Abstract: The medial amygdala (MeA) is an important substrate for the detection and actioning of threat-based stress responses. For example, the MeA is robustly activated by pheromones from novel conspecifics and predator odours, including enzymes extracted from the saliva of cat or rats. Our focus has been to determine how the MeA control hypothalamic-pituitary-adrenal (HPA) axis responses to these threat-related stimuli, with the hypothesis that this might involve a direct projection from the MeA to the hypothalamic paraventricular nucleus (PVN). To achieve this aim we used combinations of viral tracing, CLARITY, optogenetics and electrophysiology to probe the existence of a direct glutamatergic projection from the MeA to corticotrophin-releasing-factor (CRF) cells – the group of neuroendocrine cells that control the HPA axis. To validate the existence of direct projections from the MeA to the PVN, we made injections of AAV5-hSyn-YFP into the MeA of *CRF-Cre::td-tomato* mice or injections of retrograde AAV2-Ef1a-DIO-YFP into the PVN of *Vglut2-Cre* mice (n = 3-5/group). Using CLARITY and confocal imaging we found robust YFP expression in the PVN of animals that received MeA AAV5-hSyn-YFP, with terminals making close apposition to CRF-td-tomato +ve neurons. In *Vglut2-Cre* mice injections of retrograde AAV2-Ef1a-DIO-YFP in the PVN resulted in substantial numbers of MeA neurons that displayed YFP expression, confirming the existence of direct projections from MeA to PVN neurons. Next we used a transgenic mouse line that expressed Cre-recombinase under the control of the single-minded-one (Sim-1) gene promoter. Sim-1 is fundamental to the development of the PVN and is expressed in a discrete population of cells that migrate from the diencephalon to telencephalon into the MeA. We targeted the *Sim-1 Cre*-expressing neurons with injections of AAV5-DIO-ChR2-YFP and made electrophysiological recordings from putative CRF cells in PVN. We identified that 100% of photostimulation light-evoked inputs (13 cells from 7 mice) onto putative CRF cells were CNQX-sensitive but picrotoxin-insensitive; confirming that *Sim-1*+ve neurons in the MeA provide glutamatergic input to the PVN and excite putative CRF cells. Together these findings identify a functionally patent projection from glutamatergic Sim-1+ve neurons in the MeA that project to the PVN with the capacity to regulate HPA axis responses to predator threats.

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Poster

156. Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

Location: SDCC Halls B-H

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Program #/Poster #: 156.08/UU21

Topic: F.04. Stress and the Brain

Support: National Natural Science Foundation of China (31371115, 81527901)
Tsinghua IDG/McGovern Institute for Brain Research and Center for Brain-Inspired Computing Research.

Title: A potential role of non-lemniscal auditory thalamic inputs to lateral amygdala in diverse sound-induced defensive behaviors

Authors: *M. LIU[1], Y. WANG[3], D. CAI[4], F. XIE[6], L. YOU[2], Y. YUE[2], K. YUAN[5]
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Abstract: Amygdala is essential for formation and storage of the memory associated with fearful or rewarding environment stimuli. Lateral amygdala (LA) is a major input region of the amygdala. Decades of studies have shown the importance of LA in auditory fear conditioning, and it was proposed that the unconditioned-stimulus (US) and acoustic conditioned-stimulus (CS) converge in this region. Once the animal is conditioned, the acoustic CS, which is believed to be mainly relayed from the non-lemniscal auditory thalamus, triggers freezing behavior. In present study, we investigated whether the non-lemniscal auditory thalamus→LA pathway itself would be involved in defensive behaviors without being paired with US. We found that selective optogenetic activation of the non-lemniscal auditory thalamus→LA pathway led to avoidance and anxiety-like behavior, rather than freezing. Specifically, during real-time-place-avoidance test, the tested mice would run away from and mostly stay out of the laser-on chamber once they have explored that chamber for a few times. Therefore, they spent most of their time in the laser-off chamber. Control mice spent almost equal time in the two chambers. During open-field test, activation of this pathway increased the chances of rearing-up and reduced the entrance of central area. In contrast, control mice demonstrated normal exploring behavior. To our knowledge, this is the first study demonstrating independent involvement of non-lemniscal auditory thalamus→LA pathway in avoidance and anxiety-like behaviors. Our results suggested that, although the non-lemniscal auditory thalamus→LA pathway alone was not sufficient to mediate freezing behavior, this pathway might still play an important role in other sound-induced defensive behaviors.

Disclosures: M. Liu: None. **Y. Wang:** None. **D. Cai:** None. **F. Xie:** None. **L. You:** None. **Y. Yue:** None. **K. Yuan:** None.

Poster

156. Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 156.09/UU22

Topic: F.04. Stress and the Brain

Support: C.C. HI17C2665
NRF-2017R1A2B4006535

Title: Altered mPFC-amygdala circuits of the extinction impaired animal model of PTSD following fear conditioning and extinction

Authors: *K. PARK, C. CHUNG
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Abstract: Post-traumatic stress disorder (PTSD) is a debilitating psychiatric disorder occurring after experiencing one or more terrifying accidents. However, the pathophysiology and the molecular changes underlying its development remain largely unknown. The key symptoms often observed in PTSD patients are hyperarousal, recurrent visits of stressful memories and avoidance. A strain of mouse, 129S1/SvImJ (129S1) exhibits selective impairments in the fear extinction thus has been proposed as an animal model of PTSD. Subregions in the medial prefrontal cortex (mPFC), prelimbic (PL) and infralimbic (IL) cortex have been reported to be involved in fear conditioning and extinction, respectively. PL and IL project to basolateral amygdala (BLA) which also has been known to have important roles in fear conditioning and extinction. Furthermore, mPFC-BLA circuits have been suggested to alter after fear expression and inhibition. However, how mPFC-BLA circuits of extinction impaired animal models change upon fear conditioning and extinction has been unknown. Here, by combining optogenetics and electrophysiology we measured excitatory/inhibitory (E/I) ratios in the PL-BLA and IL-BLA circuits of 129S1 and C57BL/6 following fear conditioning and extinction. After fear conditioning, 129S1 showed elevated E/I ratio in the PL-BLA circuit, which is comparable to that of C57BL/6. Following fear extinction, 129S1 exhibited a prolonged increase of E/I ratio in the PL-BLA circuit, while E/I ratio in C57BL/6 with successful extinction became decreased back to the level comparable to that of baseline. Interestingly, E/I ratio of IL-BLA circuitry in C57BL/6 decreased after successful extinction, while that of 129S1 remained unaltered. Our data explain how mPFC-BLA circuits of an extinction impaired model alter following fear expression and extinction and provide brain circuits underlying PTSD-like behaviors.

Disclosures: K. Park: None. **C. Chung:** None.

Poster

156. Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

Location: SDCC Halls B-H

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Program #/Poster #: 156.10/VV1

Topic: F.04. Stress and the Brain

Support: NIH Grant MH107435 (R01)
NIH Grant MH114363 (F31)

Title: Physiology and function of amygdalo-cortical endocannabinoid signaling

Authors: *D. MARCUS, G. BEDSE, A. GAULDEN, A. HAYMER, S. PATEL
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Abstract: Stress exposure is a ubiquitous risk factor for the development of psychiatric illnesses such as generalized anxiety disorder, major depression, and post-traumatic stress disorder. However, a causal link between stress-induced neuropathologies and resultant pathological behavioral states remains elusive. Stress induces a wide variety of morphological and neurochemical adaptions in the prelimbic prefrontal cortex (plPFC), a key nodal structure that is implicated in the top down regulation of emotional behavior. The plPFC has strong reciprocal glutamatergic connectivity with the basolateral amygdala (BLA), and several studies have suggested that reciprocal excitatory coupling between these two regions is involved in generating cognitive, behavioral, and autonomic responses to stress. Interestingly, the anxiolytic properties of cannabinoid agonists appear to be mediated at least in part by the activation of CB1 receptors on glutamatergic axon terminals in the medial prefrontal cortex.
Using optogenetic projection targeting combined with retrograde labeling, we show that glutamatergic input to the plPFC from the BLA is highly regulated by endocannabinoid (eCB) signaling in a laminar and output specific manner. Furthermore, we show that exposure to traumatic stress impairs both tonic and phasic eCB regulation of the BLA-plPFC reciprocal circuit. This impairment of eCB signaling is occluded by inhibiting 2-Arachindonoyl Glyercol synthesis with the Diacylglycerol Lipase (DAGL) inhibitor DO34, and is rescued by inhibiting 2-AG breakdown with the Monoacylglyercol Lipase (MAGL) inhibitor JZL184. These data suggest that stress exposure impairs 2-AG regulation of the BLA-plPFC reciprocal circuit. To investigate whether impairment of eCB signaling within this circuit is causally related to the generation of pathological behavioral states, we used the INTERSECT approach to selectively delete the CB1 receptor from BLA cells that project to the plPFC. We demonstrate that selective deletion of CB1 from this circuit induces a ‘stress-like’ state, characterized by heightened basal anxiety. These data demonstrate a critical role for eCB signaling in regulating a neural circuit implicated in the pathophysiology of affective disorders.

Disclosures: D. Marcus: None. **G. Bedse:** None. **A. Gaulden:** None. **A. Haymer:** None. **S. Patel:** None.

Poster

156. Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 156.11/VV2

Topic: F.04. Stress and the Brain

Support: NIH grant MH059911

Title: Glucagon-like peptide 1 receptor (GLP1R) signaling promotes excitation of central amygdala (CEA) neurons innervating the bed nucleus of the stria terminalis (BST)

Authors: *N. V. POVYSHEVA[1], H. ZHENG[2], L. M. RINAMAN[3]
[1]Neurosci., Univ. of Pittsburgh Dept. of Neurosci., Pittsburgh, PA; [2]Dept Psychology, [3]Dept. of Psychology, Florida State Univ., Tallahassee, FL

Abstract: Stress-activated GLP1 neurons in the hindbrain innervate stress-sensitive neurons within the CEA, BST, and hypothalamic paraventricular nucleus (PVN). We previously demonstrated that GLP1 activates PVN-projecting neurons in the ventral BST through intrinsic and synaptic mechanisms (Povysheva et al., 2016). Here we test the hypothesis that GLP1 signaling also will activate CEA neurons that innervate the dorsal BST. To test this hypothesis, red fluorescent retrobeads were stereotaxically injected into the dorsal BST in young adult male Sprague-Dawley rats. One week later, whole-cell recordings were made in labeled neurons in brain slices containing the CEA. The synthetic GLP1 analogue Ex-4 (400 nM) was bath applied to activate GLP1Rs. To assess Ex-4 effects on neuronal excitability, cells were recorded in current-clamp mode and shifts in baseline were quantified. Ex-4 produced an upward baseline shift in labeled neurons ($p<0.01$; $n=11$). In 4 of these 11 neurons, Ex-4-induced depolarization resulted in spontaneous firing. Ex-4 effects on synaptic properties also were assessed. Ex-4 increased the amplitude of sEPSCs (-70 mV holding potential, 10 μM gabazine in Cs-based intracellular solution; 10.54±0.96 pA vs. 14.9±1.9 pA, $p<0.05$; $n=5$), but did not affect the frequency of the events (4.6±2.3 Hz vs. 5.5±4.8 Hz, $p>0.1$; $n=5$). Thus, GLP1R signaling promotes activation of dBST-projecting CEA neurons through depolarizing effects and increased excitatory drive, consistent with GLP1 effects to promote centrally mediated stress responses.

Disclosures: N.V. Povysheva: None. **H. Zheng:** None. **L.M. Rinaman:** None.

Poster

156. Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 156.12/VV3

Topic: F.04. Stress and the Brain

Support: NIH Grant MH090297 (JHU; WFC)
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Title: Inhibition of the BLA to BST pathway induces behavioral resilience to restraint stress

Authors: *M. BOMPOLAKI[1], J. A. ROSENKRANZ[2], W. F. COLMERS[4], J. H. URBAN[3]
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Abstract: Neuropeptide Y (NPY) is a potent, endogenous anxiolytic agent which buffers the effects of stress and promotes resilience. NPY mediates this stress buffering primarily by decreasing the excitatory output of the basolateral amygdala (BLA). We previously demonstrated that activation of NPY Y1 receptors (Y1r) hyperpolarizes BLA pyramidal neurons by inhibiting their resting H current, carried by the hyperpolarization-activated cyclic nucleotide-gated channel subunit 1 (HCN1; Geisbrecht et al., 2010). In addition to the acute behavioral effects, repeated application of NPY in the BLA induces lasting behavioral stress resilience, assessed by the social interaction (SI) test (Sajdyk et al., 2008). The current study was designed to examine the role of BLA projections to the bed nucleus of the stria terminalis (BST) and central amygdala (CeA) in modulating SI and behavioral stress resilience in male rats. We hypothesized that target-defined BLA projections will express Y1r and HCN1 channels and that inhibition of these projections will mimic the NPY effect on SI in control and stressed animals. First, we examined whether BLA neurons projecting to the BST or CeA express NPYr and HCN1. The retrograde tracer, Fluorogold (FG), was injected unilaterally into dorsolateral BST (dlBST) or CeA. After 1 week retrograde transport, BLA sections were processed for Y1r and HCN1 immunoreactivity. BLA neurons backfilled from either dlBST and CeA expressed immunoreactivity for Y1r and HCN1. Next, retrograde delivery of cre-recombinase (Cav2-cre), combined with cre-driven DREADD-G_i expression was used to selectively inhibit BLA neurons. Thus, CAV2-cre was bilaterally injected into either the dlBST or the CeA and cre-DREADD-G_i was bilaterally injected into the BLA. Following at least 2 weeks post-injections, systemic injection of CNO (1mg/kg, 45 minutes prior to the behavioral assay) elicited increases in SI behavior. Subjecting animals to a 30 min restraint stress significantly decreased SI. This decrease in SI behavior was prevented when the BLA to BST projection was inhibited by DREADD-G_i. This finding demonstrates that, with respect to SI, this pathway is active and further excited subsequent to restraint stress. Inhibition of the BLA to CeA projection had no behavioral effects in similar

experiments, suggesting that this projection is not recruited under the same circumstances. This work provides evidence for the role of specific BLA projections in behaviors such as SI and resilience, and also broadens our understanding of the underlying NPY circuitry to these behaviors.

Disclosures: M. Bompolaki: None. **J.A. Rosenkranz:** None. **W.F. Colmers:** None. **J.H. Urban:** None.

Poster

156. Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

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Topic: F.04. Stress and the Brain

Support: NIH Grant R01MH082802
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Title: Altered miRNA expression in amygdala mediates learned helplessness behavior through Wnt signaling

Authors: *B. ROY, M. DUNBAR, J. AGRAWAL, L. A. ALLEN, Y. DWIVEDI
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Abstract: As epigenetic modifier, microRNAs (miRNAs) present novel regulators of gene expression in brain. Recent studies suggest that miRNAs can participate in depression pathogenesis by altering a host of genes that are critical in neuronal functions. Amygdala is part of the limbic system involved in memory modulation, fear and stress response, as well as emotional learning. The present study focused on examining whether alterations in miRNA networks in amygdala is associated with susceptibility (learned helplessness: LH) or resiliency (non-learned helplessness: NLH) to develop depression in rodents. miRNA-based next generation sequencing (NGS) was used to identify dysregulated miRNA transcriptomics across LH, NLH, and control groups. Bioinformatic tools were applied to understand the target gene set enrichment and altered pathways resulting from overall miRNA dysregulation. Furthermore, miRNA-specific target interaction was determined using in-vitro transfection assay in neuroblastoma cell line. Results from group wise comparison identified 8 (miR-128-3p, 132-3p, 342-3p, 425-5p, 431, 674-3p, 674-5p and 873-3p) significantly upregulated and one downregulated (miR-34c-5p) miRNAs in LH compared to NLH group. Bioinformatic analysis showed that a majority upregulated miRNAs targeted genes enriched for Wnt signaling pathway.

Expression analysis of target genes including Wnt 3, Wnt 3a, Dvl1 and Lef1 were significant downregulated in amygdala of LH rats compared with NLH or control rats. In-vitro transfection assay using the most significantly upregulated miR-128-3p showed marked decrease in the expression of Dvl1 and Lef1 from Wnt signaling pathway. Altogether, our study suggests that miRNAs may play an important role in depression susceptibility, which could be mediated through disrupted Wnt signaling pathway.

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Poster

156. Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

Location: SDCC Halls B-H

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Topic: F.04. Stress and the Brain

Support: KAKENHI grant 25430079
The Osaka Medical Research Foundation for Intractable Diseases

Title: MARCKSL1 in the amygdala controls the HPA axis and anxiety behaviors

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Abstract: Myristoylated alanine-rich C-kinase substrate like 1 (MARCKSL1) is a group of acidic proteins localized to the plasma membrane. MARCKSL1 is known to be a primary substrate of protein kinase C, and it regulates membrane-cytoskeletal plasticity by altering the actin cytoskeleton. The molecular features of MARCKSL1 imply an important function in structural maintenance and neuronal plasticity. However, the precise physiological role of MARCKSL1 in the adult brain and its subsequent effects on behaviors remain largely unclear. Although MARCKSL1 is predominantly expressed in the immature brain, it remains localized in adult limbic regions associated with emotional processing. Here, using overexpression and knockdown techniques in vivo, we analyzed the role of MARCKSL1 in brain function, focusing on mood and anxiety. MARCKSL1 transgenic (Tg) mice exhibited anxiety-like behaviors dependent on corticotropin-releasing hormone. MARCKSL1 increased spine formation in the central amygdala, and downregulation of MARCKSL1 in the amygdala normalized both increased hypothalamic-pituitary-adrenal (HPA) axis activity and elevated anxiety-like behaviors in Tg mice. Our findings suggest that MARCKSL1 regulates amygdala circuitry to control the activity of the HPA axis, as well as induces anxiety-like behaviors in mice.

Disclosures: T. Tanaka: None. **S. Miyata:** None.

Poster

156. Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

Location: SDCC Halls B-H

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Topic: F.04. Stress and the Brain

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PASPA-DGAPA-UNAM

Title: Conservation of retinohypothalamic, hippocampal and amygdalar PACAPergic circuits in mouse and rat

Authors: L. ZHANG[1], S. Z. JIANG[2], V. S. HERNÁNDEZ[3], E. WEIHE[4], P. T. LINDBERG[5], M. K. H. SCHÄFER[6], J. W. MITCHELL[5], C. BEAULE[5], M. U. GILLETTE[5], *L. E. EIDEN[2]
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Abstract: The distribution of mRNA encoding PACAP and its cognate receptor PAC1 was studied in selected areas of mouse brain using RNAScope, an in situ hybridization histochemical technique allowing clear delineation of PACAP and PAC1 mRNA-expressing cells at the light microscopical level, and co-expression with markers for the excitatory and inhibitory neurotransmitters glutamate and GABA, respectively. The purpose of the study was to compare neurochemical and anatomical divergence between the distribution of PACAPergic circuits in phylogenetically distinct species potentially revealing evolutionary divergence, as well as conservation, of PACAP function between the two rodent species. PACAP and PAC1 distribution within the hypothalamus are relatively well conserved between rat and mouse. In particular the retinohypothalamic tract projecting to the suprachiasmatic nucleus co-expresses PACAP and the glutamate marker VGluT2 in the mouse, as previously reported in rat. Functionally this is consistent with PACAP modulation of glutamate-dependent phase advance, but not phase delay, in circadian regulation of electrophysiological activity of the mouse SCN. Functional ex vivo experiments conducted with mouse SCN-containing tissue confirmed PACAP dependence of glutamate-induced late-night phase advance observed previously in experiments

conducted in vivo. PACAP innervation of amygdala and hippocampus, however, diverge greatly in mouse and rat. We report here that the distribution of PACAPergic neurons of the central amygdala of the mouse, unlike that reported for rat, is sparse compared to basolateral amygdala. Hippocampal expression of both PACAP and PAC1 mRNA are markedly different in the mouse compared to the rat, and this was confirmed independently here by comparison between mouse and rat brains processed in parallel for in situ hybridization histochemistry using chromogenic RNAScope, and including elucidation of excitatory and inhibitory neurotransmitter (i.e. VGluT and VGABAT mRNA) co-expression in these neurons. These findings have clear functional implications for mechanistic interpretation of the behavioral effects of PACAP reported in rat and mouse.

Disclosures: L. Zhang: None. **S.Z. Jiang:** None. **V.S. Hernández:** None. **E. Weihe:** None. **P.T. Lindberg:** None. **M.K.H. Schäfer:** None. **J.W. Mitchell:** None. **C. Beaule:** None. **M.U. Gillette:** None. **L.E. Eiden:** None.

Poster

156. Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 156.16/VV7

Topic: F.04. Stress and the Brain

Support: NIH R01 DA019921

Title: Chronic amphetamine treatment and withdrawal increases anxiety-like behaviors in association with altered corticotropin-releasing hormone systems in the rat extended amygdala

Authors: *J. D. HEINZE[1], J. L. SCHOLL[3], J. M. KOPELMAN[6], K. KELLY[1], M. ARNOLD[1], K. J. RENNER[4], M. J. WATT[7], G. L. FORSTER[5], C. A. LOWRY[2]
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Abstract: Psychostimulant use and withdrawal increases anxiety-like behavior; one mechanism through which psychostimulant use and withdrawal may increase anxiety-like behaviors is through effects on central corticotropin-releasing hormone (Crh) systems in the extended amygdala. The current study examined whether chronic amphetamine treatment (2.5 mg/kg i.p. for 14 days) and two weeks of withdrawal increased anxiety-like behavior as measured using elevated plus-maze (EPM; 10 min) behavioral testing in adult male rats. In addition, a 2 (amphetamine treatment and withdrawal versus vehicle) x 2 (EPM exposure versus home cage control conditions) experimental design was used to assess the effects of treatment on c-Fos

expression in Crh-expressing neurons in the bed nucleus of the stria terminalis (BNST) and central nucleus of the amygdala (CeA), measured 2 h following onset of exposure to the EPM. Chronic amphetamine treatment followed by a two-week withdrawal period had anxiogenic effects as measured on the EPM. Amphetamine-treated rats had an increased latency to enter the open arms, decreased number of open arm entries, and decreased time spent in the open arms during EPM testing ($p < 0.05$ versus vehicle group; Student's t-test, N = 22; n = 11 for VEH group, n = 11 for amphetamine (AMP) group). The specific combination of amphetamine withdrawal and exposure to the EPM increased c-Fos expression in Crh-immunoreactive (ir) neurons within the BNST and CeA ($p < 0.001$, $p < 0.05$, respectively, versus AMP/-EPM group; BNST and CeA: $p < 0.001$ versus VEH/+EPM group, Fisher's least significant difference (LSD) tests; N = 41; n = 10 for VEH/-EPM group, n = 10 for VEH/+EPM group, n = 10 for AMP/-EPM group, n = 11 for AMP/+EPM group). These results are consistent with the hypothesis that expression of anxiety states associated with amphetamine withdrawal are driven by increased activity and reactivity of Crh systems within the extended amygdala.

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Poster

156. Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 156.17/VV8

Topic: F.04. Stress and the Brain

Support: NIH R01MH104373

Title: Noradrenergic activation of patterned inhibitory synaptic transmission in the basolateral amygdala

Authors: *X. FU, J. G. TASKER
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Abstract: The basolateral amygdala (BLA) is a central integrative component of the neural circuit for fear learning and anxiety. The concerted patterned population activity in the BLA has been shown to be critical for the encoding and retrieval of fear memory. It also has been well established that the release of norepinephrine (NE), a stress-associated neurotransmitter, increases in the BLA during stress and facilitates the consolidation of fear memories. Norepinephrine thus may modulate the activity of neuron ensembles to regulate BLA neural output and fear memory. Using whole-cell patch clamp recordings in amygdala slices, we found that NE induces two stereotyped patterns of bursts of inhibitory postsynaptic currents (IPSCs) in

BLA principal neurons through activation of presynaptic interneurons in an α1 adrenoreceptor-dependent manner. Dual recordings revealed that both types of IPSC bursts were synchronized between pairs of BLA principal neurons. Experiments with selective calcium channel blockers and cell-specific chemogenetic activation revealed that the two NE-induced patterns of IPSC bursting were generated by presynaptic inhibitory CCK- and parvalbumin-expressing interneurons. These findings suggest that NE may be able to synchronize BLA network activity by generating patterned inhibitory synaptic inputs from CCK and parvalbumin interneurons to the principal neurons. This work was supported by NIH R01MH104373.

Disclosures: X. Fu: None. **J.G. Tasker:** None.

Poster

156. Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 156.18/VV9

Topic: F.04. Stress and the Brain

Title: Voluntary exercise or systemic propranolol ameliorates maladaptive behavior following trauma in intact female rats

Authors: *C. CHRIST[1,2], M. M. CAHILL[1], S. J. ALDRICH[1], E. TAYLOR-YEREMEEVA[1], S. ROBINSON[1]
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Abstract: Fifty percent of the population will experience at least one traumatic event in their lifetime, with up to twenty percent developing PTSD or PTSD-like symptoms. Despite its prevalence, current treatment strategies for PTSD and PTSD-like symptoms are limited and substantial portions of affected individuals remain resistant to treatment. To address this the present studies investigated the effects of two underexplored therapeutic approaches, voluntary exercise and administration of the adrenergic beta-receptor antagonist, Propranolol, on trauma-related maladaptive behaviors. In Experiment 1, trauma was induced in 4 groups of female rats by exposing them to a modified version of the single-prolonged stressor paradigm that included anesthesia, restraint, forced swim, exposure to predator scent and tone-shock conditioning. Rats in the Exercise group were given a 4-week access to exercise wheels immediately after trauma. Rats in the Propranolol group underwent four days of re-exposure therapy that included Propranolol (5 mg/kg) administration. Rats in the Exercise+Propranolol group received both treatments. Control groups included untreated rats that were either exposed to trauma or were not. Trauma-associated maladaptive behavior was assessed using the elevated plus and open field mazes, the forced swim test and fear conditioning. Cognitive ability was assessed using a novel odor recognition task. A main effect of exercise on behaviors related to anxiety, depression and resilience was observed, but no main effect of Propranolol nor a Propranolol by exercise

interaction was observed. Neither trauma nor treatment influenced recognition memory. In contrast, in Experiment 2, in which the timing and dosage of Propranolol (0.25 - 2.0 mg/kg), and the number of re-exposure sessions were altered, Propranolol produced both a reduction in anxiety as well as resilience to a subsequent trauma. The results are consistent with reports of exercise-mediated relief for human female trauma victims. Furthermore, the findings support the view that in pre-clinical models, voluntary exercise, which bolsters hippocampal function and Propranolol, which affects amygdala-dependent memory reconsolidation and peripheral adrenergic signaling, can ameliorate PTSD-like symptoms.

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Poster

157. Emotion: Human Emotion II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 157.01/VV10

Topic: G.03. Emotion

Title: Aggressiveness and impulsive behaviors in different contexts in adolescence: How far can we expect them and when it goes beyond the limit?

Authors: *A. R. WILLHELM[1], A. S. PEREIRA[2], F. R. CZERMAINSKI[2], R. B. VOLPATO[2], R. M. DE ALMEIDA[3]
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Abstract: Adolescence is a phase of human development commonly characterized by diminishing self-control impulsive behaviors, which may make young people more susceptible to engaging in risky behaviors such as alcohol and drug use, aggressive behavior, and criminal activity (Spear, 2000). So, this period is also marked initiation and escalation of alcohol and drugs use, setting the stage for addiction (Spear, 2013). According to the dual system model, risk behaviors in adolescence are stimulated by a rapid and dramatic increase in dopaminergic activation within the social emotional system at this point in life, which increases the quest for reward (Steinberg, 2010). Studies have shown, however, that intense sports practice may be a protective factor for the use of alcohol and other drugs among adolescents, and there is no consensus in the literature on this subject. In this context, this study aimed to compare impulsivity, aggressiveness and alcohol and drug use in 115 male adolescents, aged 14 to 17 years: 1) juvenile offenders (n = 30); 2) regular students of public education (n = 30); 3) athletes of a soccer team (n = 30) and 4) students of militarized school (n = 25). The instruments used were: questionnaire about the beginning of drug use, Barratt impulsiveness scale-youth (BIS-youth) and State-Trait Anger Expression Inventory for children and adolescent (STAXI-CA).

The results indicated that the group of juvenile offenders had higher levels of anger feelings (inside anger and anger expression, p < 0.05), impulsivity and aggressiveness, in comparison to other groups. Although the groups did not differ in terms of alcohol experimentation, those who had already consumed alcohol presented higher scores of impulsivity and aggressiveness. Likewise, adolescents who had already tried drugs scored higher on the variables impulsivity for non-planning and not expressed anger (p < 0.05). In addition, intense sports practice was associated with a lower level of anger and studying in a militarized school was associated with lower motor impulsivity, compared to juvenile offenders. In conclusion, the results of this study demonstrated that both aggressiveness and impulsivity may be risk factors in youth, and when added to other factors, may increase the incidence of violent behavior, drug use and involvement with crime.

Disclosures: A.S. Pereira: None. **F.R. Czermainski:** None. **R.B. Volpato:** None. **R.M. De Almeida:** None.

Poster

157. Emotion: Human Emotion II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 157.02/VV11

Topic: G.03. Emotion

Support: German Research Foundation (DFG) WO733/15-1

Title: Cortisol promotes the neural network underlying cognitive emotion regulation

Authors: V. L. KINNER, C. J. MERZ, *O. T. WOLF
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Abstract: The ability to regulate emotions during or after stressful events is a major prerequisite to healthy psychosocial functioning. Acute stress and the concurrent release of neuroendocrine stress mediators, however, are believed to impair the functional integrity of prefrontal-based neural systems underlying emotion regulatory processes. Increased levels of the stress hormone cortisol, on the other hand, have been also related to mood-buffering effects and dispositional emotion regulation capacities. The present functional magnetic resonance imaging study therefore sought to explore the impact of cortisol on the neural correlates and subjective efficacy of two different emotion regulation strategies. To this end, 64 healthy men and women received either an oral dose of cortisol or placebo 90 min before they were asked to downregulate their emotional responses towards aversive pictures using cognitive reappraisal and distraction. Cortisol enhanced regulatory activity in the ventrolateral prefrontal cortex and at the same time reduced emotion-related activations in the amygdala and insula when participants applied cognitive reappraisal or distraction to downregulate their negative emotions. On the behavioral

level, cortisol further diminished the subjective experience of negative emotions in men, which was reflected by an overall increase of dorsomedial prefrontal cortex activity during reappraisal and negative picture viewing, whereas regulatory activation in this region was reduced after cortisol treatment in women. Together, these findings provide first evidence for a glucocorticoid-induced facilitation of cognitive emotion regulation processes that might be beneficial for restoring emotional stability in the aftermath of stressful events. The sex-specific cortisol effects on subjective emotional responses and prefrontal activity moreover suggest that sex and stress hormones interact in complex ways to modulate emotion regulatory processes which may ultimately translate into different vulnerabilities for stress- and mood-related disorders in men and women.

Disclosures: V.L. Kinner: None. **C.J. Merz:** None. **O.T. Wolf:** None.

Poster

157. Emotion: Human Emotion II

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Topic: G.03. Emotion

Support: Japan Society for the Promotion of Science [KAKENHI Grant Number JP15K19828]
Japan Society for the Promotion of Science [KAKENHI Grant Number JP15K09365]

Title: Resting-state networks associated with the sensory perception of dyspnea induced by effort breath

Authors: A. YORITA, T. KAWAYAMA, T. KINOSHITA, H. ODA, Y. TOKUNAGA, Y. SAKAZAKI, H. KIDA, T. HOSHINO, *T. TANIWAKI
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Abstract: Several studies have mapped brain regions associated with respiratory control and respiratory perception. However, its effect to resting state networks is unknown. Our objective was to determine the resting state networks during effort breathing. Resting-state functional magnetic resonance imaging (rs-fMRI) data was collected for 19 healthy volunteer with or without effort breathing. Using FMRIB Software Library (FSL) tools (Melodic ICA, dual regression, FSL Nets), we investigated the direct comparison between normal and effort breathing, and the correlation with modified Borg scale. Direct comparison showed no significant difference in neither intra-network connectivity nor inter-network connectivity during effort breath. Inter-network connectivity between the basal ganglia and the dorsal attention networks were significantly associated with more modified Borg scales, whereas inter-network connectivity between the insular and the visual networks was associated with less modified Borg

scales. These results suggest that the sensory perception of dyspnea induced by effort breath may associate with cortico-limbic circuity.

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Poster

157. Emotion: Human Emotion II

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Topic: G.03. Emotion

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Title: Music and emotion in Alzheimer's disease

Authors: *A. M. BELFI[1], A. RESCHKE-HERNANDEZ[2], E. GUZMAN-VELEZ[4], D. TRANEL[3]
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Abstract: Music listening has garnered media attention for its seemingly remarkable effects on patients with Alzheimer’s disease (AD). However, the extent to which music influences emotions in individuals with AD - either positively or negatively - remains unclear. We have shown in previous research that individuals with AD can experience long-lasting feelings of sadness and happiness after an emotion-inducing event (e.g., film clips), even when they cannot remember the event that triggered the emotion. Here, we similarly examined the effects of music listening on emotions in patients with AD. Participants consisted of patients with probable AD (N=20) and normal, healthy comparison participants (NC; N=20). Before beginning the study, participants provided musical pieces likely to induce happy and sad feelings (10 self-selected happy pieces, 10 self-selected sad pieces). During the experiment, participants first completed a set of brief emotion measures as a baseline. Next, participants listened to five minutes of self-selected “sad” music. Following this musical mood induction, participants completed the same emotion measures at three times post-induction. In addition to the emotion measures, participants completed recall and recognition memory tasks 10 minutes post-induction. This procedure was then repeated with the “happy” musical pieces. Electrodermal activity (skin conductance) was recorded during the experiment. Patients with Alzheimer’s disease showed impaired explicit memory for the music: Both recall and recognition memory scores were significantly lower in

the AD than the NC group. However, the AD group showed preserved signs of implicit memory for music: Emotion ratings were significantly higher than baseline for up to 30 minutes following the music listening, and skin conductance responses distinguished between previously heard and unheard pieces during the recognition memory task. This indicates that music can induce feelings in patients with AD that persist beyond explicit memory for the music itself. These results have important implications for the use of music listening as an intervention that could help improve the quality of life in patients with AD.

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Poster

157. Emotion: Human Emotion II

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Program #/Poster #: 157.05/VV14

Topic: G.03. Emotion

Support: JSPS KAKENHI 15K06731

Title: Functional MRI study of the human amygdala using multi-echo multi-band EPI at 7 Tesla

Authors: U.-S. CHOI[1,2], T. TANAKA[1], M. HARUNO[1,2], *I. KIDA[1,2]
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Abstract: Amygdala is one of the important brain regions regarding with emotional face process and it contains multiple nuclei. Although functional magnetic resonance imaging (fMRI) has been mostly used to investigate the function of amygdala in the human brain, the function in the sub-region of amygdala has not been fully elucidated by using fMRI at conventional magnetic field because of a low contrast to noise ratio (CNR) and signal to noise ratio (SNR). FMRI at ultra-high field (UHF) can provide a higher CNR and SNR, but the ventral region including amygdala is suffered from severe magnetic field inhomogeneity at UHF. To overcome the limitation, several studies have applied fMRI with multi-echo sequence at UHF. Here, we performed the multi-echo multi-band EPI sequence with a high temporal resolution (repetition time = 1000 ms) at 7 Tesla fMRI for an emotional face image discrimination task. Averaged echo images were calculated from three different echo images by using a weighted summed approach and general preprocessing were performed. The preprocessed data were registered to anatomical data acquired by magnetization-prepared rapid acquisition gradient echoes and normalized into MNI space for a group analysis. Masks of amygdala nuclei were applied to our normalized brain images and the time courses in the regions were extracted to estimate the event-related averaged blood oxygenation level-dependent responses. We found that the multi-echo multi-band EPI sequence with the amygdala sub-region mask approach at 7 Tesla obtained the

different temporal characteristics including initial-dip in the multiple nuclei of amygdala. The results suggested that it should be careful to apply the hemodynamic response function models to accurately estimate statistical parametric mapping using a general linear model in the amygdala regions.

Disclosures: U. Choi: None. **T. Tanaka:** None. **M. Haruno:** None. **I. Kida:** None.

Poster

157. Emotion: Human Emotion II

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Topic: G.03. Emotion

Support: Government of the Russian Federation (grant No 14.Z50.31.0027; E.L.G., Principal Investigator).

Title: Cross-modal emotion processing in children raised in institutional settings: An event-related potentials study

Authors: *I. OVCHINNIKOVA[1], A. DAVYDOVA[1], M. ZHUKOVA[1], M. PETROV[1], T. LOGVINENKO[1], I. GOLOVANOVA[1], S. KORNILOV[2,3,4], E. GRIGORENKO[1,2,3,4,5]
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Abstract: The lack of caregivers' resources or the provision of insufficient, impoverished input during early sensitive periods are associated with atypical developmental trajectories (Walker et al., 2007), an example of such conditions are baby homes. Early institutional experience affects both structural and functional development of the brain (Dobrova-Krol et al., 2008). Multiple developmental domains have been reported to be affected in this population, ranging from socio-emotional development to general cognition to language. Although published studies suggest that processing of emotional information communicated through facial expression is impaired in children with the early institutionalization experience (Nelson et al., 2013; Tarullo, Youssef, & Gunnar, 2016), to the best of our knowledge, cross-modal integration of emotional information using facial expression and prosodic information has not been investigated in this population. A total of 48 children in the age range from 20 to 40 months (M = 30, SD = 5.6) participated in the study - 24 individuals (M = 29,7; SD = 5,6; 11 boys) raised in institutional settings (IC) and 24 (M = 30,2, SD = 5,6; 13 boys) individuals raised in biological families (BF). The groups did not differ with respect to age or gender distributions. All children were raised in the only-Russian-speaking environment. We used the modified version of experimental paradigm proposed by Grossmann, Striano, & Friederici (2005) to elicit event-related potentials associated with cross-

modal emotional processing. The EEG data were collected as the participants were viewing photographs (Olszanowski et al., 2015) of female faces with three different emotional states (Happy, Angry, and Neutral) and listening to the pseudowords recorded by a native speaker with different prosody (Happy, Angry, and Neutral) at the same time. The data was recorded using the actiCHamp EEG amplifier with 64 active Ag/AgCl electrodes placed in a 10-20 montage cap. The signal was re-referenced to a common average, and conventional pre-processing procedures were used. Statistical analysis showed that the amplitude of the midline-central N4 component was expressed more in the BF group in the 400 - 600 ms in the congruent 'angry' condition, $F(42,1) = 6.174$, $p = .02$). Also, in the 90 - 250 ms timeframe after the 'happy' congruent stimuli BF group showed more negative amplitude in all three parietal clusters in comparison to IC group ($F(46,1) = 6.329, 4.003, 4.904$; $p = .02, .05$ and $.03$, respectively). Thus, the results indicate that children who had been institutionalized had a decreased neural response to different prosody stimuli than children raised in biological families.

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Poster

157. Emotion: Human Emotion II

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Program #/Poster #: 157.07/VV16

Topic: G.03. Emotion

Support: Programa de becas de posgrado CONACYT: Reg. 249807, LXCP

Title: Emotional discrimination of female caregivers of palliative cancer patients

Authors: *X. CORTIJO-PALACIOS[1], E. ACOSTA-MARI[2], B. REYES-BAEZ[3], E. DIAZ-DOMINGUEZ[4], A. ESCALANTE-VARELA[3], B. BERNAL-MORALES[5], T. CIBRIAN-LLANDERAL[6]
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Abstract: Objective: the purpose of this study was to examine the presence of stress, anxiety and depression and determine if there are differences in the assessment of images from the International Affective Picture System (IAPS) between female family caregivers (FC) of palliative cancer patients and a control group (CG). Participants and methods: two groups of 20 participants each. The FC was recruited at the Palliative Care Unit of the Doctor Miguel

Dorantes Mesa Cancer Center (CECan) in the State of Veracruz, Mexico (FC and CG; mean age 30) for CG; the age, gender and scholarship matched to the FC group. Assessment of psychological variables: we used the Perceived Stress Scale (PSS), Beck Anxiety Inventory (BAI) and Beck Depression Inventory (BDI) to identifying stress, anxiety and depressive symptoms respectively. Pictures task: the pictures were selected from IAPS database (Lang et al., 2008). The images were set in a power-point presentation and showed individually during 10 sec followed by 10 sec of transition between images. Emotional response (valence) and time to response (latency) was recorded immediately after watching each image using the Self-Assessment Manikin (SAM; Bradley et al., 1994). Results: the caregivers were in their youthful and active economic age, dominated by daughters (80%). 55% of caregivers crossed primary and secondary levels of education, the mean of time care was 35.6 months. 40% of caregivers had stress, 50% had moderate and severe anxiety levels and 70% had moderate and severe depression levels. We found significant differences of the dimension of valence between FC and CG groups in the 5% of slides numbered pictures evaluated: 4559 (p=0.018); 4647 (p=0.049); 4694 (p=0.023); 5836 (p=0.046), Mann-Whitney U test. In addition, significant differences were found in the latency evaluation between the groups in the images: 1675 (p=0.024); 7055 (p=0.027); t-test. Conclusions: The findings demonstrate that despite the presence of stress and symptoms of anxiety and depression, coupled with the time of care and the confliction of being caregivers of palliative patients, there is no exists differences in the processing of emotional content between both groups.

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Poster

157. Emotion: Human Emotion II

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Topic: G.03. Emotion

Support: CONACYT Grant 1840
CONACYT Grant 445171

Title: Self-assessment scale proposal for the measurement of emotional recognition

Authors: *T. CIBRIAN-LLANDERAL[1], O. ROMERO-MOLINA[2], M. CADENA-BARAJAS[2], T. C. CIBRIAN-LLANDERAL[3,2], A. CARDOSO-VILLEGAS[3]
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Abstract: Introduction: Self-assessment methods are employed in emotion research for the collection of subjective affective ratings. The Self-Assessment Manikin (SAM), is still among the most popular self-reporting tools. In collaboration with graphic design experts, we developed a Pictographic Scale (PS), for the measurement of pleasure. Is a self-reporting tool composed of graphic face characters horizontally arranged, according to a 9- point scale. Objective: The aim of the study consisted of systematic comparison between our PS and SAM ratings (mexican validation from Madera-Carillo et al., 2015) collected through a task that involved the emotional assessment of a series of images taken from the International Affective Picture System (IAPS), a standardized database containing pictures representing a wide range of semantic categories. Participants and Methods: We designed an experiment where 268 students from two different universities were asked to rate two sets of 60 pictures from IAPS. The images were set in a power- point presentation and presented individually during 5 sec following by 5 sec of transition between images. Emotional response was recorded immediatly after watching each image using the PS or the SAM in the measurement of pleasure. Participants were equally assigned to one of the two experimental conditions. The statistical analysis included descriptive statistic and Wilcoxon rank-sum test, using R program. Results: Mean age was 20.28 years (SD=1.96), 67% were female and 33% male. It was found that all the images showed p-values > 0.05, therefore, there is no statistical evidence to suggest that the hypothesis of equality between the values of the medians reported by previous studies and the data obtained in this investigation should be rejected. Conclusion:Our results show that de PS is equivalent to SAM in the self-assesment of pleasure with the advantage of a novel design with more understandable expression features.

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Poster

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Topic: G.03. Emotion

Support: NIH Grant MH113347

Title: LPFC representations support goal-oriented responses during emotional processing

Authors: *R. C. LAPATE, M. HECKNER, J. MARTIN, J. WU, M. D'ESPOSITO
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Abstract: The ability to override reflexive responses to emotional events and respond in a context-appropriate manner is paramount to mental health and wellbeing. While abstract rule

representations in mid-lateral prefrontal cortex (LPFC) have been suggested to support the implementation of context-guided behavioral responses in cognitive control tasks (e.g. Cole, Ito & Braver, 2015), it is unclear whether and how goal representations in LPFC similarly promote behavioral regulation in the face of prepotent emotional events. Here, we ask whether LPFC representations of task rule during the processing of emotional stimuli are associated with successfully overriding emotional-stimulus driven behavioral responses. In addition, we examine whether the strength of task-rule representations in LPFC and behavioral performance relied on LPFC functional interactions with amygdala circuitry. To do so, in the fMRI scanner, human subjects (18-30 y old, 56% female) performed an event-related Affective Go/No-Go task, which captured a prepotent, approach/withdrawal bias in behavior as evidenced by faster reaction times (RTs) in response to positive compared to negative stimuli (happy vs. fearful faces). We applied a linear classifier to multivoxel patterns of activation in prefrontal cortex, and found that the cross-validated decoding accuracy of task rule exceeded chance in mid-LPFC. Moreover, the magnitude of task-rule classifier performance in mid-LPFC was associated with reduced emotional-stimulus driven bias in behavior across individuals, i.e., smaller Δ [Negative − Positive] RTs. Finally, greater mid-LPFC decoding of task rule and reduced bias in behavior were associated with greater functional connectivity between the amygdala and mid-LPFC as well as ventrolateral PFC during the processing of negative (compared to positive) stimuli. Collectively, these results suggest that task-relevant goals dynamically represented in mid-LPFC interact with the amygdala to promote goal-oriented behavior in the face of emotional events, shedding light on how LPFC function contributes to adaptive emotional responding.

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Poster

157. Emotion: Human Emotion II

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Program #/Poster #: 157.10/VV19

Topic: G.03. Emotion

Support: University of Minnesota UROP

Title: Sexually dimorphic autonomic responses to affective manipulation as reflected by heart rate variability

Authors: R. J. HJELLE[1], J. HAMBRICK[1], *R. L. LLOYD[2]
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Abstract: Porges' Polyvagal Theory postulates that one vagus nerve nucleus (nucleus ambiguous) interacts with brain stem nuclei regulating facial musculature. Porges postulates a

face-heart connection which has evolved in mammals with emergent properties of a social engagement system. This would result in social interactions regulating visceral states (2009). The majority of axons of the vagus nerve are ascending and sensory in nature, with information rapidly proceeding to limbic tissue (per vagus nerve stimulation for depression). Previous research finds that differential oral-buccal articulation mimicking smiles and frowns influenced affective responses to humorous material.

Presently, subjects' reactions to two two-minute humorous videos (previously judged by an independent sample to be of equivalent humor) were assessed while the subject was holding a pen in their mouth, either between the teeth or between the lips, counter balanced for order. Following each video, subjects rated the humor of the video on a 9 point scale. The subject's EKG and GSR were recorded during each video recording, as well as during each of two preceding baseline intervals. Three minutes elapsed between exposure to the first video and recording of the second baseline.

There was a differential parasympathetic response to humorous videos as a function of sex. Parasympathetic tone, as reflected in the PNN50, significantly and strongly predicts reactions to the humorous material in women but not men ($r^2 = 0.367$, $p = 0.027$). In addition, Levenson Self-Report Psychopathy Scale Scores (LSRP, 1995) are inversely related to the subjective response to humorous material in females ($r = -0.397$, $p = 0.0465$, 1-tail). In females there was a significant elevation in parasympathetic tone in response to oral-buccal articulation involving the lips but not the teeth ($p = 0.045$, $\eta^2 = 0.21$). In general across conditions, females were found to have higher parasympathetic tone. Findings as varied as measures of psychopathy (Levenson) and responses to stress (Taylor, 2001) indicate that women have a greater propensity towards social interaction. The present data are consistent with the social engagement implications of the Porges Poly-Vagal Theory, and our sexually dimorphic data are consistent with that of Levenson and of Taylor.

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Poster

157. Emotion: Human Emotion II

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Program #/Poster #: 157.11/VV20

Topic: G.03. Emotion

Support: NIH Grant MH103324

Title: Altered neural regulation of emotional conflict in resilient trauma-exposed individuals

Authors: *C. A. CORNELSSEN, R. N. WRIGHT, C. DE LOS ANGELES, A. NRUSIMHA, A. TULSEJA, J. JIANG, C. MILLS-FINNERTY, R. EDELSTEIN, B. ROSENBERG, Y.

ZAIKO, A. ETKIN
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Abstract: Most literature on the neural effects of trauma exposure has been conducted on Post-Traumatic Stress Disorder (PTSD) patients. The executive function (EF) and emotion regulation (ER) circuits have been shown to be dysregulated in PTSD patients (Etkin and Wagner, 2007), and have been implicated in resilience in healthy trauma-exposed individuals (Caston and Mauss 2011). Differences in ER and EF activity between non trauma-exposed healthy individuals (NTHCs) and trauma-exposed healthy individuals (TEHCs) remain largely unexplored. Our work explored the EF/ER circuits for NTHCs and TEHCs by looking at ROIs known to be within the circuits in an emotional conflict fMRI task.
34 NTHCs and 26 TEHCs, all right-handed, matched on education (p =0.947, n.s.), age (p=0.3042, n.s.), gender (p = 0.5221, n.s.), underwent functional magnetic resonance imaging (fMRI), while performing an emotional conflict stroop task (Etkin et al., 2006). In this task, participants indicate whether a face is fearful or happy while attempting to ignore an overlaid word that is either congruent or incongruent with the expression. An incongruent trial preceded by another incongruent trial is shown to elicit less conflict (and thus high conflict resolution) than if it is preceded by a congruent trial (more conflict and low conflict resolution). Brain regions involved in conflict resolution, such as the dACC, insula, and dlPFC, are less active in conditions of high conflict resolution, and more active for low conflict resolution.
We found significantly less activation in TEHCs compared to NTHCs of the R dlPFC (p=0.004*) and L dlPFC (p=0.04*) for the low>high conflict resolution task contrast.
These results indicate an interesting finding for the implications for trauma exposure on emotion regulation, even as it is unrelated to the development of psychiatric symptoms. This can help to understand the differences between ER/EF circuits in resilient and non-resilient individuals. Future research in this area will examine these differences in non-trauma exposed and trauma-exposed patient populations. Clinical interventions for PTSD can be created that will target these circuits specifically, resulting in more effective treatments.

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Poster

157. Emotion: Human Emotion II

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Program #/Poster #: 157.12/VV21

Topic: G.03. Emotion

Title: Dynamic modeling and brain decoding of internal thoughts and emotions

Authors: *B. E. KIM[1], C.-W. WOO[2]
[1]Ctr. for Neurosci. Imaging Res., Suwon-si, Gyeonggi-do, Korea, Republic of; [2]Univ. of Colorado Boulder, Boulder, CO

Abstract: Internal dynamics of thoughts, memories, concepts, and feelings are an essential part of one's self-identity and personality. However, no tasks and brain models are currently available for the study of these internal dynamics. Here we developed a novel task, named the Free Association Semantic Task (FAST), for functional magnetic resonance imaging (fMRI) experiment with the aim of developing brain decoding models for internal dynamics of spontaneous thoughts and emotions. The task consists of three parts: We first ask participants to report 40 consecutive words that came to their mind when thinking of the previous word, starting from a given seed word. We collect total 160 self-generated words from four experiment runs. Second, we show the self-generated words one by one and ask to think about their personal meaning while recording their brain activity. After the scan, we show the words again and ask to rate their valence, self-relevance, time, vividness, and safety/threat. The behavioral results (N = 63) using a Markov chain model show that the probability of generating negatively valenced concepts is strongly predictive of individual difference measures of general negative affect. We also develop fMRI-based brain signatures for multiple dimensions of internal concepts and their dynamics using single-trial level brain activation maps, which could then be used to decode individual's spontaneous thoughts and emotions during resting. This study can contribute to the understanding of how internal cognitive and affective contexts are represented in the brain and provide new predictive tools for mental health and clinical use.

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Poster

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Topic: G.03. Emotion

Title: Facial emotional expression as a predictor of moral decision making

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Abstract: Emotions are critically involved in the way we interact with each other and drive our choices. Previous research has shown that manipulating emotions influences the way in which we make moral judgments. Emotions are also quite specific, studies conducted in the past have

shown that different kinds of moral transgressions elicit different kind of emotions on the spectator in a systematical way. An altered emotional reactivity has been linked to emotion processing deficits and failure to comply with social conventions and moral norms. This leads us to think that emotional reactivity might be playing an instrumental role in driving moral behavior.
In this study we will explore the link between emotional reactivity, the type of emotion expressed by participants while reading a moral dilemma, and their choice on the same task. To do it, participants were presented with a standardised set of 26 moral dilemmas, each one depicting a different kind of moral conflicting situation in which they were asked to make a choice. Simultaneously, their facial expressions were recorded and analysed with a facial emotion recognition software that followed the Facial Action Coding System (FACS) proposed by Ekman and Friesen (1978) to classify their emotions.
We found that participants who showed a stronger emotional reactivity when reading the dilemma, tended to choose the less harm inflicting options when harm was done in an indirect way. But, chose the greater harm over the direct harm option (deontological choice), in comparison to participants with less emotional reactivity, who did not show show this trend. Our findings go in accordance with other studies that have shown a direct influence of emotional states in moral choices, here we show that emotional reactivity modulates moral choices during a moral dilemma task. This results contribute to the ongoing debate about the possible regulatory role of emotions during moral behavior; and add up to the hypothesis that emotions are critically involved in moral judgements, and may be instrumental in deterring transgressions and promoting prosocial behavior.

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Poster

157. Emotion: Human Emotion II

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Program #/Poster #: 157.14/WW1

Topic: G.03. Emotion

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NRF Korea Grant 2017M3C7A1041823

Title: Reduced empathy associated with medical experience is perspective-dependent

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Abstract: Empathy, the ability to share and understand feelings of others, is pervasive and naturally occurring human experience. Previous studies showed that empathic responses can be modulated by various factors such as dispositional traits and personal experience. Especially experts who are required, by the nature of their work, to cognitively control empathic responses often report reduced empathy. Less is known, however, whether this empathy reduction would depend on perspective-taking context. This study investigated how medical and nonmedical students' empathic neural responses differed depending on perspective-taking instructions. Nineteen medical students who completed the third year medical curriculum and twenty-three non-medical matching student controls participated in this functional neuroimaging study. Inside the scanner, participants read a series of written scenarios of three sentences depicting interactions between a doctor and a patient. Two versions of scenarios existed: an empathy version (Emp) describing an interaction between a suffering patient and a careless doctor, and a neutral version (Neu) describing nonemotional interaction between a patient and a doctor. Participants were asked to read the scenarios either from the perspective of the doctor (Doc) or the patient (Pat) with equal frequency. Three sentences of each scenario were presented sequentially, one at a time, which respectively lasted 7 s, 7 s and 8 s. Then participants rated the valence of emotion elicited by each scenario on a Likert scale ranging from -7 (very positive) to +7 (very negative) for 6 s. Behavioral results indicated that, overall, participants felt worse for empathy scenarios relative to neutral scenarios. Neuroimaging results revealed that, relative to controls, medical students showed decreased activity in empathy related neural regions including the superior and middle temporal gyrus, inferior parietal gyrus, supramarginal gyrus, and inferior frontal gyrus when reading empathy scenarios vs. neutral scenarios from the perspective of a patient. Whereas, two groups showed no differences in neural activation when reading scenarios from the doctor's perspectives. These results indeed indicate that medical experience can reduce empathic responses but, more interestingly, we show that this reduction was perspective dependent.

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Poster

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Topic: G.03. Emotion

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the National Program for Support of Top-notch Young Professionals

Title: The effect of transcranial direct current stimulation over The right temporal parietal junction on microexpression training

Authors: *R. SU, Y. GE, C. LIU
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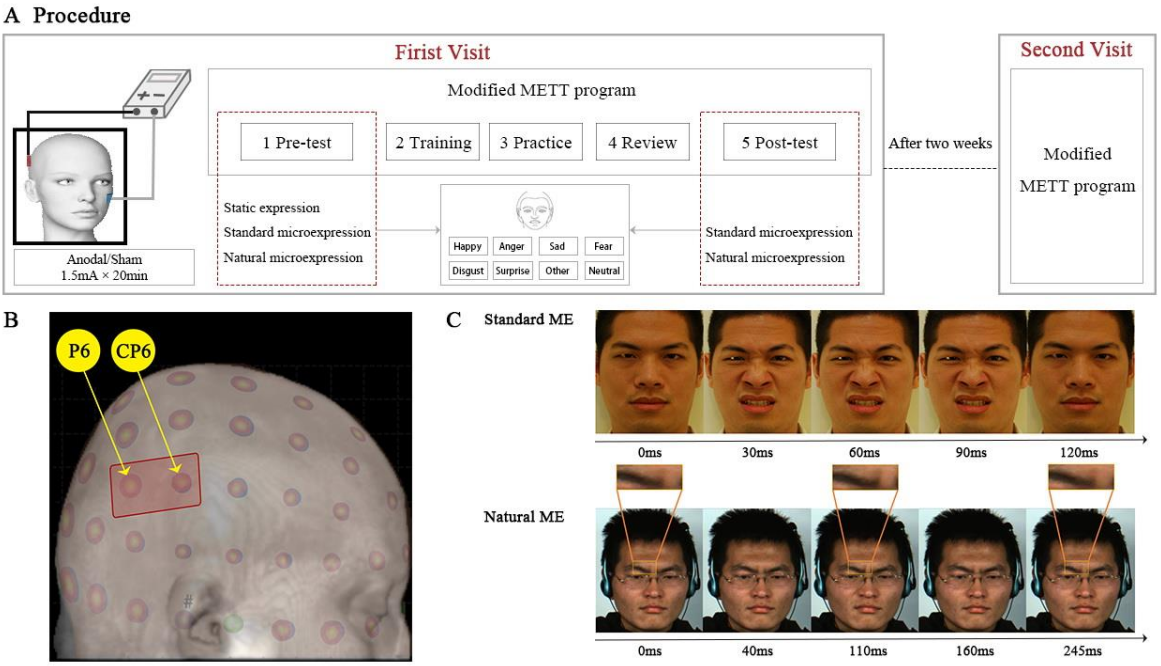
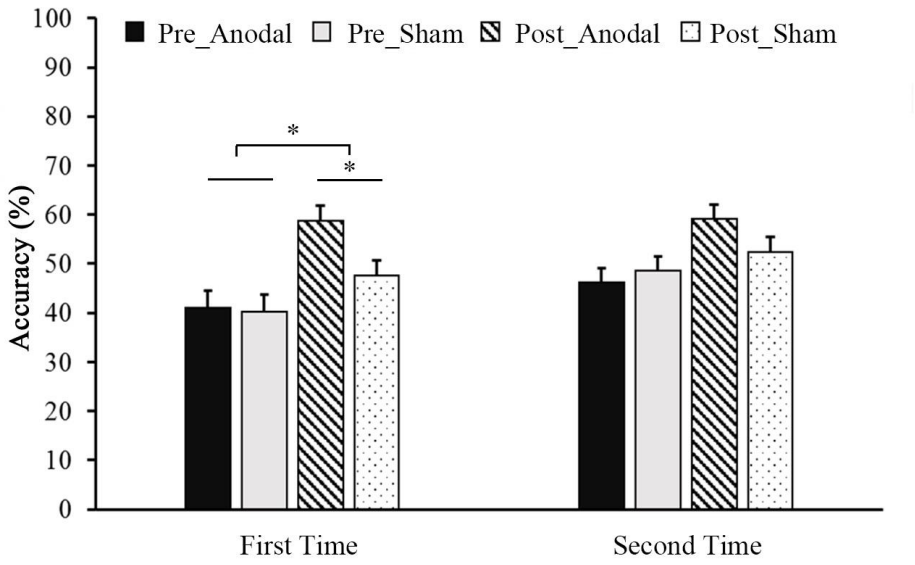
Abstract: Microexpression recognition as a rare but trainable skill can improve interpersonal interaction and social communication that attracts public attentions. Previous imaging studies suggested that the right temporal parietal junction (rTPJ) activity was closely associated with the explicit process of the facial emotion recognition which was emphatically trained in the METT (microexpression training tool) program. To explore the effect of tDCS over the rTPJ on the microexpression training and whether the influence on the standard and natural microexpressions was different, 58 participants were randomly assigned into two groups, where 30 participants received real tDCS (1.5 mA for 20 min) and 28 participants received sham stimulation (1.5 mA for 30 s). All participants made two visits. During the first visit, they accepted corresponding stimulation according to different groups, then completed the modified METT paradigm and finally finished the empathy assessment with the Interpersonal Reactivity Index (IRI) questionnaire. After two weeks, they only accomplished the modified METT paradigm during the second visit. We found that both standard and natural microexpression recognition could be improved by METT in two visits. Anodal stimulation over the right TPJ only enhanced the training effect of the natural microexpressions, particular for the fear natural microexpressions. These findings may provide another intervention to treat fear expression identify deficits.





Disclosures: R. Su: None. **Y. Ge:** None. **C. Liu:** None.

Poster

157. Emotion: Human Emotion II

Location: SDCC Halls B-H

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Program #/Poster #: 157.16/WW3

Topic: G.03. Emotion

Title: Emotional faces incongruity interferes with word affective evaluation

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Abstract: Everyday people express emotions, both, through verbal and non verbal language, which must be congruent in order to transmit a clear message. Emotional incongruity between both modalities could affect information transmission and therefore social interaction. The aim of this study was to evaluate the effects of emotional faces and affective words incongruity in the amplitude of N2 and P3 components. Eighteen women performed a pleasant and unpleasant word evaluation task, at the presence of congruent or incongruent happy and angry faces; scrambled faces were used as a neutral condition. Behavioral results showed that response times were longer and, accuracy was lower for the incongruent compared to the congruent condition.

N2 amplitude at anterior locations for both congruent and incongruent conditions was lower than for the neutral condition; meanwhile P3 amplitude for the congruent condition was larger than the neutral condition at posterior locations. These findings suggest that the implicit processing of an emotionally incongruent face, difficult the evaluation of the affective value of a word at different processing stages.

Disclosures: V. Chavez: None. **J. Ramos-Loyo:** None.

Poster

157. Emotion: Human Emotion II

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Program #/Poster #: 157.17/WW4

Topic: G.03. Emotion

Title: Comparison of automated and manual systems for coding pain-related facial expressions

Authors: *J. QUINDE[1], M. D. FAILLA[2], P. A. DEPALMA[1], C. J. CASCIO[2]
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Abstract: Facial expressions are a transient but quantifiable form of nonverbal communication that can be used to interrogate more stable characteristics like personality traits. The amygdala and midcingulate cortex are two subcortical structures thought to be involved in facial expression production and perception. Exploring emotion through facial expression behavior also provides an indirect and non-invasive method to assess emotion processing. With the advent of automated facial coding (AFC) software, emotion research has exponentially increased in popularity, with applications ranging from clinical to marketing research. Manually-coded facial action coding system (FACS) is considered a 'gold standard' but is very time-consuming and laborious. Rapid developments in computer science have advanced AFC reliability and accessibility and recent findings suggest that this technique is a promising tool for developing tailored treatments targeting emotion processing deficits in clinical populations. However, to successfully employ AFC tools in place of FACS, there needs to be a standard for agreement between the two systems. To this end, a handful of studies have explored matching between the *de facto* measurement of emotional facial expression (FACS) and its AFC competitors. These studies focus on matching FACS emotion labels using multiple algorithms and have found that some engines perform better or worse depending on the emotion involved. However, matching emotion labels is a coarse level of analysis. Facial emotions can be broken down into action units (AUs) that reflect activity of individual facial muscles. To date, there has not been a study investigating agreement at the more basic level of AUs. The purpose of this study is to validate Affectiva's Affdex (2009) engine using a database on pain facial expressions, the UNBC-McMaster Shoulder Pain Expression Archive Database. While the FACS system codes AU intensity on a 0-5 scale, the Affdex engine assigns continuous likelihood scores ranging from 0-

100. To assess agreement between AU labels for each image across the two platforms we applied percentile thresholds to continuous data generated by Affdex. Preliminary results suggest that agreement depends on which percentile threshold (50,70,90,99th) is applied to the Affdex scores. Matching for scores in the 50^{th} percentile is lower than for 70^{th} percentile thresholding while agreement is greater for the extreme threshold (99%). In efforts of standardizing AFC and FACS comparisons, matching scores at these extremes indicate that a balance between allowing false positives (50^{th}) and false negatives (99^{th}) in Affdex scores need to be further explored.

Disclosures: J. Quinde: None. **M.D. Failla:** None. **P.A. DePalma:** None. **C.J. Cascio:** None.

Poster

157. Emotion: Human Emotion II

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Program #/Poster #: 157.18/WW5

Topic: G.03. Emotion

Support: Swerve Fitness

Title: Exercise-induced changes in delta, theta, alpha, and beta frequencies predict improvements in general positive affect

Authors: *J. C. BASSO[1], C. E. O'BRIEN[1], C. CROSTA[1], A. DAS[1], S. SEWNAUTH[2], E. NEAL[2], J. ELLEN[2], D. J. OBERLIN[1], W. A. SUZUKI[1]
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Abstract: Exercise causes a range of morphological, physiological and neurochemical changes in the brain including increases in neurogenesis, angiogenesis, synaptogenesis, long-term potentiation, and growth factors including BDNF. These exercise-induced brain changes also result in a range of behavioral outcomes, with the strongest and most consistent benefit on affective state. However, few studies to data have examined how exercise-induced changes in brain state relate to the changes in affect. Here, we not only focused on the mood outcomes of a 3-month exercise regime, but analyzed the prominent mood shifts relative to changes in baseline electroencephalography (EEG) data. Previously sedentary and low-fit subjects were recruited from the New York City area. Subjects were randomized into a sedentary, control group (n=17; video game playing) or an exercise group (n=27; indoor cycling classes). Participants engaged in 3, 45-minute sessions per week for 3 months. Before and after the 3-month intervention, subjects were tested on aerobic capacity (VO_2 max) as well as a range of self-reported affective measures. In addition, baseline brain activity was captured during 10 minutes of both an open- and closed-eyes electroencephalography recording session; time-frequency analysis was conducted using Matlab and EEGlab. Compared to a sedentary experience, long-term exercise improved affective

behavior in three significant ways: 1) by increasing the motivation to exercise, $F(1,26)=8.261$, $p=0.008$, partial $\eta^2=0.241$; 2) by decreasing negative body attitudes, $F(1,26)=4.456$, $p=0.045$, partial $\eta^2=0.146$; 3) and by increasing general positive affect, $F(1,26)=5.268$, $p=0.030$, partial $\eta^2=0.168$. In addition, long-term exercise significantly increased relative power in delta (1-4 Hz; $t(38)=3.948$ $p<0.001$), theta (4-8 Hz; $t(38)=8.455$, $p<0.001$), and alpha (8-12 Hz; $t(38)=7.882$, $p<0.001$) frequencies, but decreased relative power in the beta (12-35 Hz; $t(38)=-7.889$, $p<0.001$) frequency. Finally, the exercise-induced change in delta ($R=0.419$, $p=0.046$) and theta ($R=0.506$, $p=0.014$) power was positively associated, and the decrease in beta ($R=-0.549$, $p=0.007$) power was negatively associated with the change in general positive affect. Multiple regression indicated that exercise-induced changes in delta, theta, alpha, and beta frequencies predicted the improvement in general positive affect, $F(4,18)=2.943$, $p=0.049$, adj. $R^2=0.395$.

Disclosures: J.C. Basso: None. **C.E. O'Brien:** None. **C. Crosta:** None. **A. Das:** None. **S. Sewnauth:** None. **E. Neal:** None. **J. Ellen:** None. **D.J. Oberlin:** None. **W.A. Suzuki:** None.

Poster

157. Emotion: Human Emotion II

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Topic: G.03. Emotion

Support: NIH/NIA R01AG057204
NIH/NIA R01AG052496
NIH/NIA K23AG040127

Title: Apolipoprotein ε4 carrier status impacts affective and cognitive empathy

Authors: *T. E. CHOW[1], I. J. SIBLE[1], E. KOSIK[1], S. DATTA[1], J. S. YOKOYAMA[1], A. KARYDAS[1], K. P. RANKIN[1], J. H. KRAMER[1], G. COPPOLA[2], B. L. MILLER[1], W. W. SEELEY[1], V. E. STURM[1]
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Abstract: The Apolipoprotein ε4 (*APOE*E4*) allele confers one of the greatest genetic risks for developing Alzheimer's disease, with some estimates indicating that a single copy of the allele can increase the likelihood of disease by three to four times relative to non-carriers (Bertram & Tanzi, 2008). While patients with Alzheimer's disease often demonstrate emotional symptoms (Spalletta et al., 2010) and alterations in empathy (Dermody et al., 2016; Sturm et al., 2013), it is unknown whether cognitively healthy *APOE*E4* carriers may differ from non-carriers in this regard. This study was conducted to help address whether *APOE*E4* relates to individual differences in empathy in a sample of healthy, cognitively normal older adults. A total of 374

cognitively healthy older participants, including 89 *APOE*E4* carriers with either one or two copies of the allele (mean 69.8 years old; 45 females) and 285 *APOE*E4* non-carriers (mean 70.3 years old; 167 females), were recruited from the UCSF Hillblom Healthy Aging Network. All participants underwent a multidisciplinary diagnostic evaluation, which included neuropsychological testing and an assessment of daily functioning, and were determined to be cognitively normal. Informants completed the Interpersonal Reactivity Index (IRI), a multidimensional measure of empathy, and rated participants' current levels of cognitive and affective empathy (Davis, 1983). The Empathic Concern (EC) and Personal Distress (PD) IRI subscales measure affective empathy, the ability to experience others' emotions, while the Perspective Taking (PT) and Fantasy (FS) IRI subscales measure cognitive empathy, the ability to understand others' perspectives. Analyses conducted on participants' IRI scores revealed significant differences in empathy between *APOE*E4* carriers and non-carriers. *APOE*E4* carrier status predicted measures of EC, PT, and FS (all p-values < 0.05) such that *APOE*E4* carriers had significantly lower affective (EC + PD subscales; $p = 0.02$) and cognitive (PT + FS subscales; $p < 10^{-2}$) empathy than *APOE*E4* non-carriers. Whereas in *APOE*E4* non-carriers cognitive empathy was greater than affective empathy ($p < 10^{-5}$), in *APOE*E4* carriers there was no such differentiation ($p = 0.35$) and both forms of empathy were low. Taken together, these results suggest that *APOE*E4* plays a role in socioemotional behavior and that studies of empathy may offer new inroads into the very early stages of Alzheimer's disease.

Disclosures: T.E. Chow: None. **I.J. Sible:** None. **E. Kosik:** None. **S. Datta:** None. **J.S. Yokoyama:** None. **A. Karydas:** None. **K.P. Rankin:** None. **J.H. Kramer:** None. **G. Coppola:** None. **B.L. Miller:** None. **W.W. Seeley:** None. **V.E. Sturm:** None.

Poster

157. Emotion: Human Emotion II

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Program #/Poster #: 157.20/WW7

Topic: G.03. Emotion

Support: Internal Support; Hope College Frost Grant

Title: A hopeful heart: The impact of hopeful imagery on heart rate variability and emotion

Authors: *L. ROOT LUNA[1], C. V. O. WITVLIET[2], F. J. RICHIE[3], N. BERNAL[2]
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Abstract: Past research has identified a relationship between self-regulatory processes and parasympathetic nervous system function (Geisler, Vennewald, Kubiak, & Weber, 2010). Like self-regulation, the emotion of hope involves motivation, finding pathways toward a goal, and practicing self-control (Vohs & Schmeichel, 2002). Although hope has received attention for its

mental health benefits (Ai, Peterson, Tice, Bolling, & Koenig, 2004), its connections with peripheral physiology have not been explored experimentally. In this study, we examined the impact of hopeful thinking on cardiovascular, psychological, and linguistic indicators.
Sixty undergraduate students (25 males, 35 females; age M = 19.5, SD = 0.99) participated in an incomplete repeated measures experiment with three imagery conditions. Participants first thought about a specific, personal hoped-for outcome (e.g., academic, professional, relational) and then imagined their hoped-for outcome being fulfilled, remaining unfulfilled, and a neutral control condition. Changes in blood pressure, heart rate, respiration, and high-frequency heart rate variability (HF-HRV) were analyzed. Following each trial, participants completed self-report measures of hope, flourishing, and subjective emotion.
As expected, hope-fulfilled imagery was positively related to positive emotions (joy, gratitude, peace) and inversely related to negative emotions (sadness, anger), ps < .001. Participants also reported greater levels of flourishing in the hope-fulfilled condition. Linguistic analyses indicated that participants used more hopeful, social, positive emotion, and benefit-finding language in the hope-fulfilled condition (ps < .004). The main effect of imagery condition was not statistically significant for the physiological measures. However, an interaction of trait hope and imagery condition revealed that for participants with high trait hope, HF-HRV was negatively impacted by the hope-unfulfilled condition (p = .018).
Results demonstrated that hopeful thinking prompted increased state hope, flourishing, positive mood, and positive language whereas hopeless thinking induced opposite effects. Furthermore, hopeless thinking came with a cardiac cost (i.e., decreased HF-HRV) for people with high trait hope. Provided that goals are appropriate, imagining hopes fulfilled yields emotional benefits and buffers cardiac response for high hope people.

Disclosures: L. Root Luna: None. **C.V.O. Witvliet:** None. **F.J. Richie:** None. **N. Bernal:** None.

Poster

157. Emotion: Human Emotion II

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Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 157.21/WW8

Topic: G.03. Emotion

Support: NIH MH108705

Title: The effects of cortisol administration on emotion, stress reactivity, and brain activity in depression

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Abstract: Introduction:Circulating cortisol levels rise when people experience a variety of physical and psychological stressors. Cortisol levels are also elevated and dysregulated in individuals experiencing symptoms of depression. It is widely assumed that cortisol plays a role in generating the subjective feeling of emotions that accompany both stress and depression. It is also possible that cortisol is elevated in stress and depression to coordinate physiological processes that are unrelated to subjective feelings. The assumptions that cortisol promotes feelings of stress and symptoms of depression have rarely been formally tested. This series of studies aims to test how cortisol administration influences the subjective experience of emotion and the neural correlates of emotion it healthy individuals and medication-free individuals experiencing symptoms of depression. MethodsThis series of studies was structured as a double-blind placebo-controlled crossover study. Participants were randomly assigned to the 0.65mg/kg oral hydrocortisone (cortisol) condition or a matched placebo pill on the first study day and vice-versa on the second study day. Participants 1) rated their emotional reactions to emotion-inducing pictures/videos 2) rated their emotional reactions to undergoing the socially evaluated cold-pressor test (SECPT) 3) completed a fMRI scan to quantify the subgenual cingulate response to sadness. Participants included 33 healthy individuals and 17 individuals experiencing symptoms of depression (BDI-II, range 16-47).ResultsCortisol administration did not produce robust effects on 1) the emotional response to emotion-inducing pictures/videos 2) the emotional responses to the SECPT. However, cortisol did produce reductions in sadness-induced subgenual cingulate activity.DiscussionThese data suggest that cortisol may not be responsible for generating the subjective feelings of stress or depression. Instead, cortisol may be modulating brain activity patterns that do not directly impact subjective emotional reactions.

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Poster

157. Emotion: Human Emotion II

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Program #/Poster #: 157.22/DP10/WW9

Topic: G.03. Emotion

Title: The role of accessible and inaccessible rewards in eliciting emotional states such as happiness and anger

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Abstract: While it seems intuitive that the achievement or non-achievement of goals would result in different emotions being elicited, a systematic model based approach to investigate this link is incomplete. One popular approach is to investigate the correlation of prediction errors

with emotional states. However, prediction errors, defined as the difference between the predicted and actual rewards, are only meaningful at those states and/or time instants at which a reward is predicted to be present. Moreover, most of these studies consider only positive and negative valence as a proxy for emotions. The objectives of the research are (1) to develop an experimental paradigm in the form of an interactive game, whereby emotional states such as anger and happiness are readily elicited and (2) to model the actions using an artificial agent to gain insights into the factors underlying the elicitation. We develop an experimental paradigm in which 50 participants (16 female) control the movement of an agent in a 2d environment with a number of obstacles and goal states, such that some goals are accessible whereas others are not. Initially the agent 'sees' only a part of the environment, predefined by a radius of vision around the agent, and as the agent moves additional states are revealed. A variety of different environments are constructed with differing number of obstacle and goal states. At the end of each environment the participant is asked rate his/her happiness and anger using an emoji scale. We observe that anger is rated significantly higher in environments with all goals being inaccessible, followed by a majority inaccessible, followed by a minority inaccessible. The actual number of accessible and inaccessible goals appears to be irrelevant for the anger rating. However, the number of goals is a significant factor for the happiness rating, with accessible goals having a positive influence and inaccessible negative. We further develop an iterative Q-learning reinforcement learning model, with obstacles having a negative reward, goals a large positive reward, and unexplored states a small positive reward. The change in value function at every step the agent takes is computed. We propose that the mean of the change of the value function could be one of the underlying factors governing emotional responses. On normalizing with respect to the maximum value and averaging across all moves the agent makes, we observe a similar trend as the experimental results for the different scenarios. These results suggest that an individual's internal perception of changes in value of the environment could be the basis for emotion elicitation.

Disclosures: R.T. Philips: None. **X. Gu:** None.

Poster

158. Emotion: Positive and Negative Emotional States

Location: SDCC Halls B-H

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Program #/Poster #: 158.01/WW10

Topic: G.03. Emotion

Support: DARPA Grant W911NF-14-2-0043

Title: Decoding natural, spontaneous emotional behavior from human fronto-temporal mesolimbic circuits

Authors: *M. BIJANZADEH, M. DESAI, H. E. DAWES, E. F. CHANG
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Abstract: The identification of neural features correlated with behavioral symptoms of mood disorders, such as Major Depressive Disorder, could provide objective measures for diagnosis, risk-assessment and recovery tracking. fMRI imaging studies utilizing behavioral paradigms stated that brain regions including limbic circuits, frontal cortex and temporal cortex are involved in affective displays, such as laughter, and other emotional states. Although such behavioral paradigms afford experimental control, they may engage neural circuits which are distinct from those underlying endogenous changes in emotional state. Here we study the neural correlates of spontaneous emotional behavior, defined to include affective displays (e.g., smiling) and relevant reflexive statements (e.g., “I’m anxious”), in the absence of an experimental paradigm. Instances of positive and negative emotional behavior were hand-annotated from 24-hour audio and video recordings of consented patients implanted with intracranial electrodes for seizure localization. Annotations were time-aligned with intracranial electroencephalography (iEEG) recordings in mesolimbic structures including amygdala, hippocampus, insula, cingulate and orbitofrontal cortex. Neural features in conventional EEG frequency bands were extracted from 1 minute of data centered on each annotated instance of emotional behavior. The same neural features were randomly sampled from times in which no emotional behaviors were annotated (neutral behavior). We utilized support vector machine (SVM) classifiers using power features from mesolimbic channels to develop decoders for emotional behavior within each patient. With feature selection methods such as information gain prior to the classification, we ranked the top 10 features and then trained SVM classifiers with 10-fold cross validation. The decoder was able to differentiate between (1) positive and neutral emotional behavior (n=8 subjects); (2) negative and neutral emotional behavior (n=4); and (3) positive and negative emotional behavior (n=3), with average accuracy above 70% in all three comparisons. Classifier accuracies were significantly higher than random models (~50%) that were trained on shuffled labels. Across subjects, high-gamma and theta/alpha powers were dominant in distinguishing positive/negative emotional behavior from neutral behavior. High-gamma power was greater for positive behaviors than negative, while lower-frequency power was seen to correlate with negative emotional behavior. These results could inform biomarker identification for objective symptom assessment in the treatment of severe mood disorders.

Disclosures: M. Bijanzadeh: None. **M. Desai:** None. **H.E. Dawes:** None. **E.F. Chang:** None.

Poster

158. Emotion: Positive and Negative Emotional States

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Program #/Poster #: 158.02/WW11

Topic: G.03. Emotion

Support: NIH R01MH097320

Title: EEG decoding of emotional states:Neural substrates revealed by simultaneous EEG-fMRI

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Abstract: Multivariate pattern analysis (MVPA) has been applied to both EEG and fMRI data. Although Rrelative to MVPA decoding of fMRI data, MVPA decoding of EEG data offers the advantage of being able to temporally resolve the formation and evolution of different brain states, but it has the limitation of not being ableed ability to provide information on the relevant neuroanatomical substrate. We hypothesized that aAppropriate fusion of the two recording modalities holds the key to solving this problem. We tested the idea by recording Ssimultaneous EEG-fMRI was recorded from healthy human subjects who passively viewinged unpleasant (mutilation, human violence, attacking animals) and neutral (house hold scenes, people) pictures selected from the International Affective Picture System (IAPS). On each trial the picture was shown for 1000ms. The inter-trial interval (ITI) varied randomly from 6000 to 9000ms. Applying the support vector machine (SVM) technique to single-trial EEG and BOLD responses, we spatially and temporally decoded unpleasant versus neutral brain statescontent. Function connectivity between amygdala and visual cortex was further computed to yield a deeper understanding of the neural mechanisms underlying affective picture processingaid with interpretation. The following results were found. First, starting at ~200ms after picture onset, EEG decoding became significantly above chance level, which lasted until ~the end of the trial.1800ms after picture onset. Second, fMRI decoding was significantly above chance level in all retinotopic visual areas. Third, fMRI decoding accuracy in visual areas were positively correlated with EEG decoding accuracy during two time periods: 400ms-800ms and 1100ms-1500ms. Fourth, functional connectivity between amygdala and high-order ventral visual cortex predicted fMRI decoding accuracy in ventral visual cortex as well as EEG decoding accuracy in the time period of 1100ms-1200ms. These results suggest that EEG decoding of emotional states elicited by affective pictures can be decoded from EEG datal content reflects distinct neural representations in visual cortex and the the formation and development of the of these neural representations of these emotional states may involve dynamic interactions between cortical and subcortical structures.

Disclosures: K. Bo: None. **S. Yin:** None. **Y. Liu:** None. **A. Keil:** None. **M. Ding:** None.

Poster

158. Emotion: Positive and Negative Emotional States

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 158.03/WW12

Topic: G.03. Emotion

Title: Premenstrual syndrome and anhedonia: Evidence from the reward process

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Abstract: Introduction Premenstrual syndrome (PMS) encompasses a wide variety of luteal phase symptoms that are bothersome and usually disappear or greatly improve shortly after the onset of bleeding (Dueñas et al., 2011). PMS has higher comorbidity with other emotional disorders, particularly Major Depression Disorder (MDD; Carine, Elisabeth, Christine, & Sibil, 2011), which indicated PMS and MDD may share some mechanisms. Anhedonia, which is defined as the diminished capacity to experience pleasure, is one of the most important diagnostic criteria and phenotypes for MDD (APA, 1994; Hasler, Drevets, Manji, & Charney, 2004). Empirical researches also indicated that women with Premenstrual Dysphoric Disorder (PMDD, the severe form of PMS) reported significantly lower positive affect (Petersen et al., 2016). Furthermore, in the premenstrual (vs. postmenstrual) phase, PMDD subjects, compared with asymptomatic subjects, showed a decreased ventral striatum response to positive vs. neutral stimuli (Protopopescu et al., 2008). That is to say, PMS also associated with anhedonia, which is caused by the alter function of reward circuitry. In this study, we investigated the reward circuitry in women with PMS. **Method** A monetary incentive delay task was presented to 16 unmedicated women with PMS and 16 healthy comparison women during fMRI scanning in the premenstrual (late luteal) phase. The score of Chinese Premenstrual Syndrome Scale of the participants in PMS group were higher than that of healthy group ($t(30)=20.73$, $p<0.001$), and the two groups were not different in length of menstrual cycle and length of menstrual flow ($ps>0.05$). **Results** We estimated a first-level general linear model (GLM) per subject, modeling feedbacks (reward and punishment) and motion regressors included in the design matrix. On the second level, we estimated a full factorial design of 2 (group: PMS/health) × 2 (feedback: reward and punishment) mixed measure analysis of variance (ANOVA). The whole brain results indicated that there was a significant main effect of group in bilateral super parietal gyrus (left: [36 -60 54], k=354, $Z=3.92$, $p<0.005$ GRF correction; right: [-36 -15 24], k=469, $Z=3.66$, $p<0.005$ GRF correction). Then, we defined a region of interest (ROI) of striatum by combining the caudate and putamen within WFU Pickatlas. The ROI results showed the bilateral striatum (left: [-9 18-3], k=12, $Z=2.76$, $p<0.005$ GRF correction; right: [9 6 6], k=27, $Z=2.93$, $p<0.005$ GRF correction) were activated stronger in health group than PMS group. **Conclusion** The results revealed that regardless of feedback received, the PMS group had a blunted reward processing, resulting in the anhedonia of PMS women.

Disclosures: L. Hou: None. **R. Zhou:** None.

Poster

158. Emotion: Positive and Negative Emotional States

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 158.04/WW13

Topic: G.03. Emotion

Title: How short-term contemplative practices with vocalization alter psychological states in an unexperienced sample

Authors: *H. MIYATA[1], A. TANAKA[2], Y. SASE[2], N. SUZUKI[2], D. OH[2]
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Abstract: Traditional contemplative practices including yoga and meditation have been shown to cause plastic changes to the neurophysiological, psychological, and somatic functions in adult humans. How can desirable changes in psychological states occur when unexperienced participants engage in short-term contemplative practices? The present study examined changes in emotional and relevant psychological states associated with instructed contemplative practices that involve continuous vocalization lasting for multiple minutes. Ten Japanese university undergraduate students (6 females and 4 males, age: 20-22 [mean age = 20.7] years) with no daily training of yoga or meditation were assigned to a practice group, and participated in a session of contemplative practices lasting for approximately 75 minutes. Specific content of the practice included mantra meditation with vocalization (10 min) and "overtone chanting," i.e., a traditional contemplative practice originated in the Nyingma school of Tibetan Buddhism in which participants continuously vocalize vowels in a group (30 min). The practice session was guided by a professional yoga instructor (female; 50 years). In addition, another 18 undergraduates (12 females and 6 males, age: 20-22 [mean age = 20.7 years]) who were also naïve to meditation were assigned to the control group, and engaged in a 75-min free description regarding the content of the lecture in which these students participated. Both prior to and immediately after the session/task, participants from these two groups completed an identical battery of questionnaires concerning positive/negative affect (PANAS), state anxiety (STAI-S), and degree of relaxation (1-to-10 scale). For the practice group, scores of anxiety significantly decreased and those of relaxation significantly increased after the practice session. By contrast, statistically significant changes in the scores failed to be observed for the control group. These results support the notion that short-term contemplative practices that involve continuous vocalization can cause desirable changes to the psychological states such as reducing anxiety and enhancing relaxation, regardless of prior experience with the contemplative training. Future studies may further focus on the effect of each specific form of practice, long-term effects of repeated practice sessions, as well as potential changes at the neurophysiological level including autonomic nervous system activities and cerebral hemodynamics.

Disclosures: H. Miyata: None. **A. Tanaka:** None. **Y. Sase:** None. **N. Suzuki:** None. **D. Oh:** None.

Poster

158. Emotion: Positive and Negative Emotional States

Location: SDCC Halls B-H

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Program #/Poster #: 158.05/WW14

Topic: G.03. Emotion

Support: INPRFM Project NC123340.1

Title: Emotional facial expressions in healthy women during REM sleep

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Abstract: Introduction: While motor activity is actively inhibited during REM sleep, specific activations of the facial mimetic musculature have been observed during this stage, which may be associated with greater emotional dream mentation. Nevertheless, no specific biomarker of emotional valence or arousal related to dream content has been identified to date.
Objective: To explore, in healthy women subjects, the association between the voltage, number, frequency and duration of facial muscle contractions (FMC) from the corrugator and zygomaticus muscles during REM sleep and EDM reported in experimental awakenings from REM sleep, and to determine whether or not they represent specific emotional dream enacting behaviors.
Method: Two 8 h sleep standard PSG recording were obtained from 12 female healthy volunteers, ages 20-30. Facial EMG recordings were obtained from right and left corrugator and zygomatic major muscles. Experimental awakenings exploring EDM (through narration, rating of a dream scale, and Dream Content Questionnaire) were performed during *in vivo* REM sleep stages that lasted at least three minutes. Awakenings were determined by a FMC. Following sleep recordings, FMC were quantified, mean voltage (μV) per muscle during REM sleep was acquired and differences were statistically evaluated. EDM global scores were obtained by the analysis among 5 blind judges of dream mentation in which a number is obtained in a scale of both valence and arousal of emotion(s) present. A linear regression analysis was performed

between the individual FMC variables as the response, and the prevalence of the four emotional subscales as predictors.
Results: Emotions were mentioned in 80.4% of dream reports. The voltage, number, density, and duration of facial muscle contractions were greater for the corrugator muscle than for the zygomaticus muscle, while high positive emotions predicted the number (R^2 0.601, p=0.0001) and voltage (R^2 0.332, p=0.005) of the zygomaticus.
Conclusion: We confirm the activation of corrugator and zygomaticus facial muscles during REM sleep, which occurs when the incidence of emotional dream mentation is high. For the first time, we have shown that high positive
emotional dream mentation strongly affects both the number of FMCs and the voltage of the right and left zygomaticus muscles. Our findings suggest that zygomatic activity during REM sleep could be considered to be a specific emotional DEB, and that in addition to being associated with emotional arousal, this activity is related to emotional valence as well.

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Poster

158. Emotion: Positive and Negative Emotional States

Location: SDCC Halls B-H

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Program #/Poster #: 158.06/XX1

Topic: G.03. Emotion

Support: Center for Happiness Studies via the Center for Social Sciences at Seoul National University (No. 0404-20160001).
SMG-SNU Borame Medical Center (No. 03-2018-24)

Title: Predictors of life and health satisfaction among elderly Koreans: A machine-learning approach

Authors: *S. LEE[1], I. CHOI[1,2], W.-Y. AHN[2], B. OH[3]
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Abstract: Life satisfaction measures how people evaluate their life as a whole and is often used as an index for happiness or subjective well-being. South Korea is one of the rapidly aging nations with life satisfaction scores below the OECD average. As numerous studies indicate its association with physical health and longevity, life satisfaction is expected to decline in elderly people as their health conditions deteriorate. Since life and health satisfaction among the elderly population has become an important issue in Korea, it is necessary to develop a panel to identify

their significant determinants. The present study investigates these predictors using a data-driven approach. We used the Korean Longitudinal Study of Aging (KLoSA), a nationally representative longitudinal panel database of the Korean elderly population. Information on demographics, physical performance, social connections, socioeconomic status, cognitive function, depression, and household income was collected from 6,089 respondents aged 54 or above. To analyze this data, a machine learning method called an elastic net was used to identify multivariate data patterns and to predict life and health satisfaction. Our findings indicate that life satisfaction and health satisfaction share common elements, but they are determined by different components in spite of the high correlation between them. Both variables are affected by the respondent's level of education, depression, and subjective social class. However, the machine learning approach identifies different patterns for health satisfaction vs. life satisfaction: whereas cognitive/emotional components as well as physical conditions are stronger predictors of health satisfaction, overall life satisfaction is better predicted by social and economic components. These results show that multiple components predicting health or life satisfaction need to be considered to facilitate healthy and successful aging.

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Poster

158. Emotion: Positive and Negative Emotional States

Location: SDCC Halls B-H

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Program #/Poster #: 158.07/XX2

Topic: G.03. Emotion

Support: The William K Warren Foundation

Title: Evidence indicating no effects of neighborhood affluence on brain functions and behaviors of positive/negative valence systems among mood/anxiety disorders

Authors: *K. L. FORTHMAN[1,2], C. FENG[1], R. KUPLICKI[1], H.-W. YEH[1], M. PAULUS[1]
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Abstract: There is a growing recognition that mental health conditions are substantially influenced by neighborhood characteristics, independent of factors at an individual level. For instance, neighborhood socioeconomic disadvantage has been shown to be strongly related to increased anxiety and depression symptoms. However, little is known about whether neighborhood factors contribute to brain and behavioral functions as they pertain to mental health. We addressed this by examining whether neighborhood affluence impacts brain and behavioral functions of positive/negative valence systems among mood/anxiety disorders. Subjects with mood/anxiety disorders (n = 240) underwent fMRI scanning during which they performed a monetary incentive delay task probing anticipation of positive (reward) and negative

(loss) events. Moreover, the tendency to experience positive and negative affect was collected for each subject using an extended positive affect negative affect schedule (PANAS-X). The subjects' neighborhood affluence was determined in two steps: (1) the tract-level affluence (and four other factors) was determined by factor analysis using data from the American Community Survey collected by the U.S. Census, (2) neighborhood affluence score was assigned to each subject based on the resident tract. Effects of neighborhood affluence on brain responses to reward/loss anticipation and positive/negative affect were estimated after controlling for individual income, age, and sex. Bayes factor (BF) was used to quantify evidence in favor of alternative against the null hypothesis (neighborhood affluence is effective vs. not effective). Region-of-interest analyses revealed that neighborhood affluence did not account for neural responses of nucleus accumbens (NAcc) to reward anticipation (left NAcc: $\beta = 0.067$, log (BF) = -1.44, where natural logarithm was used henceforth; right NAcc: $\beta = -0.016$, log (BF) = -1.92) or anterior insula (AI) responses to loss anticipation (left AI: $\beta = -0.010$, log (BF) = -1.94; right AI: $\beta = 0.034$, log (BF) = -1.83). Whole-brain analyses further showed that there were no associations between neighborhood affluence and neural responses in other reward-related (e.g., ventromedial prefrontal cortex) and loss-related (e.g., dorsal anterior cingulate cortex) regions. Finally, neighborhood affluence was not associated with subjective negative ($\beta = -0.076$, log (BF) = -1.30) or positive ($\beta = 0.10$, log (BF) = -0.78) affect. Our findings thus indicate that there was no effect of neighborhood affluence on brain and behavioral functions associated with positive/negative valence systems among mood/anxiety disorders.

Disclosures: K.L. Forthman: None. **C. Feng:** None. **R. Kuplicki:** None. **H. Yeh:** None. **M. Paulus:** None.

Poster

158. Emotion: Positive and Negative Emotional States

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Topic: G.03. Emotion

Support: The William K Warren Foundation

Title: Neighborhood affluence accounts for inter-individual variations in the left insula volume among mood/anxiety disorders

Authors: *C. FENG[1], K. FORTHMAN[1,2], H.-W. YEH[1], R. KUPLICKI[1], M. PAULUS[1]
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Abstract: Neighborhood factors can have profound effects on mental health conditions ranging from anxiety and depression to substance use and schizophrenia. However, it remains unknown whether these neighborhood effects manifest as differences in brain structure in regions related

to mental health. Here, we address this issue by investigating whether neighborhood affluence accounts for structural changes among mood/anxiety disorders, particularly in regions engaged by three primary domains in the Research Domain Criteria framework: positive/negative valence systems (nucleus accumbens[NAcc], caudate, amygdala); cognitive system (dorsolateral prefrontal cortex[dlPFC]), and arousal system (dorsal anterior cingulate cortex [dACC], insula). High-resolution anatomical images were acquired from 255 subjects with mood/anxiety disorders. Anatomical images were processed using Freesurfer version 6.0.0 to obtain volumes for regions of interest. The subjects' neighborhood affluence was determined in two steps: (1) the tract-level affluence (and four other factors) was determined by factor analysis using data from the American Community Survey collected by the U.S. Census, (2) neighborhood affluence score was assigned to each subject based on the resident tract. Effects of neighborhood affluence on brain structures were estimated after controlling for individual income, age, and sex. Bayes factor (BF) was used to quantify evidence in favor of alternative against the null hypothesis (neighborhood affluence is effective vs. not effective). In the positive/negative valence systems, evidence indicated no effect of affluence on left NAcc volume ($\beta = 0.066$, log (BF) = -1.27, where natural logarithm was used henceforth), and there was no evidence that affluence accounted for right NAcc volume ($\beta = 0.094$, log (BF) = -0.62), caudate volumes (left: $\beta = 0.012$, log (BF) = 0.20; right: $\beta = 0.097$, log (BF) = -0.59), or amygdala volume (left: $\beta = 0.89$, log (BF) = -0.76; right: $\beta = 0.11$, log (BF) = -0.0027). In the cognitive system, affluence was not related to dlPFC volume (left: $\beta = 0.032$, log (BF) = -1.76; right: $\beta = 0.042$, log (BF) = -1.61). In the arousal system, affluence correlated with left insula volume ($\beta = 0.14$, log (BF) = 1.28), but not with right insula volume ($\beta = 0.085$, log (BF) = -0.65) or dACC volumes (left: $\beta = 0.046$, log (BF) = -1.72; right: $\beta = 0.046$, log (BF) = -1.71). Our findings thus indicate that neighborhood affluence is related to structural changes in the left insula, a key region of the arousal system, but not in other areas of the arousal, positive/negative and cognitive systems.

Disclosures: C. Feng: None. **K. Forthman:** None. **H. Yeh:** None. **R. Kuplicki:** None. **M. Paulus:** None.

Poster

158. Emotion: Positive and Negative Emotional States

Location: SDCC Halls B-H

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Program #/Poster #: 158.09/XX4

Topic: G.03. Emotion

Title: Association between reward-related electrocortical activity and gambling behavior

Authors: *E. TUNISON, IV, R. SYLVAIN, V. HILEY, J. CARLSON, J. DAAR
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Abstract: The reward-related positivity (RewP) is an event-related potential (ERP) with a positive amplitude occurring approximately 250-350 ms post-feedback at frontocentral electroencephalogram (EEG) electrode sites. The RewP amplitude is relatively larger for positive vs negative outcomes. Although individuals exhibit a relatively more positive RewP for monetary gains, relative to non-monetary gains, the RewP is observed for positive, nonmonetary, feedback as well. However, individual differences in previous gambling experience might affect RewP reactivity; especially in nonmonetary conditions. We hypothesized that the RewP response would be weaker for individuals who had previously gambled for relatively larger sums of money. To address this question, a 64-channel EEG cap was used to record reward-related electrocortical activity at frontocentral electrodes during a simple gambling task (N = 30). During each trial two doors were presented: one on each side of the screen. On every trial, one door contained either an increase in points or a decrease in points. Participants were instructed to choose one door on each trial. After choosing a door, they received feedback as to whether they gained or lost points on that trial. The amount of wins and losses was equally divided across 160 trials. After the task, participants answered a gambling questionnaire to assess gambling behavior and risk for problem gambling. The RewP was maximal at electrode FCz with a relatively larger amplitude for wins M = 3.061 ± 0.769 μV compared to losses M = 1.982 ± 0.627 μV, $p < .05$). Amplitudes for wins (r = -0.37, p = 0.047) and losses (r = -0.38, p = 0.04) negatively correlated with the amount of money gambled in day-to-day life. After adjusting for this effect, the RewP difference score (win - loss) positively correlated with risk of problem gambling (r = -0.38, p = 0.04). In sum, RewP amplitudes were larger for positive (nonmonetary) feedback relative to negative feedback at frontocentral electrode sites—indicates that monetary reward is not necessary to elicit the RewP. Amplitudes for wins and losses negatively correlated with the amount of money gambled in day-to-day life. Yet, the difference between win and loss amplitudes correlates with risk of problem gambling.

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Poster

158. Emotion: Positive and Negative Emotional States

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Topic: G.03. Emotion

Support: 15H03125
KMU consortium

Title: Role of dopamine in the primate caudate for the dexision making under different emotional context

Authors: *Y. UEDA, M. YASUDA, K. NAKAMURA
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Abstract: While the decision making is often based on the expectation of rewards, aversive information may also influence the process. However, the behavioral and neuronal course of mediating decision making through analyses of both appetitive and aversive information is poorly understood. To answer this question, we developed a saccade task where two monkeys (*Macaca fascicularis*/*mulatta*) made choice under different task context with and without appetitive or aversive cues. First, monkeys were conditioned to associate three fractal images to a drop of juice (R), a tone with a small drop of juice (T), or an airpuff (A). After fixating on a central fixation point (FP), a pair of two images: R-T, R-A, or T-A, appeared in the left and right of the FP. The monkeys then chose one of two images by saccades to obtain a reward and/or to avoid a punishment. By repeating the same image pair for 15-20 trials as a block, monkeys performed the task under specific emotional context. We previously reported that the inclusion of 'A' (R-A, T-A) significantly increased non-optimal choices (chose A), even though another counterpart was rewarding (R-A). The non-optimal choice occurred often at the beginning of the block and gradually decreased as the number of trials increased. We further found that more than half of analyzed single caudate neurons exhibited differential activity depending on the pair of cues; 42 and 32% of neurons showed differential activity depending on the inclusion of appetitive and aversive cues, respectively. The differential activity developed as the number of trials increased. Dopamine (DA) neurons respond to appetitive/aversive cues/outcomes, and they project to the striatum. We hypothesized that the DA in the caudate may be involved in the behavioral changes depending on the task context. To this end, we performed local injections of DA D1 (SCH23390 10μg/μL, n=9) or D2 (Eticlopride hydrochloride 6μg/μL, n=7) antagonists in the caudate during the task. After injection, the monkey showed difficulty in 1) controlling of response execution (too slow or fast/premature responses) and 2) decision making (slower decrease in the non-optimal choice rate within a block (D1) or the non-optimal choice remained high (D2)). These effects were found only in the task contexts with 'A', suggesting the context-dependent effect. These results suggest that decision process in different emotional context depends partly on dopaminergic modulation of neuronal activity in the caudate.

Disclosures: Y. Ueda: None. **M. Yasuda:** None. **K. Nakamura:** None.

Poster

158. Emotion: Positive and Negative Emotional States

Location: SDCC Halls B-H

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Program #/Poster #: 158.11/XX6

Topic: G.03. Emotion

Title: A new method to evaluate emotional valence and arousal of each visual stimulus in monkeys

Authors: *H. IWAOKI, K. NAKAMURA
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Abstract: Recent human studies have shown that the amygdala plays an important role in the evaluating of emotional significance of environmental stimuli. However, it remains unclear how emotional valence and arousal of each sensory stimulus is encoded by neuronal activity in the amygdala. We should have a method to quantitatively evaluate emotional valence and arousal of each stimulus in monkeys to reveal how amygdala neurons encode emotional valence and arousal of a specific stimulus in detail. Here, we developed a new behavioral method. The monkey sat in front of a CRT monitor and performed a simple two-way choice task. We prepared twelve categories of visual stimulus (e.g. infant monkey face, male monkey face, fearful monkey face, threatening monkey face, female monkey breast, female monkey hip, human face, scene, tool, food, abstract symbol, and snake) and a gray circle as the control stimulus and seven reward conditions. At first, a visual stimulus was presented for 50ms on the monitor. Then two targets were presented horizontally on the monitor. The monkey chose one of two targets; Target 1 was followed by Reward 1, and Target 2 was followed by Reward 2 and the same stimulus previously presented. Our hypothesis is that if the stimulus had positive emotional valence for the monkey, they chose Target 2 more frequently than Target 1, even Reward 2 was smaller than Reward 1 because the stimulus had reinforcement value. On the other hand, if the stimulus had negative emotional valence, they choose Target 1 more frequently than Target 2. The seven reward conditions were as follows; (Reward 1: Reward 2) were (0.58ml: 0.43ml), (0.55ml: 0.45ml), (0.53ml: 0.48ml), (0.5ml: 0.5ml), (0.48ml: 0.53ml), (0.45ml: 0.55ml), and (0.43ml: 0.58ml). By changing the balance of Reward 1 and Reward 2 and by comparing choice rate, we calculated emotional valence of each stimulus. We applied the task to two Japanese monkeys (3-4 years old). We found infant face, monkey hip and scene were positive and snake, threatening face, and male face were negative, and fearful face, food, human face, and abstract symbol were neutral. In addition, we could estimate the magnitude of emotional arousal of each stimulus as amount of reward. Using our new task, we could evaluate both emotional valence and arousal of each stimulus for each monkey. We believe that our method is useful for future emotional neuroscience research.

Disclosures: H. Iwaoki: None. **K. Nakamura:** None.

Poster

158. Emotion: Positive and Negative Emotional States

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Title: Rats will aid a distressed conspecific independent of social reward

Authors: *S. COX[1], C. M. REICHEL[2]
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Abstract: Prosocial behaviors, such as social interaction and empathy, are imperative for an adaptive social structure by the allowance for personal understanding of the perceived valence of others. Further, these behaviors may play a role in the underlying pathology of some cognitive disorders, such as substance use disorder (SUD). There is evidence to suggest animal species will behave prosocially in the hopes of receiving social reward, but only recently has it been intimated that some animals are capable of behaving empathically. Empathy can be broadly defined as the capacity for one to experience the valence of another which, in turn, generates a response more appropriate to another's emotional situation than one's own, independent of personal gain. Some studies show rats will perform a task to reduce the distress of a conspecific, and animals with previous experience of the distress will learn the task faster than if they had not. These data suggest an understanding of the valence of a conspecific and a motivation to reduce their perceived distress. Our lab has adapted a model of helping behavior that requires the animal to pull a chain to release a distressed conspecific from 100mm of water via an automated guillotine door, and implemented improvements on the task to eliminate social reward as a confound. Initially, we replicated a model of social interaction, showing male Sprague Dawley rats (n=16) will release a conspecific from water, and rats with a previous experience of the distress learned to release a conspecific faster than experience-naïve rats. Next, to eliminate social interaction as a motivator, rats (n=16) learned to release a conspecific from water into a dry area separate from their own in a three chamber apparatus. Again, in this model, rats with previous experience of the distress showed faster latency to chain pull than those without. This behavior is specific to helping a distressed rat, because latency to chain pull is significantly increased if the conspecific is removed or replaced with an 'imposter rat' (a fake rat). Future studies include immunohistochemical detection of c-fos following task performance to elucidate the role of brain regions such as the insula and anterior cingulate, and assessments of empathic responding between animals in a withdrawal state from heroin and/or methamphetamine self-administration compared to controls. Our lab's model allows for an evaluation of empathic behavior in rats, as well as an elucidation of the neural underpinnings of empathy and how they are affected by cognitive disorders like SUD.

Disclosures: S. Cox: None. **C.M. Reichel:** None.

Poster

158. Emotion: Positive and Negative Emotional States

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 158.13/XX8

Topic: G.03. Emotion

Title: Responses to positive and aversive stimuli in female rats treated with estradiol + progesterone and housed in seminatural environment: Effetcs of yohimbine and chlordiazepoxide

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Abstract: The behavioral effects of putative anxiolytic and anxiogenic drugs are usually evaluated in highly standardized tests. The external validity of these tests is unknown. In the present experiment, we determined the effects of the classical anxiolytic drug chlordiazepoxide and the purportedly anxiogenic adrenergic α_2 antagonist yohimbine in mixed sex groups of rats housed in a seminatural environment. Different kinds of emotional events were introduced into the environment. Exposure to lavender odor, music (Mozarts sonata for 2 pianos) and chocolate pellets were positive stimuli whereas a 90 dB white noise and fox odor were aversive. Eight groups of 7 rats (4 ovariectomized females and 3 males) were housed for 8 days in a seminatural environment consisting of a large open area and a complex burrow system. On day 5, the females were given 18 μg/kg of 17β-estradiol benzoate. On day 7, all females received 1 mg/rat of progesterone. Three hours later they were injected either with saline, chlordiazepoxide (2 mg/kg), or yohimbine (1 mg/kg). Thirty minutes after drug treatment, the stimuli mentioned above were presented in sequence. Each stimulus lasted for 15 - 30 min. They were separated by a 50 min interval. Sociosexual and exploratory behaviors were quantified from the video record. Lavender and chocolate increased social and sexual behaviors, while exposure to music, white noise and fox odor decreased them. White noise reduced the time spent in as well as the number of visits to the open area. Concerning drug effects, females treated with yohimbine had a higher lordosis quotient than females given saline. This was the only significant drug effect. We then evaluated the structure of behavior with an analysis of co-occurrence followed by a descending hierarchical analysis. This made it possible to identify behaviors typical of each treatment, and of each of the stimuli employed. It turned out that yohimbine clearly distinguished itself from both saline and chlordiazepoxide, while the latter two treatments belonged to the same cluster. Interestingly enough, yohimbine was associated with the male behaviors of mounting and pursuit of the female. Thus, yohimbine-treatment had rendered the females more attractive. Among the stimuli, white noise and chocolate availability formed two separate clusters, whereas lavender odor, music and fox odor formed another cluster. Chocolate was associated with eating and grabbing the pellets and rejecting the males. White noise was associated with startle and hiding.

In the seminatural environment, none of the drugs seemed to affect behaviors related to fear or anxiety. Yohimbine made the females more attractive and more receptive to the males.

Disclosures: O. Le Moene: None. **M. Kvalheim:** None. **A. Agmo:** None.

Poster

158. Emotion: Positive and Negative Emotional States

Location: SDCC Halls B-H

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Program #/Poster #: 158.14/XX9

Topic: G.03. Emotion

Title: Sex differences in suppression of conditioned fear during a safety cue in a fear-safety-reward cue discrimination task

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Abstract: The inability to distinguish safety from fear is a biomarker of Post-Traumatic Stress Disorder (PTSD). Despite the higher diagnosis of PTSD in women, research using female subjects in animal studies has been lacking. In our laboratory, we have a well-established discriminative conditioning (DC) task in which male rats readily learn to discriminate among fear, safety and reward cues. In the DC task, rats are exposed to pairings of a) a fear cue with shock, b) a safety cue with no shock, c) a reward cue with sucrose, and most importantly, d) a compound fear+safety cue with no shock. In the current study, we investigated for possible sex differences in learning the DC task. As is typical, males in this study (n=16) showed significant suppression of freezing during the fear+safety cue compared to the fear cue, demonstrating conditioned inhibition of fear in the presence of a safety cue. Females, however, showed equitable levels of freezing to the fear and fear+safety cues, indicating they are not learning conditioned inhibition. But, interestingly, when 'darting behavior' (Gruene et al, 2015) was assessed, females did show significant suppression of darting behavior during the fear+safety cue compared to the fear cue. This implies females are showing a different fear profile compared to males under fearful and safe conditions. Females also exhibited significantly higher reward seeking behavior during the reward cue in early DC sessions, suggesting they may be more reward responsive. Differences in female rats' learning of safety and reward signals suggest the need for research on the underlying neurobiological processes, which may lead to gender-specific treatment techniques for disorders involving anxiety and/or addiction.

Disclosures: M.R. Norris: None. **E. Greiner:** None. **I. Mueller:** None. **K.H. Ng:** None. **S. Sangha:** None.

Poster

158. Emotion: Positive and Negative Emotional States

Location: SDCC Halls B-H

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Program #/Poster #: 158.15/XX10

Topic: G.03. Emotion

Support: Alexander von Humboldt Foundation

Title: Adolescent conditioning affects fear expression and rate of safety learning during adult discriminative conditioning

Authors: *I. MUELLER[1,2], A. L. BRINKMAN[1], E. M. SOWINSKY[1], S. SANGHA[1,2]
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Abstract: Stimuli that are paired with threats or rewards gain a predictive value and induce fear or reward seeking, respectively. Both behaviors are modulated by safety cues, signalling the non-occurrence of a threat. It is well known that the influence of previous experiences on later fear- and reward learning is highest when taken place during sensitive developmental periods, such as adolescence. But the impact of specific fear and reward preconditioning on the interaction of different conditioned emotions and conditioned safety is poorly understood. In this exploratory study, we randomly assigned male Long Evans rats (n=16/group) to one of four groups. Rats were either exposed to adolescent (postnatal day 30) fear or reward conditioning, to foot shocks explicitly unpaired to the future safety cue or to the later conditioning context only. All groups underwent discriminative conditioning (DC, Sangha et al., 2013) in adulthood (postnatal day 70). In this DC task, rats simultaneously learned to distinguish among fear, reward and safety cues. Freezing and port entries, as readouts for fear and reward-seeking behaviors, respectively, were assessed manually by trained experimenters blind to group affiliation. A reduction of freezing, when the fear cue was co-presented with the safety cue compared to the fear cue alone indicated learned safety. We hypothesized that preconditioning would strengthen the respective conditioned emotion and modulate the interaction with others during adulthood. We show that reward preconditioning did not affect later reward learning, but instead accelerated safety learning. Fear preconditioning accelerated later fear and reward-seeking behaviors but delayed safety learning. Together, our results suggest that the rate of safety learning can be influenced by adolescent priming of different emotions. Since adolescent experiences can significantly affect later responses to stress, investigating this process explicitly could help unravel the complexity of psychiatric disorders, such as posttraumatic stress disorder, where safety learning is diminished.

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Poster

158. Emotion: Positive and Negative Emotional States

Location: SDCC Halls B-H

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Program #/Poster #: 158.16/XX11

Topic: G.03. Emotion

Support: P50MH106428
T32DA07278

Title: Next-gen sequencing of TRAP/RiboTag mRNA from serotonergic raphe neurons identifies a small subset of stress-sensitive genes

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Abstract: Serotonin is a primary mediator of stress, anxiety, and depression. Drugs that regulate serotonin reuptake and metabolism have been critical for treating anxiety and depressive disorders. However, currently available medications target rather few mechanisms within these neurons and can induce numerous side effects, highlighting the importance of identifying new molecular targets within the serotonergic neurons. A major challenge in identifying novel molecular targets in serotonergic neurons is the diversity of other cell types that are also located in the midbrain. In this study, we conducted a cell-specific gene expression analysis of serotonergic neurons in the raphe utilizing the translating ribosome affinity purification (TRAP) method. To express HA-tagged RPL22 protein (RiboTag) specifically within serotonergic neurons, we crossed Cre-dependent floxed RiboTag mice with serotonin-specific Cre driver mice (Pet1-CRE). The resulting cross allowed for isolation and next-gen sequencing of ribosome-associated mRNA transcripts specifically from serotonergic neurons. Using both male and female mice, we isolated ribosome-bound mRNA from midbrain punches of Pet1-CRExRiboTag mice 4h after a two-day repeated swim stress were compared to that of unstressed mice.
We detected significant enrichment of expected serotonergic-neuron gene markers in the cell-specific immunoprecipitated RNA (IP) relative to the general mRNA expression in the tissue punch of the dorsal raphe (Input). By comparing RNAseq data from both input and IP RNA samples, we were able to assess the relative enrichment and signal to noise parameters for all genes. Over 2,000 novel genes enriched in Pet1-neurons also had a high signal to noise expression pattern. We identified differentially expressed genes (DEGs) between male and female mice in both unstressed and stressed conditions. There were a subset of DEGs in unstressed males compared to unstressed females, but only a small subset of genes changed in both sexes in response to stress. RNAseq analysis identified 10 differentially expressed genes (DEGs) that were regulated by stress, specifically in serotonergic neurons; most were confirmed

by a combination of qPCR, FISH, and IHC. Current experiments are investigating how CRISPR knockout of these stress-sensitive genes in 5-HT neurons alters the vulnerability to stress.

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Poster

158. Emotion: Positive and Negative Emotional States

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Topic: G.03. Emotion

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HDRF

Title: Microendoscopy in the dorsal raphe nucleus reveals functionally-distinct subpopulations of serotonergic neurons

Authors: *G. PAQUELET[1], K. CARRION[3], C. LACEFIELD[1], P. ZHOU[2], L. PANINSKI[2,1], R. HEN[1,3], B. MILLER[3]
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Abstract: The mammalian serotonergic system has been implicated in a number of psychiatric disorders, including major depression and anxiety, as well as in emotional behaviors, such as social interaction, punishment, and reward. This functional diversity may result from cellular heterogeneity within the brainstem's raphe nuclei, whose serotonergic neurons project to targets throughout the limbic system. We hypothesize that distinct subpopulations of serotonergic neurons in the dorsal raphe nucleus are recruited during distinct emotional behaviors. To test this hypothesis, we used microendoscopy and calcium imaging to record serotonergic activity with single-cell resolution in freely-moving mice. As a survey of emotional behaviors, we employed well-established paradigms that measure anxiety (elevated plus maze), reward (sucrose consumption), punishment (footshock), and social behavior (free interaction with a novel mouse). While the population of serotonergic neurons collectively responded to each measure, we found that individual cells varied significantly in their responsiveness to reward, punishment, social interaction, and anxiogenic environments. By tracking individual cells across multiple behavioral tests, we have further demonstrated that the population of cells responding during

each behavior is largely different than that recruited by the others, thus supporting our hypothesis of functionally distinct subpopulations in the dorsal raphe nucleus.

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Poster

158. Emotion: Positive and Negative Emotional States

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Topic: G.03. Emotion

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Title: Cortical involvement in RMTg-mediated aversive signaling

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Abstract: The rostromedial tegmental nucleus (RMTg) is a small GABAergic nucleus that exerts inhibitory control over midbrain dopamine neurons. Exposure to aversive stimuli is associated with enhanced activity within the RMTg and activation of the RMTg facilitates aversive responding. The medial prefrontal cortex (mPFC) provides input to the RMTg and activity within this region has also been implicated in many of the same endpoints. However, little is known about the anatomy and function of this projection. To better characterize the density of the mPFC input to the RMTg, adult male Long-Evans rats were injected with the retrograde tracer cholera toxin B (CtB) into the RMTg. Two weeks after injection, cell body labeling was apparent throughout the entire medial and orbital walls of the PFC as well as the entire rostrocaudal extent of the region. Labeling was restricted primarily to layer V of the mPFC, though a small number of cells were also consistently observed in the deepest portion of layer VI. Quantification of CtB labeled neurons relative to NeuN labeling found that approximately 14.21% ±0.38 of layer V prelimbic neurons projected to the RMTg. This indicates that there is a substantially greater relative proportion of mPFC projections compared to other subcortical projections from the prelimbic cortex including the amygdala, ventral tegmental area,

and periaqueductal gray. Using in vivo optogenetics, we found that stimulation of RMTg-projecting mPFC inputs produced significant real-time place avoidance, the magnitude of which was similar to that produced by stimulation of lateral habenula inputs to the RMTg ($p \leq 0.01$) indicating that activation of this pathway can induce aversive responding. In addition, rats presented with a shock or a tone predictive of shock exhibited a significant increase in cFos expression in RMTg-projecting mPFC neurons compared to rats exposed to neutral stimuli ($p \leq 0.05$). Using whole-cell patch-clamp slice electrophysiology, we found that exposure to a single episode of 10 consecutive footshocks resulted in a significant decrease compared to unshocked controls in the frequency of evoked firing in RMTg-projecting prelimbic neurons ($p \leq 0.0001$). Together, these results demonstrate involvement of mPFC input to the RMTg in the behavioral response to aversive stimuli, and further reveal significant plasticity as a result of exposure to aversive stimuli. Alterations within this neural circuit may be critically involved in neuropsychiatric illnesses associated with disruption of the balance between signaling of rewarding and aversive outcomes including addiction and mood disorders.

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Poster

158. Emotion: Positive and Negative Emotional States

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Program #/Poster #: 158.19/XX14

Topic: G.03. Emotion

Support: ERC Grant #311701

Title: Corticolimbic interactions in emotional behaviors

Authors: *D. KARGL[1], J. KACZANOWSKA[1], F. GROESSL[1], M. PASIEKA[2], P. OPRIESSNIG[2], J. ZINNANTI[2], W. E. HAUBENSAK[1]
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Abstract: Emotions are an inherent component of our mental self, they attribute meaning and significance to our environment. To adequately respond to potential reward or threat, we have to assign valence to neutral sensory cues, a fundamental task to survive and a major driving force of motivational behavior. Interestingly, valence-specific activity can be found in diverse brain areas, including cortical and subcortical structures, hence valence information is represented on multiple hierarchical levels. Therefore, a key question in neuroscience is how valence information is distributed throughout diverse brain areas and to what extent the interaction between those is required for emotional learning.

To assess interregional dependencies between abovementioned levels, we lesioned various candidate structures of a literature-based emotional control network in the mouse, including the amygdala, primary sensory and association cortices and performed a resting-state fMRI study. Subsequently these animals were subjected to a discriminative Pavlovian conditioning paradigm, allowing us to correlate behavioral performance with alterations in the emotional control network quantified by BOLD signals and network measures. We delineate a corticolimbic network in which BOLD signals correlate with behavioral phenotypes like impairments in Pavlovian learning or cue discrimination. Our approach of linking fMRI and behavioral phenotyping allowed us to construct correlative networks that identify key hubs in the brain responsible for emotional learning and behavior.
To investigate inter-regional dependencies between identified network components, we applied opto- and chemo-genetic techniques to achieve uncoupling of identified regions. Strikingly, functional disconnection of determined corticolimbic nodes during Pavlovian fear and reward acquisition resulted in a significant reduction in behavioral responses towards conditioned stimuli during recall. In summary, we identified specific interactions within the corticolimbic network that are required for proper emotional learning in the positive and negative valence domain.

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Poster

158. Emotion: Positive and Negative Emotional States

Location: SDCC Halls B-H

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Topic: G.03. Emotion

Support: Cornell University Department of Design and Environmental Analysis graduate student dissertation funding

Title: Neural instantiation of regulatory processes for dynamic emotional stimuli

Authors: *Y. HAO[1], L. YAO[1], D. M. SMITH[2], E. SOREL[3], A. K. ANDERSON[1], E. H. SCHUMACHER[2], G. W. EVANS[1]
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Abstract: Most studies of emotional responses depend on static stimuli, failing to capture the underlying mechanisms of emotional transitions. The temporal context of emotional stimuli likely influences emotional regulation. We investigate the brain mechanisms underlying responses to dynamic emotional stimuli (e.g. emotional images changing from neutral to

negative, or from negative to neutral), by comparing left and right intra-hemispheric fronto-posterior coherence of EEG beta band (Reiser et al., 2012). We also explore the chronic stress as a moderator of the expected difference between dynamic and static and subsequent executive functioning (EF) with the Flanker task. The sample size was 33.
With chronic stress, the coherence of the fronto-posterior region to negative stimuli was blunted in the right hemisphere; whereas left hemisphere coherence is elevated, bearing the burden longer after the removal of negative stimuli. Response and regulation were executed with different mechanisms in the brain based on different chronic stress conditions. The right hemisphere was responsive to negative stimuli with low chronic stress; however, the left hemisphere was responsive with high chronic stress. The overall dynamic conditions evoked less coherence than static conditions. The subsequent EF was hindered through preceding brain activities, especially in high chronic stress individuals. On the other hand, static condition generating more coherence, boosted the subsequent EF in high chronic stress individuals.
In the follow-up study (N=20) that we are currently analyzing we will explore the influence of cognitive reappraisal on brain activity and EF in response to dynamic stimuli conditioning among high and low, chronic stress individuals. We expect to see an increased coherence and moderation effect with chronic stress. Overall the results indicate that emotion is processed dynamically with hemispheric functions associated with self-regulation, that are dependent on chronic stress levels and potentially on cognitive reappraisal ability. More research on self-regulation under conditions of dynamic emotional stimulation is needed.

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Poster

158. Emotion: Positive and Negative Emotional States

Location: SDCC Halls B-H

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Topic: G.03. Emotion

Support: Lallemand, Inc

Title: Neurobiological effects of probiotic-supplemented diets in chronically stressed male Long-Evans rats: Evidence of enhanced resilience

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Abstract: Recent evidence suggests that the microbiome-gut-brain axis modulates neural, endocrine and behavioral functions—producing psychobiotic effects (e.g., anxiolytic and antidepressant responses; Sarkar et al., 2016; Wallace & Milev, 2017). In the current study, 24

male Long-Evans rats were assigned to either a chronic unpredictable stress (CUS) or no-stress group with half of each group further assigned to the probiotic-supplement (PB) group receiving a combination of *L. rhamnous* and *L. helveticus* or a control group receiving maltodextrin (MD); n=6 per group. Following a week of habituation to the laboratory conditions, the PB/MD diets commenced and continued through the duration of CUS for the next two weeks. For behavioral analyses, the animals were assessed in the open-field test (for anxiety) and a problem-solving challenge task (cognitive uncertainty); further, the two CUS groups were assessed in the forced swim task as part of the CUS exposure. Open-Field data indicated that the PB groups exhibited significantly fewer signs of anxiety [i.e., decreased freezing (p=.004) and increased time in the center (p=.05)] than the MD groups. In the problem-solving task, the PB rats visited the reward zone more frequently than the MB groups (p=.007); further, a significant interaction indicated that the PB animals exhibited a reduced latency to leave the start position (p=.014; evidence of task-directed attention in PB animals). In the swim task, the PB animals floated significantly more than the MB stressed animals (p=.048), a response interpreted as conserving energy (Hawley et al., 2014). Fecal samples were collected for corticosterone (CORT) and dehydroepiandrosterone (DHEA) assays at baseline, mid-way through stress exposure, and at the end of the study. At mid-point, the PB animals had significantly lower CORT than the MD animals (p=.04). Further, the DHEA/CORT ratio (a marker of emotional resilience) was higher in the PB animals (p=.05). Following stress exposure, brains were processed for brain-derived neurotrophic factor (BDNF) and microglia immunoreactivity (ir) as measures of plasticity and neuro-immune functions, respectively. Although no effect was observed in BDNF-ir, PB rats had less microglial activation (i.e., Iba1-ir) in the basolateral amygdala (p=.008). Corroborating previous studies, these behavioral, neural and endocrine results provide further evidence of psychobiotic effects in PB-treated rodents.

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Poster

158. Emotion: Positive and Negative Emotional States

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Topic: G.03. Emotion

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NIH Grant DA025634

Title: Kappa opioid receptor-mediated depressive-like states and suppression of nucleus accumbens dopamine release are blunted in female rats

Authors: S. CONWAY[1], D. PUTTICK[2], S. OSMOND[2], M. F. ROITMAN[1], *E. H. CHARTOFF[2]

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Abstract: The neuropeptide dynorphin activates kappa opioid receptors (KORs) in neural reward and stress circuits to produce depressive-like states (e.g., anhedonia). It is thought that KOR-mediated suppression of dopamine release is one underlying mechanism. Using intracranial self-stimulation (ICSS), in which a stimulating electrode is implanted in the medial forebrain bundle (MFB) at the level of the lateral hypothalamus, we previously found that gonadally intact female rats are less sensitive than males to the depressive-like effects of the KOR agonist U50,488—regardless of estrous cycle stage. To examine the activational effects of gonadal hormones on aversive responses to U50,488, we gonadectomized male and female rats that had previously been trained in ICSS. After five weeks, during which plasma sex hormones decreased (measured with ELISA), baseline ICSS responding was similar across groups (male and female, gonadectomized and sham; N=6-7 per group). Rats were treated with U50,488 (0.0, 2.5, 5.0, and 10.0 mg/kg, IP) and stimulation thresholds compared. No significant differences to U50,488-induced increases in ICSS thresholds were detected between sham and gonadectomized rats. These data suggest that sex differences in KOR-mediated depressive-like states are not due to circulating gonadal hormones. Using quantitative real-time RT-PCR, we found no sex differences in mRNA levels for either dynorphin or KOR in the nucleus accumbens or ventral tegmental area (VTA). However, the level of tyrosine hydroxylase (TH) mRNA was significantly higher in the female compare to the male VTA. This suggests that females have an increased capacity to produce dopamine, which might in turn protect them from the depressive-like consequences of KOR activation by blunting the magnitude of KOR-mediated suppression of dopamine release. Using fast scan cyclic voltammetry in intact male and female rats (N=7 and 5, respectively), we stimulated the medial forebrain bundle at different frequencies (5 - 60 Hz) to mimic the ICSS procedure. We recorded dopamine release in the nucleus accumbens after systemic treatment with the KOR agonist U50,488 (0.0 or 5.0 mg/kg). There were no sex differences in baseline stimulated dopamine release. However, as predicted, U50,488-induced suppression of dopamine release was significantly blunted in female compared to male rats. Neither sex nor U50,488 altered dopamine reuptake. Taken together, these data suggest that females are less sensitive to KOR-mediated suppression of dopamine release and are therefore less sensitive to the depressive-like effects of KOR activation.

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Poster

158. Emotion: Positive and Negative Emotional States

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Topic: G.03. Emotion

Support: NIMH R01 MH107239
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Title: Basolateral amygdala (bla) activity during an approach-avoidance conflict task

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Abstract: When foraging, animals weigh the need to attain food against an increased risk of predation. This approach-avoidance conflict requires the selection of appropriate behavioral responses to stimuli that predict rewards in the face of threats. To shed light on how the amygdala regulates such risky decisions, we recorded BLA neurons in rats presented with conditioned light stimuli that predicted a reward (CS-Rs) or a footshock (CS-Ss) in a rectangular arena. The position of the light stimuli indicated whether they signaled rewards (behind the left or right wall) or footshocks (under one of three floor sectors). During conflict trials, the CS-R and CS-S were adjacent and therefore called for incompatible responses: rats could either retrieve the reward while tolerating a shock or forego the reward to actively or passively avoid a shock. Pseudo-conflict and no-conflict trials served as controls. In pseudo-conflict trials, a CS-R and a CS-S were simultaneously presented but their relative position was such that rats could retrieve the reward without getting shocked. No-conflict trials included either a CS-R or CS-S, presented alone. The rats' behavioral strategies varied based on trial type. On conflict relative to pseudo- or no-conflict trials, rats showed more taunting behavior, characterized by neck elongations directed toward the conflicting stimuli but without stepping on the shock sector to retrieve the reward. Also, the incidence of escape behavior and the latency to actively avoid increased during conflict trials, indicating that rats were engaging in risk-taking. Nevertheless, due to the heightened risk level on conflict trials, reward seeking behaviors occurred less frequently. There were marked individual variations in the rats' propensity for risk-taking with some rats approaching the reward port despite the impending shock on as many as 35% of conflict trials and others rarely (8%). On conflict trials, the responses of BLA cells to joint presentations of a CS-R and CS-S, as assessed during the first 0.4 sec, were lower than expected from the cells' responses to these stimuli presented separately. However, the same pattern was observed for pseudo-conflict trials. This suggests that the neural correlates of conflict processing happen after the initial excitation of BLA cells by CS inputs.
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Disclosures: P. Kyriazi: None. **D.B. Headley:** None. **D. Pare:** None.

Poster

158. Emotion: Positive and Negative Emotional States

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 158.24/YY5

Topic: G.03. Emotion

Support: ONR MURI N000141310672
ONR MURI N000141612829

Title: Involvement of the anterior insular cortex in empathic response in rats

Authors: M. CONTRERAS[1], *J.-M. FELLOUS[2]
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Abstract: The neurophysiological mechanisms underlying empathy have been the subject of intense research in the last decade. Recent imaging studies suggest that the insular cortex, in particular the anterior insula, plays an important role in human empathy. However, very little is known of how neurons of this region respond to affective and social signals that are relevant for empathic responses. We have previously shown that the rat anterior insular cortex activity correlated with empathic responses to conspecific distress in an operant task. Animals were trained to obtain food pellets by pressing either of two cued levers in an operant chamber. During the empathy test, one of the levers was programmed to also deliver a footshock (0.5 mA, 0.5 sec) to a conspecific animal which was placed in full view, in an adjacent chamber. Single lever (forced-choice trial) or both levers (free-choice trial) were cued throughout the course of testing. We also showed that the rat empathic responses were modulated by the familiarity of the other rat and by ICV oxytocin and vasopressin injections. Here, we extend this work by conducting several additional electrophysiological analyses to gain a better understanding of the role of anterior insular cortex neurons in rat empathy. Our findings may have relevance for the treatment of neuropsychiatric conditions associated with deficit in empathy and related anti-social behaviors.

Disclosures: M. Contreras: None. **J. Fellous:** None.

Poster

158. Emotion: Positive and Negative Emotional States

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 158.25/YY6

Topic: G.03. Emotion

Support: NIH Grant AG004085-26

Title: The association of bmi, cognitive restraint, and stress with confusion

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Abstract: There is considerable interest in the early development of variables associated with risk factors of metabolic syndrome. Low levels of cognitive restraint, higher BMI, and high stress are known to be associated with the development of metabolic syndrome. There is currently a lack of research into how metabolic syndrome might be associated with neuropsychological processes such as mood states. Thus, we sought to better understand how cognitive restraint for eating behavior, BMI, and stress might be associated with the mood state of confusion. Participants were 33 adults. The POMS subscale of confusion was used to assess confusion. Cognitive restraint was measured on the Three Factor Eating Questionnaire. BMI was calculated from measures of height and weight. Perceived stress was measured with the perceived stress scale, BMI ($r = .387$, $p <..05$), cognitive restraint ($r = -.35$, $p < .05$), and stress ($r = .41$, $p < .05$) were significantly associated with the mood state of confusion. A univariate general linear model of confusion, controlling for all possible interactions, revealed that BMI was the most significant predictor, followed by perceived stress, then cognitive restraint. The BMI X Cognitive Restraint revealed the most significant interaction and the greatest risk factor for confusion. Since BMI, stress, and cognitive restraint are all potentially modifiable risk factors the present study suggests the potential utility of early intervention of metabolic syndrome.
Supported by NIH grant # AG004085-26 from the National Institute on Aging to CM.

Disclosures: P. Wheeler: None. **C. Murphy:** None. **M. Marvin:** None.

Poster

158. Emotion: Positive and Negative Emotional States

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Topic: G.03. Emotion

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NARSAD 23687
The Ellison Medical Scholar Award

Title: Tac2 controls a brain state induced by stress

Authors: *M. ZELIKOWSKY, M. HUI, T. KARIGO, A. CHOE, B. YANG, M. R. BLANCO, K. BEADLE, V. GRADINARU, B. E. DEVERMAN, D. J. ANDERSON
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Abstract: Chronic social isolation causes severe psychological effects in humans, but their neural bases remains poorly understood. We investigated the effects of prolonged social isolation stress (SIS) on behavior and the brain. We identified the neuropeptide tachykinin 2 (Tac2)/neurokinin B (NkB) in the control of the brain state produced by SIS. Systemic administration of an Nk3R antagonist prevented virtually all of the behavioral effects of chronic SIS. Conversely, enhancing NkB expression and release phenocopied SIS in group-housed mice, promoting aggression and converting stimulus-locked defensive behaviors to persistent responses. Multiplexed analysis of Tac2/NkB function in multiple brain areas revealed dissociable, region-specific requirements for both the peptide and its receptor in different SIS-induced behavioral changes. Thus, Tac2 coordinates a pleiotropic brain state caused by SIS, via a distributed mode of action. These data reveal the profound effects of prolonged social isolation on brain chemistry and function, and suggest potential new therapeutic applications for Nk3R antagonists.
Keywords: Tac2, neuropeptides, social isolation, stress, aggression, fear

Disclosures: M. Zelikowsky: None. **M. Hui:** None. **T. Karigo:** None. **A. Choe:** None. **B. Yang:** None. **M.R. Blanco:** None. **K. Beadle:** None. **V. Gradinaru:** None. **B.E. Deverman:** None. **D.J. Anderson:** None.

Poster

158. Emotion: Positive and Negative Emotional States

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Topic: G.03. Emotion

Support: NIH Grant U01MH105982
NIH Grant U19MH114830
HHMI International Student Research Fellowship

Title: Characterization of cell-types in the ventromedial hypothalamus which mediate innate social behaviors

Authors: *D.-W. KIM[1], L. LO[2,3], T. KIM[4], Z. YAO[4], K. A. SMITH[4], L. T. GRAYBUCK[4], O. FONG[4], L. YI[2], S. SHAH[2], N. KOULENA[2], L. CAI[2], L. PACHTER[2], B. TASIC[4], H. ZENG[4], D. J. ANDERSON[2,3]
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Abstract: Innate social behaviors such as aggressive and sexual behaviors are highly conserved across a wide range of species, and their appropriate control is extremely critical for animal survival and reproduction. Recently, our lab identified the small hypothalamic region called ventrolateral subdivision of the ventromedial hypothalamic nucleus (VMHvl), which is responsible for aggression in mice. Furthermore, the follow-up study found that the subpopulation of VMHvl neurons which expresses estrogen receptor 1 (Esr1) was also indispensable for aggression in male mice, and more surprisingly, depending on the intensity of stimulation on $Esr1^+$ VMHvl neurons, evoked social interaction could be progressed from appetitive (close investigation/mounting) to consummatory phases (attack).
To identify which neuronal subpopulations within VMHvl are involved in specific innate social behaviors, we first classified 33 clusters from over 3,200 neurons in VMHvl and their surrounding areas by using SMART-Seq based, single-cell RNA sequencing (scRNA-seq). Specifically, based on the region-specific gene expressions in VMH from Allen Brain Atlas RNA ISH data, there were 17 VMHvl clusters (1869 neurons), and among them Esr1 was highly expressed in 6 clusters. Next, we validated the clusters based on 1) the expressions of marker genes in each cluster by using multiplexed single molecule fluorescence RNA in situ hybridization method called seqFISH, 2) computational comparison with different clustering algorithms, and 3) comparisons with new transcriptomic dataset in VMHvl obtained from another scRNA-seq method, 10x Chromium, which enable massive processing of single-cell transcriptional profiling in a highly scalable manner.

Disclosures: D. Kim: None. **L. Lo:** None. **T. Kim:** None. **Z. Yao:** None. **K.A. Smith:** None. **L.T. Graybuck:** None. **O. Fong:** None. **L. Yi:** None. **S. Shah:** None. **N. Koulena:** None. **L. Cai:** None. **L. Pachter:** None. **B. Tasic:** None. **H. Zeng:** None. **D.J. Anderson:** None.

Poster

158. Emotion: Positive and Negative Emotional States

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Program #/Poster #: 158.28/YY9

Topic: G.03. Emotion

Support: Helen Hay Whitney Postdoctoral Fellowship

Title: Models of fear-related persistent neural activity in VMHdm

Authors: *A. KENNEDY, P. S. KUNWAR, L. LI, D. J. ANDERSON
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Abstract: Predators and predator-related stimuli elicit acute defensive behaviors in mice, as well as changes in behavior that persist after stimuli themselves are removed. We previously showed that activation of SF1+ neurons located in the dorsomedial and central part of ventromedial hypothalamus (VMHdm/c) is sufficient to elicit persistent fear behavior that lasts for tens of seconds following the end of stimulation. In subsequent fiberphotometry and microendoscopic imaging studies, we have also observed persistent activation of SF1+ neurons in VMHdm/c following exposure to multiple predator-related sensory cues. Our microendoscopic imaging experiments furthermore revealed diverse and stimulus-dependent response dynamics among imaged SF1+ neurons. In the current study, we construct several possible network models that can account for persistent population activity, and contrast model neuron responses to responses of individual imaged SF1+ neurons. We show that features of neural responses, such as their temporal structure, their tuning to different stimuli, and their trial-to-trial variability, can be used to distinguish between alternative models of persistent activity. By examining the distribution of these features among imaged SF1+ neurons, we are able to further constrain the space of models that can account for persistent VMHdm/c activity, and point to a family of models that best fits the observed data.

Disclosures: A. Kennedy: None. **P.S. Kunwar:** None. **L. Li:** None. **D.J. Anderson:** None.

Poster

158. Emotion: Positive and Negative Emotional States

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Program #/Poster #: 158.29/YY10

Topic: G.03. Emotion

Support: NIH Grant DA031389
HHMI

Title: Male interneurons that underlie reward learning associated with mating in *Drosophila*

Authors: *E. D. HOOPFER[1], D. J. ANDERSON[1,2]
[1]Div. of Biol. and Biol. Engin., Caltech, Pasadena, CA; [2]Howard Hughes Med. Inst., Pasadena, CA

Abstract: Animals have evolved brain systems that control states of motivation and reward to ensure they seek out what they need to survive and reproduce. Understanding the neural mechanisms that underlie these natural reward systems has important relevance to human health, since drugs of abuse and addiction involve maladaptive changes in these brain systems. Many of

the neuromodulatory systems that underlie reward and the actions of addictive drugs are conserved from *Drosophila* to humans. However, studies of reward in *Drosophila* have focused largely on reward related states that involve resources deprivation (e.g., starvation), which may engage different neural mechanisms than intrinsically rewarding behaviors such as mating. In *Drosophila*, successful male mating has been shown to induce a long-lasting appetitive memory, suggesting that mating may be rewarding in fruit flies. As an entry point into the neural circuits underlying the reward systems associated with mating behavior in flies, we focus on a population of interneurons involved in courtship initiation. Using associative learning assays, we show that optogenetic activation of this population promotes a lasting place preference for the zone in which neuronal activation occurs. Furthermore, optogenetic activation of this population can impart a conditioned preference for a neutral odor, which is stable for at least thirty minutes. These results show that activity of these interneurons can induce an appetitive memory and suggest that they play a role in reward learning associated with mating behavior. This provides a framework for investigating the neural mechanisms of innate reward states associated with social behaviors.

Disclosures: E.D. Hoopfer: None. **D.J. Anderson:** None.

Poster

158. Emotion: Positive and Negative Emotional States

Location: SDCC Halls B-H

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Program #/Poster #: 158.30/YY11

Topic: G.03. Emotion

Support: Mathers Foundation
Kavli Institute for Brain and Mind
Frontiers of Innovation Scholars Program

Title: In the heat of love - How mating inhibits vigilance in fruit flies

Authors: *R. SUN[1], T. KATSUKI[3,2], Y. HUANG[1], R. J. GREENSPAN[4]
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Abstract: Much of fruit fly courtship studies have been focusing on how each step of courtship and mating is controlled by genes, circuits and neuromodulations. Focusing on individual behaviors, such as courtship, and their underlying mechanisms only gives us very limited insights of how this behavior affects the internal states of the animal and other behavioral programs available to the animal. Using a combination of genetic, optogenetic and imaging tools available, we want to study the neural mechanisms underlying the mating inhibition of vigilance of fruit flies in this project. We have built a behavioral setup in which we can observe fruit flies'

escape response during mating. Our results showed that mating activity inhibits both female and male flies' vigilance, suggesting that the internal state of the animal is altered by the act of mating. The insights from this preliminary study will contribute to our understanding of the brain-mind gap, in that the internal state afforded by mating is a mental state shared by many species while escape behaviors are innate for all animals to survive. Understanding how the former modulates the latter will pave the way for elucidating links from behaviors to internal states, and then back to behaviors.

Disclosures: T. Katsuki: None. **Y. Huang:** None. **R.J. Greenspan:** None.

Poster

159. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms I

Location: SDCC Halls B-H

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Program #/Poster #: 159.01/YY12

Topic: G.08. Drugs of Abuse and Addiction

Support: NHMRC Grant 1079893

Title: Projections of RXFP3 positive cells from the zona incerta

Authors: *C. J. PERRY[1], A. B. SIMON[2], A. J. LAWRENCE[3]
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Abstract: RXFP3 is the cognate receptor for relaxin-3. This highly conserved neuropeptide is implicated in wide range of behavioural patterns including stress responses, emotional regulation, circadian rhythms, and motivated behaviour and addiction. Application of the selective RXFP3 antagonist R3(B1-22)R decreases alcohol seeking behaviours when administered either centrally (icv) or locally into the BNST or central amygdala. Conversely, following administration into the dorsal part of the lateral hypothalamus/zona incerta, alcohol-preferring iP rats show an increase in alcohol self-administration. This suggests that in this region specifically, RXFP3 expressing neurons act to inhibit alcohol-seeking. In order to better understand this effect, we interrogated the connectivity of RXFP3 containing neurons in the dorsal lateral hypothalamus/zona incerta. Transgenic RXFP3-Cre reporter mice were injected into the dorsal lateral hypothalamus/zona incerta with a cre-dependent anterograde tracer pAAV-hSyn-FLEXmGFP-2A-Synaptophysin-mRuby. This tracer produces GFP expression within axonal arborisations of Cre+ cells, as well as mRuby expression in presynaptic contacts in target regions. Target regions were then confirmed using retrograde tracing. Choleratoxin subunit b conjugated to Alexofluor 488 or 555 was injected into target regions of reporter mice, and the hypothalamus/zona incerta examined for backfilled neurons. Using this approach, three main pathways were identified, extending rostrally to the lateral septum, dorsally to the lateral

habenula, caudally to the periaqueductal grey. Since the projection profile of hypothalamic/zona incerta RXFP3+ neurons has not previously been defined, these experiments are the first to document the efferent connectivity of the lateral hypothalamic/zona incerta RXFP3+ system throughout the brain.

Disclosures: A.B. Simon: None. **A.J. Lawrence:** None.

Poster

159. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

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Topic: G.08. Drugs of Abuse and Addiction

Support: DGAPA-PAPIIT Grant IA205218
DGAPA-PAPIIT Grant IN215218
DGAPA-PAPIIT Grant IN217918

Title: Potential role of lateral habenula CB2 expression on impulsive behaviours and alcohol consumption in rats

Authors: *Y. A. ALVARADO RAMÍREZ, B. M. ROMERO TORRES[1], D. A. RANGEL RANGEL[1], A. E. RUIZ-CONTRERAS[2], O. PROSPERO-GARCIA[3], M. MENDEZ DIAZ[4]
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Abstract: During adolescence there are a disparity between the functioning of prefrontal cortex and nucleus accumbens, that may be causing adolescents exhibit impulsive and risky behavior, i.e. use of drug of abuse. The activity of the LHb inhibits behaviors leading to obtaining a reinforcer. CB1 and CB2 expressed in other brain structures regulate motivated behaviors, depending on the age of the subject. However, there is no expression of CB1 in the LHb. Our goal is to assess whether there is a relationship between impulse control, alcohol consumption and the expression of CB2 receptor in the LHb. Three groups of Wistar rats, adolescents (PND42), adults (PND120) and aged (PND390), were utilized. They were submitted to delay discounting reinforcer (DDR) protocols and alcohol conditioned place preference (CPP). Three additional groups were used to evaluate CB2 expression in the LHb by means of immufluorescence. Results. DDR showed that adolescents rats were unable to wait for the major reinforcer (chocolate flavored pellets) delivered with a delay of 16 s vs. adults and aged rats. On the other hand, during CPP, adolescents consume more alcohol (g of alcohol / g of body weigh) that adults and aged rats and develop a stronger alcohol CPP. In addition, CB2 expression in LHb was confirmed. This evidence suggests that adolescent rats exhibit a greater amount of

times impulsive behavior. Likewise, they exhibit a great preference for alcohol consumption. CB2 in LHb may be involved in regulating impulsive behavior and reward system.

Disclosures: Y.A. Alvarado Ramírez: None. **B.M. Romero Torres:** None. **D.A. Rangel Rangel:** None. **A.E. Ruiz-Contreras:** None. **O. Prospero-Garcia:** None. **M. Mendez Diaz:** None.

Poster

159. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms I

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Title: D5 dopamine receptor in ethanol-induced behavioral disinhibition

Authors: *I. MERCADO, K.-A. HAN
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Abstract: Alcohol affects multiple behaviors such as motor coordination, learning and memory, cognition, and disinhibition. Previous studies have demonstrated that dopamine, a major neuromodulator, mediates several ethanol-induced behaviors including hyper-locomotor activity, behavioral disinhibition and sensitization. The goal of this research is to identify the role of DAMB, the insect ortholog of the mammalian D5 dopamine receptor, in ethanol-induced behaviors in the fruit fly *Drosophila melanogaster*. *Drosophila* and mammals have comparable dopamine biosynthetic enzymes, receptors and transporter and exhibit similar behavioral responses to ethanol. We used the FlyPub assay to monitor disinhibited sexual behaviors under the influence of ethanol. We found that the flies deficient in DAMB showed enhanced courtship disinhibition and the phenotype was rescued by reinstating the receptor in the mushroom body but not in other neurons known to be important for dopamine functions. This study reveals a novel function of the D5 dopamine receptor for the ethanol-induced behavioral response. This work was supported by the NIH grants: NIMHD 2G12MD007592, NIAAA R15AA020996 and NIAAA R15AA020996-01S1.

Disclosures: I. Mercado: None. **K. Han:** None.

Poster

159. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms I

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Program #/Poster #: 159.04/YY15

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant 4T32 GM008471-24
NIH Grant 4T32 DA7234-30

Title: Cell-specific targeting of individual nicotinic acetylcholine receptor subunits in alcohol and nicotine reward

Authors: *J. K. MOEN[1,2], J. C. TOUCHETTE[3], J. J. MAERTENS[3], A. M. LEE[3]
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Abstract: Nicotinic acetylcholine receptors (nAChRs) in the mesolimbic reward system are the major target of nicotine, the main psychoactive component of tobacco. Additionally, recent evidence points to a critical role of neuronal nAChRs in mediating the rewarding properties of alcohol. nAChRs mediate alcohol enhancement of dopamine (DA) neuron excitability and alcohol-induced DA release in the nucleus accumbens. Drugs targeting nAChRs can also modulate alcohol physiology and consumption patterns, as infusion of the nonspecific nAChR antagonist mecamylamine directly into the ventral tegmental area (VTA) reduces alcohol consumption and attenuates alcohol-induced dopamine release in rodents, while the nAChR partial agonist varenicline reduces alcohol consumption in both animal models and humans. Studies to date have utilized a combination of genetic knockout mice alongside pharmacological agents to investigate the role of specific nAChR subunits in alcohol reward and consumption. While useful for delineating the global contribution of nAChRs, these techniques cannot provide information on how nAChR subunits in different cell populations mediate alcohol's rewarding mechanism. High-affinity nAChR subunits, such as α4 and α6, are present on both DA projection neurons and local inhibitory GABA neurons in the VTA, and their relative contributions to the rewarding properties of alcohol remain unknown. We have developed a gene delivery approach that allows for targeted genetic knockdown of specific nAChR subunits in select cell populations. Here, we show that viral delivery of Cre-dependent short-hairpin RNA (shRNA) into the VTA of mice expressing Cre selectively in DA neurons (DAT-Cre mice) reduces α6 nAChR subunit mRNA expression without impacting α4 transcript levels compared to mice injected with a control scramble virus (n=8-9 mice/group). Additionally, our preliminary data indicate that this manipulation is sufficient to modulate chronic, voluntary alcohol and nicotine consumption in both male and female mice (n=3-4 mice/sex/group). Our approach provides a novel strategy for investigating the cell-specific role of nAChR subunits in reward circuitry. This technique will provide new and fundamental information on the pharmacological

mechanism of alcohol reward, as well as identifying potential nAChR targets for alcohol cessation drugs.

Disclosures: J.K. Moen: None. **J.C. Touchette:** None. **J.J. Maertens:** None. **A.M. Lee:** None.

Poster

159. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms I

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Topic: G.08. Drugs of Abuse and Addiction

Support: ZonMw Grant 912.14.093

Title: Investigating the neural mechanisms of compulsive reward seeking in rats

Authors: A. M. MINNAARD[1], J. A. S. SMEETS[1], H. M. B. LESSCHER[1], G. M. J. RAMAKERS[2], R. A. H. ADAN[2], *L. J. VANDERSCHUREN[1]
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Abstract: Substance addiction is a chronic relapsing brain disorder, characterised by loss of control over substance use. Restoring control over substance use is a promising treatment strategy for addictive disorders. However, the neural underpinnings of loss of control are incompletely understood. In animal studies, loss of control over substance intake has been operationalised as seeking behaviour that is resistant to punishment. Importantly, the negative consequences of substance use are typically unpredictable in humans. Therefore, we aimed to develop a novel paradigm that incorporates unpredictable negative consequences to study compulsive reward seeking in rats. To this aim, rats were trained to lever press for alcohol or sucrose. They were subsequently tested in a task in which presentation of a tone cue signalled a probabilistic footshock upon responding. We observed reduced reward seeking in sessions during which the tone cue was presented compared to baseline sessions without a tone cue. Since previous studies have implicated dysfunction of the prelimbic prefrontal cortex (PrL) in compulsive reward seeking, we examined the effect of pharmacological inactivation of the PrL in reward seeking under threat of adversity. Indeed, inactivation of the PrL inhibited suppression of responding for alcohol, suggesting that suppression of responding under threat of adversity depends on functional activity in the PrL. In ongoing experiments, we further study the neural underpinnings of reward seeking under threat of adversity.

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Poster

159. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms I

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Program #/Poster #: 159.06/YY17

Topic: G.08. Drugs of Abuse and Addiction

Support: ZonMw Grant 91214093

Title: Habitual alcohol seeking in rats: Individual differences and the role of the dorsolateral striatum

Authors: *H. M. LESSCHER[1], J. A. S. SMEETS[1], A. M. MINNAARD[1], G. M. J. RAMAKERS[2], R. A. H. ADAN[2], L. J. M. J. VANDERSCHUREN[1]
[1]UU, Fac. Vet. Med., Utrecht, Netherlands; [2]Brain Ctr. Rudolf Magnus, Univ. Med. Ctr. Utrecht, Utrecht, Netherlands

Abstract: Alcohol use disorder (AUD) is characterized by excessive alcohol use and a loss of control over alcohol intake. Considering that only a subset of individuals who drink alcohol will develop AUD, it is important to investigate factors that contribute to its development. It has previously been shown that the dorsolateral striatum (DLS) is involved in the shift from goal-directed to inflexible, habitual drug seeking behaviour. We therefore examined the relation between individual variation in alcohol consumption and the development of habitual alcohol seeking in rats. We also investigated the role of the DLS in the gradual shift towards habitual alcohol seeking behaviour. To this aim, voluntary home-cage alcohol consumption was monitored in a large cohort of outbred Lister-Hooded rats during a two-month intermittent access two-bottle choice paradigm. We found considerable individual variation in alcohol consumption. Next, all animals were bilaterally implanted with cannulas aimed at the DLS and trained to self-administer alcohol. To investigate goal-directed vs habitual alcohol seeking, we performed outcome devaluation by pre-feeding animals with alcohol before operant training. Our results indicate that alcohol seeking behaviour was goal-directed after limited training, since outcome devaluation reduced responding for alcohol, which was not altered by pharmacological inactivation of the DLS. We hypothesize that animals will become insensitive to outcome devaluation after extended training, which depends on functional activity within the DLS. Furthermore, we predict that animals with a high level of voluntary alcohol intake will show a faster progression from goal-directed to habitual alcohol seeking.

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Poster

159. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 159.07/YY18

Topic: G.08. Drugs of Abuse and Addiction

Support: AA019682
AA026537
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Title: Effects of mineralocorticoid receptor antagonism on alcohol self-administration and seeking behavior in female and male rats

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Abstract: Cortisol/corticosterone and the hypothalamic-pituitary-adrenal (HPA) axis serve an important role in modulating alcohol drinking behaviors. To date most alcohol research has focused on the functional involvement of corticosterone and the glucocorticoid receptor (GR), the primary receptor for corticosterone. Recent studies have indicated that the related mineralocorticoid receptor (MR), which binds both corticosterone and aldosterone, may also play a role in alcohol drinking. Therefore, the purpose of the present study was to test the functional role of MR signaling in alcohol self-administration via pharmacological antagonism of the MR with spironolactone. Male and female Long-Evans rats were trained to self-administer a sweetened alcohol solution (15% (v/v) alcohol + 2% (w/v) sucrose). The effects of spironolactone (0, 10, 25, 50 mg/kg; IP) were tested on alcohol self-administration and under "probe extinction" conditions to measure the effects of spironolactone on alcohol-seeking. Parallel experiments in sucrose self-administration trained rats were used to confirm the specificity of spironolactone effects to an alcohol reinforcer. In female rats spironolactone (50 mg/kg) reduced alcohol self-administration and alcohol-seeking. In male rats spironolactone (25 and 50 mg/kg) reduced alcohol self-administration, but not alcohol-seeking behavior. There was no effect of spironolactone on sucrose self-administration or sucrose-seeking in male or female rats. Under different conditions spironolactone-induced reductions in locomotor behavior were observed in the alcohol self-administration groups. These studies add to growing evidence that the MR is involved in alcohol drinking, while underscoring the importance of studying both male and female animals.

Disclosures: V. Makhijani: None. **K. Van Voorhies:** None. **J. Besheer:** None.

Poster

159. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms I

Location: SDCC Halls B-H

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH grants [U01 AA10760 (JCC, ARO)
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NIH and Office of Scientific Workforce Diversity Grant RL5GM118963
NIH and Office of Scientific Workforce Diversity Grant TL4GM118965

Title: Effects of chronic chemogenetic stimulation of nucleus accumbens on binge drinking and transcriptome

Authors: *D. Y. POZHIDAYEVA[1], K. G. TOWNSLEY[2], E. J. FIRSICK[2], A. T. D. TRAN[2], O. D. IANCU[2], A. R. OZBURN[2]
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Abstract: We previously found that stimulating activity in the nucleus accumbens (NAc) reduced binge-like alcohol drinking in mice. We manipulated the NAc using clozapine-n-oxide (CNO) and Designer Receptors Exclusively Activated by Designer Drugs (DREADDs). In a subsequent study, we tested the hypothesis that chronic administration of CNO (to stimulate excitatory DREADDs expressed in the NAc) could produce lasting reductions in binge drinking (as compared with mice receiving vehicle). We observed that 4 weeks of CNO administration resulted in reductions in binge-like drinking that lasted at least 1 week. Based on these results, we hypothesized that transcriptional changes may underlie the observed behavioral plasticity. To test this hypothesis, we conducted a study to further explore the effects of NAc stimulation on behavior and gene expression in mice selectively bred to drink to intoxication (High Drinking in the Dark; HDID mice).
We stereotaxically injected AAVCre and AAV2DIO-hM3Dq into the NAc and subsequently measured ethanol intake for 6 weeks using the Drinking in the Dark (DID) paradigm. We employed 2 experimental conditions [drinking fluid (ethanol or water) and treatment (CNO or vehicle; IP)] with 11-12 mice/group. Vehicle groups were injected with 1% DMSO in saline daily for 6 weeks. For CNO groups, mice were treated with vehicle during weeks 1 (baseline) and 6 (washout) and CNO during weeks 2-5. At the end of the study we isolated NAc RNA and performed RNA Seq.
To explore quantitative changes across samples in the NAc transcriptome and determine target

genes associated with binge-like drinking, we performed Differential Expression analysis (DE) and Weighted gene co-expression analysis (WGCNA). RNA-Seq sample reads were aligned to the *Mus musculus* genome (via STAR aligner), filtered and normalized to produce a count matrix. Using DESeq2, the count matrix was used to determine significantly up or down regulated genes based on p-value threshold. WGCNA was then used to describe correlation patterns among expressed genes. To identify modules (co-regulated genes) and hubs (genes correlating strongly with a significant number of related expressed modules) the count matrix was processed with the WGCNA package in R. By using these analyses, we are working to identify changes in gene expression related to harmful binge-like drinking and CNO/DREADD-induced reductions in binge-drinking. Our results suggest that chronically increasing NAc activity (via CNO/DREADDs) can induce molecular and behavioral plasticity.

Disclosures: D.Y. Pozhidayeva: None. **K.G. Townsley:** None. **E.J. Firsick:** None. **A.T.D. Tran:** None. **O.D. Iancu:** None. **A.R. Ozburn:** 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.; Portland Veterans Affairs Medical Center.

Poster

159. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 159.09/YY20

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant P50AA010761
NIH Grant R37AA009986

Title: Ethanol inhibition of lateral orbitofrontal cortex neuron firing is mediated via D1 receptor induced release of glycine

Authors: S. NIMITVILAI[1], *J. J. WOODWARD[2]
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Abstract: Alcohol use disorders (AUDs) are associated with dysfunction of frontal cortical areas including the orbitofrontal cortex (OFC). Although most studies of alcohol dependence have focused on changes in neuronal function, data in the literature suggests that astrocyte pathology is also an important feature of AUDs. For example, astrocyte density in the OFC is reduced in rats following ethanol exposure and in human alcoholics, this decrease positively correlates with the relapse-like motivation to self-administer ethanol. Recent findings from this laboratory demonstrate that, in naïve male and female mice, ethanol reduces the intrinsic excitability of

neurons in the lateral OFC (lOFC) via activation of strychnine-sensitive glycine receptors. Following chronic intermittent ethanol (CIE) exposure, lOFC neuronal excitability is enhanced for up to two weeks following withdrawal. CIE treatment also diminishes the inhibitory effect of acute ethanol on lOFC spiking by blunting its activation of a glycine receptor-dependent tonic current. Although the mechanism linking ethanol to the release of glycine is currently unknown, several studies have identified astrocytes as a source of neurotransmitters including glycine. Interestingly, dopamine has been shown to reverse the astrocytic glycine transporter 1 (GlyT1) resulting in elevated extracellular levels of glycine. As we recently reported that ethanol, dopamine or the D1 agonist SKF81297 all increase a glycine-mediated tonic current in lOFC neurons,. we used slice electrophysiology and examined whether astrocytic glycine is involved in ethanol inhibition of spike firing of lOFC neurons and if ethanol induced release of glycine is dopamine receptor dependent. Ethanol, applied acutely, produced a significant reduction in current-evoked spiking of lOFC neurons and this inhibition was blocked by the D1 receptor antagonist SCH23390, but not by the D2 receptor antagonist sulpiride. Ethanol inhibition of spiking was also suppressed by the non-transportable GlyT1 blocker NFPS suggesting an astrocytic source of glycine. Incubating OFC slices with the Krebs cycle inhibitor fluorocitrate, that inhibits glial cell activity, dramatically decreased the inhibitory effect of acute ethanol. The results of this study suggest that D1R-mediated release of glycine from astrocytes is critical for ethanol inhibition of lOFC neuron firing. Finding ways to restore astrocyte function might thus lead to novel interventions for treatment of OFC-dependent behavioral deficits in alcohol-dependent individuals.

Disclosures: S. Nimitvilai: None. **J.J. Woodward:** None.

Poster

159. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms I

Location: SDCC Halls B-H

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant AA007611
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NIH Grant AA010721
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Title: Chronic alcohol drinking enhances basal extracellular glutamate levels within the prelimbic cortex of alcohol-preferring (P) rats

Authors: *Z. DING[1], C. INGRAHAM[2], A. M. SENTIR[2], E. A. ENGLEMAN[3], W. J. MCBRIDE[2]

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Abstract: A hyper-glutamatergic state has been associated with the development of alcohol use disorders. Our previous study indicated that chronic alcohol drinking increased basal extracellular glutamate levels within the nucleus accumbens shell and posterior ventral tegmental area. However, the effects of alcohol drinking on extracellular glutamate levels within the medial prefrontal cortex (mPFC), a key regions of the mesocorticolimbic system, remain unknown. The current study tested the hypothesis that chronic ethanol drinking would produce persistent increase of basal glutamate transmission within the mPFC. The first experiment included two groups of female alcohol-preferring (P) rats, one receiving water for 8-10 weeks and the other receiving ethanol (water vs 15% ethanol) for 8-10 weeks. Following the last drinking session, quantitative no-net-flux microdialysis was conducted to determine basal extracellular glutamate concentrations and clearances in the prelimbic (PL) region of the mPFC. The second study included two groups of P rats with one given access to ethanol (water vs 15%) for 8-10 weeks then undergoing abstinence for 2 weeks, and the other one drinking water throughout. No-net-flux microdialysis was conducted in the PL cortex following the last abstinence session. Chronic alcohol drinking by P rats significantly increased basal extracellular glutamate concentrations within the PL cortex (6.7 ± 0.5 vs 4.6 ± 0.4 µM, $p = 0.03$) compared to water drinking. In addition, the extraction fractions of glutamate, an index for glutamate clearance, were significantly reduced in ethanol-drinking rats (36 ± 3% vs 27 ± 2%, $p = 0.01$). Following abstinence, extracellular glutamate concentrations in ethanol-experienced rats (4.9 ± 0.4 µM) were similar to the levels seen in water-drinking rats (4.6 ± 0.4 µM; $p = 0.71$). However, the extraction fractions were significantly greater in ethanol-experienced rats than those in water-drinking rats (38 ± 2% vs 46 ± 2%, $p = 0.02$). These results indicate that chronic alcohol drinking by P rats increased extracellular glutamate levels within the PL cortex, whereas alcohol abstinence returned the extracellular glutamate to control levels. The changes in extracellular glutamate levels were mediated through, at least in part, alterations in glutamate clearance. Overall, this study, along with our previous findings, suggests that a hyper-glutamatergic state, represented by increased basal extracellular glutamate levels within the MCL system, may contribute to the development and maintenance of alcohol drinking. (AA007611, AA012262, AA010721, DA044242)

Disclosures: Z. Ding: None. **C. Ingraham:** None. **A.M. Sentir:** None. **E.A. Engleman:** None. **W.J. McBride:** None.

Poster

159. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms I

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Program #/Poster #: 159.11/YY22

Topic: G.08. Drugs of Abuse and Addiction

Support: BX001271

Title: Ethanol-induced presynaptic GABA release depends on internal calcium store homeostasis in the mouse central amygdala nucleus

Authors: Q. LI[1], *S. D. MOORE[2]
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Abstract: It has previously been demonstrated that ethanol can enhance GABA release in various brain regions via presynaptic mechanisms. However, the presynaptic action of ethanol on inhibitory GABA release is still not well understood. Calcium is required for neurotransmitter release from presynaptic terminals, with either external calcium influx and internal calcium store release as the primary calcium sources. In acute slice preparations, we investigated the role of internal and external calcium in the presynaptic terminal of the principal neurons within the central amygdala nucleus (CeA) in acute ethanol induced-enhancement of GABA releases in male 2 month old mice. Miniature inhibitory postsynaptic currents (mIPSCs) were isolated from CeA neurons held at -70mV in the presence of TTX (1μM), AP-5 (50μM), DNQX (20μM) and CGP55845 (1μM). mIPSCs were completely blocked with bath application of picrotoxin (75μM), confirming that the mIPSCs were mediated by $GABA_A$ receptors. Ethanol (25mM (n=12), 50mM (n=15), 75mM (n=9) and 100mM (n=5 neurons) dose-dependently increased the mean frequency of mIPSCs, though amplitudes were not altered. Ethanol (50 and 100mM) induced-increases in the frequency of mIPSCs were antagonized by ryanodine receptor (RyR) blockade by dantrolene (10 (n=11) and 100μM (n=10). Blockade of IP_3R with the antagonist 2-APB (42μM) (n=5) diminished ethanol-enhancement of mIPSC frequency. When the endoplasmic reticulum calcium pump (SERCA) was blocked with cyclopiazonic acid (CPA, 20μM), ethanol (50mM (n=8) and 100mM (n=3) failed to increase the frequency of mIPSCs. These data suggested that calcium released from internal calcium stores contributes to presynaptic release of GABA induced by ethanol. However, the external calcium influx process was apparently not involved in ethanol mediated GABA release since ethanol (50mM (n=8) and 100mM (n=7)) still enhanced the frequency of mIPSCs in the presence of non-selective calcium blocker cadmium (200 and 300μM). Furthermore, blocking calcium release from mitochondria or neurotransmitter release via exocytosis with ruthenium red (1μM, n=5) attenuated ethanol (50 and 100mM) induced enhancement of mIPSCs. Our preliminary results suggest that, by mobilizing calcium originated from various sources and by disrupting neurotransmitter exocytosis process, ethanol regulates inhibitory synaptic function within the CeA region, a critical brain area involved in drugs of abuse and addiction.

Disclosures: Q. Li: None. **S.D. Moore:** None.

Poster

159. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms I

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Diversity Supplement R01AA022448
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USC School of Pharmacy

Title: Subunit-dependent cross-talk between p2x4 and nmda receptors

Authors: *L. RODRIGUEZ[1], J. CHEN[2], T. HASAN[2], E. JEONG[2], M. RYU[2], A. GUAN[2], J. LIANG[3], L. ASATRYAN[2], J. J. WOODWARD[5], M. S. BRODIE[6], D. L. DAVIES[4]
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Abstract: Purinergic P2X4 receptors (P2X4Rs) are non-selective, cation channels that are gated by ATP, and have recently become recognized as a target for the development of drugs to prevent and/or treat alcohol use disorder (AUD). This hypothesis is derived from genetic, pharmacological and behavior¬al evidence reporting an inverse relationship between ethanol (EtOH) intake and P2X4R activity where a reduction in P2X4R expression leads to an increase in EtOH intake. EtOH also affects excitatory ionotropic glutamate (NMDA) receptors, where EtOH also leads to a reduction in cation flux. Both receptors are expressed in areas of the brain associated with 1) ethanol intake and 2) behaviors that are strongly associated with reward, memory and addiction (e.g., ventral tegmental area, nucleus accumbens, hippocampus). NMDAR ethanol sensitivity has been shown to depend on the combination of the GluN2 subunits, which have distinct physiological and behavioral functions. Much is known regarding the effects of EtOH on P2X4 and NMDA receptors -- tested individually. However, there is minimal information regarding P2X4 and NMDA receptor interactions (i.e. “cross-talk” between receptors) or how interactions of these two receptors affects receptor/ethanol-induced signaling. We hypothesized that receptor cross-talk plays an important role in modulating behavioral and/or sensorimotor functions associated with addiction. We tested this hypothesis by investigating interactions between P2X4Rs and NMDARs expressed in X. laevis oocytes using two-electrode voltage-clamp electrophysiology, site-directed mutagenesis, and cell-surface trafficking studies. We used cell imaging techniques to examine the nature of this cross-talk interaction between P2X4Rs and the different GluN2 subunits. We found that P2X4 and NMDA receptors functioned properly (e.g., expected individual channel activity, EC50, etc.) in oocytes co-expressing P2X4 and NMDA receptors. However, when applying agonists sequentially, (glutamate followed by

ATP, or vice versa) we found that ATP activity via P2X4Rs appeared to suppress signaling in NMDARs containing the GluN2A and GluN2B subunits, but not the GluN2C subunit. On the other hand, NMDAR induced signaling did not appear to significantly alter P2X4R activity. Furthermore, we found that when P2X4R stimulation precedes NMDAR stimulation, NMDAR responses were significantly reduced in a time-dependent manner. Taken together, this initial work provides preliminary insight into unrecognized signaling interactions between P2X4 and NMDA receptors. We are currently investigating the effects of EtOH on P2X4 and NMDA cross-talk.

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Poster

159. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms I

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Topic: G.08. Drugs of Abuse and Addiction

Support: GAP304-12-G069
European Union Horizon 2020 (668863; SyBil-AA)

Title: Increased brain diffusivity and reduction in microglia in a rat model of alcohol abuse

Authors: *L. VARGOVA[1,2], I. VORISEK[2], R. CICCOCIOPPO[3], W. H. SOMMER[4], S. CANALS[5]
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Abstract: Previous magnetic resonance imaging studies found changes in the brain diffusivity after chronic alcohol intake. However, the results are not consistent and the connection between alterations of brain diffusion properties and microstructural changes of the tissue is not fully understood. Using real-time iontophoresis, we measured the brain extracellular space diffusion parameters extracellular volume fraction (α) and tortuosity (λ) in alcohol preferring rats of the marchigan sardinian line (msP), a well-established model of chronic high alcohol consumption, and correlated the results with structural changes of astrocytes, microglia and expression of chondroitinsulphate proteoglycans (CSPG). Rats were allowed a 2-bottle choice between a 10% Ethanol solution (*EtOH*) and water for 4 weeks resulting in an average consumption above 5

g/kg/day; control animals had access to water only. The extracellular space diffusion parameters were determined *in vivo* right after the 4 weeks of water or alcohol drinking in the sensorimotor cortex. Structural tissue changes were evaluated in all animals and also after 1 week of abstinence (*Abs).*
Basal values in the control cortical tissue were $\lambda = 1.50 \pm 0.02$, $\alpha = 0.196 \pm 0.007$. Four weeks after alcohol drinking, we observed a decrease in λ as well as α values (1.40 ± 0.02 and 0.176 ± 0.004, respectively). Compared to controls, the most striking difference was a profound microglia reaction as demonstrated by a decrease in microglial cell number (Iba1+ cells) and atrophic processes in both *EtOH* and *Abs* groups. Evaluation of GFAP positive astrocytes revealed enlargement of average surface of an individual astrocyte in cortical layer V, which persisted in the *Abs* group with marginal significance. Number of GFAP+ cells started to decline in *EtOH* but a significant decrease we found in *Abs* group only. CSPG positivity increase in the both *EtOH* and *Abs* tissue was only marginally affected.
Overall, our results indicate that high levels of alcohol consumption induce profound alterations in the microstructure of brain parenchyma, especially in glial cells. Even though the volume of the extracellular space is smaller due to an increased astrocyte size, a strong glial atrophy translates in increased diffusivity properties that correlate with a reduction of the diffusion barriers.

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Poster

159. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms I

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Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 159.14/ZZ1

Topic: G.08. Drugs of Abuse and Addiction

Support: Radford University, Department of Psychology

Title: Fecal microbiota transplantation: Targeting the gut-brain axis in alcohol use

Authors: R. M. CAIN, H. C. STEWART, J. E. CAUGHRON, J. E. ASPELMEIER, P. A. JACKSON, *D. M. HAYES
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Abstract: According to a recent survey, over half of adult Americans are current consumers of alcohol. Alcohol use is thought to alter the intestinal microbiota and increase the permeability of the intestinal epithelial barrier in a way that elicits a gut-mediated inflammatory response. This inflammatory response is associated with heightened levels of alcohol craving. Fecal microbiota transplantation (FMT) has been shown to successfully reestablish a balanced, healthy gut

microbiota in human patients with gastrointestinal disorders. FMT may restore the distrubed microbiota in alcohol users, prevent gut-mediated inflammation, and ultimately reduce alcohol cravings. In the present study, adult, male, Sprague-Dawley rats were exposed to alcohol during multiple repetitions of a voluntary consumption, drinking-in-the-dark (DID) paradigm shown to induce relevant levels of alcohol consumption. Following DID, omeprazole was administered to increase bacterial survival in the stomach prior to FMT containing either donor fecal matter or their own. Subsequently, rats were given a two-bottle choice paradigm with water and 10% alcohol solution to determine their alcohol consumption levels. Due to the known relationship between anxiety and alcohol consumption as well as the known effects of anxiety on the gut microbiota, anxiety-like behavior was measured on an elevated plus-maze (EPM) apparatus. A 2 x 2 repeated-measures factorial ANOVA was used to test the hypothesis that alcohol consumption levels in rats originally exposed to alcohol who received donor FMT would be significantly lower than in similar rats who received their own fecal matter. Preliminary findings revealed that there was a significant effect of day on consumption levels ($p<.001$). However, there was no main effect of alcohol ($p=.817$) or FMT ($p=.061$) on two-bottle choice alcohol consumption, nor was there an interaction between FMT and alcohol ($p=.703$). Additionally, analysis of time spent in the open arms of the elevated plus-maze failed to reveal a main effect of alcohol ($p=.620$), FMT ($p=.076$), or an interaction ($p=.825$). Additional data is currently being collected from a second cohort of animals. Further analyses may reveal important interactions between alcohol and the gut-brain axis.

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Poster

159. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms I

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Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 159.15/ZZ2

Topic: G.08. Drugs of Abuse and Addiction

Support: CONACyT 82728

Title: Dose-dependent effects of ethanol on pro-enkephalin mRNA expression in the rat brain

Authors: *K. HERNANDEZ FONSECA, M. MENDEZ
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Abstract: Alcoholism is one of the main health problems over the word. Alcohol (ethanol) abuse and dependence seriously affect the function of several organs in the body, including the brain. Ethanol exhibits biphasic behavioural effects in rodents and humans. Low alcohol doses produce psychomotor activation and euphoria, whereas high doses induce locomotor inhibition and

sedation. Alcohol and opioid peptides share numerous pharmacological properties and exhibit similar behavioural effects in animals and humans. The endogenous opioid system is associated with a variety of functions, including alcohol reward and reinforcement. Ethanol reinforcing properties and high alcohol drinking behaviour may be mediated, at least in part, by the ethanol-induced activation of the endogenous opioid system. This activation may include selective alterations of opioidergic transmission at the level of opioid peptide biosynthesis, processing and release, as well as ligand binding to opioid receptors. The aim of this work was to investigate the effects of different doses of ethanol on Pro-enkephalin (Pro-enk) mRNA expression in different brain areas. Male Wistar rats were administrated with saline or different doses of ethanol (0.5, 0.75, 1.0, 1.5, 2.0, 3.0 g/kg i.p.) and 30 min later distinct brain areas were dissected: prefrontal cortex (PFC), nucleus accumbens (NAcc), anterior-medial (amCP) and medial-posterior (mpCP) regions of the caudate-putamen (CP), amygdala, hypothalamus and hippocampus. Pro-enk mRNA levels were quantitated by real time PCR. Pro-enk mRNA expression was increased by high ethanol doses in the PFC, amCP, hippocampus, hypothalamus and amygdala. In contrast, ethanol induced biphasic effects in the NAcc and mpCP: moderate doses (1.5 and 2.0 g/kg) increased, but high doses (3.0 g/kg) decreased mRNA expression. Our results indicate that ethanol-induced changes in Pro-enk mRNA expression are dose-dependent and region-specific. These alterations could be involved in both the stimulant and sedative effects of the drug.

Disclosures: M. Mendez: None.

Poster

159. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms I

Location: SDCC Halls B-H

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Program #/Poster #: 159.16/ZZ3

Topic: G.08. Drugs of Abuse and Addiction

Support: Department of Psychology and College of Arts and Sciences at Miami University

Title: A chemogenetic investigation of striatal and cortical contributions to aversion-resistant alcohol drinking

Authors: *E. A. SNEDDON, J. FRANKEL, A. NADER, K. SCHUH, J. SETTERS, A. K. RADKE
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Abstract: Alcohol use disorder (AUD) involves drinking despite negative consequences. This type of inflexible drinking can be modeled in rodents by adding quinine, a bitter tasting substance to a 15% ethanol (EtOH) solution to devalue it in a drinking in the dark (DID) paradigm. Previous literature has suggested that both striatal and cortical regions contribute to aversion-resistant alcohol-drinking. The present study researched aversion-resistant intake in

both male and female C57BL/ 6J mice using a two-bottle, limited access DID paradigm. Chemogenetic (DREADD) technology was used to inhibit or excite specific cortical and striatal regions during aversion-resistant drinking. For all experiments, the subjects self-administered EtOH (15%) in a two-bottle limited access protocol for five days a week for two weeks. In the third week, 100 μM quinine hemisulfate was added to the EtOH bottle for three consecutive days. The subjects were injected with clozapine-n-oxide (CNO) or vehicle on days 1 and 3 of quinine exposure. Experiments involving inhibition used the viral vector hSyn-hM4Di-mCherry-AAV (Addgene). For excitation, hSyn-hM3Dq-mCherry-AAV was expressed. Experiment 1 (n=12 males, n=12 females) assessed whether inhibition of the nucleus accumbens (NAc) core changed aversion-resistant intake of EtOH. Experiment 2 (n=12 males, n=12 females) looked at whether excitation of the NAc core affected aversion-resistant intake of EtOH. Experiment 3 (n=12 males, n=12 females) assessed whether inhibition of the medial orbitofrontal cortex (mOFC) affected aversion-resistant intake of EtOH. Consistent with previous experiments, we found that females consumed more EtOH than males during escalation and that both male and females exhibited aversion-resistant intake (did not decrease intake despite devaluation with quinine). Experiment 1, revealed that inhibition of the NAc core decreased aversion-resistant intake (CNO group drank less than the vehicle group). NAc core excitation and mOFC inhibition did not significantly change aversion-resistant EtOH consumption. The results of this study provide insight into the specific regions associated with aversion-resistant intake and confirm the important role of the NAc core in aversion-resistant EtOH consumption. Future experiments will further investigate the role of the NAc core in this and other models of compulsive behavior. Research supported by the Department of Psychology and College of Arts and Sciences at Miami University.

Disclosures: E.A. Sneddon: None. **J. Frankel:** None. **A. Nader:** None. **K. Schuh:** None. **J. Setters:** None. **A.K. Radke:** None.

Poster

159. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms I

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Program #/Poster #: 159.17/ZZ4

Topic: G.08. Drugs of Abuse and Addiction

Support: NIAAA Grant R00AA021417

Title: Binge ethanol drinking and the central amygdala: A possible role for a unique population of corticotropin-releasing factor neurons

Authors: *S. ARONI[1], J. M. IRVING[2], H. QADIR[3], D. R. SPARTA[4]
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Abstract: Binge alcohol drinking is a severe public health problem, that has been linked to long-term alteration in brain stress and reward circuitry, and is considered a critical first step in the development of alcoholism. Corticotropin-releasing factor (CRF) cells in the central amygdala (CeA) appear to modulate binge drinking as pharmacological blockades reduce drinking. However, the neural activity of these neurons in acute and repeated binge drinking remain poorly understood. Here we combined optogenetics and *in vivo* electrophysiology to record CeA CRF neurons and define the role of these cells in a rodent model of binge ethanol drinking. We found that CeA CRF units had higher overall firing rate and a more regular pattern of discharge when compared to non-CRF units. Furthermore, we identified four types of CRF units by their ethanol lick-responses: lick-excited (CRF-E), lick-inhibited (CRF-I), lick-predictive (CRF-P), and no response (CRF-NR). We focused our analysis on CRF-NR and CRF-P units, due to their higher prevalence. We observed that CRF-P units had a significantly higher firing rate, as well as the percentage of spikes in bursts compared to CRF-NR units, during ethanol sessions, making them uniquely responsive to alcohol consumption. Next, we further analyzed the firing/burst properties of the CRF-P and CRF-NR units by classifying them into four different groups (using a cutoff of firing rate to 4 Hz, and burst firing to 30%): low-firing/high-burst (LFHB), low-firing/low-burst (LFLB), high-firing/high-burst (HFHB), and high-firing/low-burst (HFLB). We determined that CRF-P had approximately 30% of LFHB, 25% of HFHB, and 45% of LFLB, whereas CRF-NR showed almost the same % of LFHB and HFHB (~10%), and a higher % of LFLB (~80%), suggesting that CRF-P cells are more active during ethanol consumption. We then further investigated if the distinct firing activity of CRF units changes over repeated sessions of ethanol consumption, and we noticed CRF-P units firing rates increase significantly in later ethanol sessions compared to early ethanol sessions, suggesting alcohol-induced plasticity. Finally, we examined the change in the distribution of firing/burst populations over time and found that CRF-P units showed a strong increase in HFHB units and a marked decrease in LFLB units during late sessions. Altogether, our data show an important role for a subclass of CeA CRF neurons in binge drinking and could lead to novel, specific circuit strategies for therapeutic intervention for alcohol use disorder.

Disclosures: S. Aroni: None. **J.M. Irving:** None. **H. Qadir:** None. **D.R. Sparta:** None.

Poster

159. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 159.18/ZZ5

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH AA025718
NIH AA10422

Title: Characterization of a new mouse with an ethanol insensitive alpha 2 glycine receptor

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Abstract: Introduction
Glycine receptors (GlyR) are abundant in spinal cord and brain stem, however, recently they have also been found to be expressed in upper brain regions. In our laboratory, we are studying the expression of GlyR in brain areas of the reward system, specifically evaluating the roles of the alpha 1, 2 and beta subunits of the GlyR in the nucleus accumbens (nAc). Using CRISPR gene editing, we generated a mouse with a mutation (KR385-386AA) in the intracellular loop of the GlyR α2 subunit. This mutation in the α2β receptor conformation inhibits the potentiation of ethanol, but the channel remains functional for glycine activation. Using this mouse model, denoted α2 KI, we tested the hypothesis that this mutation alters electrophysiological and behavioral responses to ethanol.
Results
Using acutely dissociated neurons from nAc of WT (C57BL/6J mice, 6-10 weeks old) and α2 KI mice, a concentration-response curve to glycine was obtained showing EC_{50} values of 32 ± 3 μM for α2 KI and 43 ± 2 μM for WT. Ethanol (50-100 mM) induced a significant potentiation of the current in most nAc neurons from the WT mice. Overall, the effect of ethanol was smaller in neurons from the α2 KI mice compared with the WT. Interestingly, a number of KI nAc neurons were still affected by ethanol (7 of 11 neurons were affected by ethanol).
In behavioral tests, the α2 KI mouse showed no differences in motor activity compared with the WT mouse (qualitative grip test, open field and accelerated rotarod). However, the KI mice recovered faster from acute ethanol exposure in the fixed speed rotarod test (2g/kg) and loss of righting reflex (LORR) (3.5 g/kg). Finally, ongoing experiments examining ethanol consumption (DID, drinking in the dark) showed that the α2 KI mice drank more ethanol as compared to WT.
Conclusions
The KR385-386AA mutation does not impair the activation of the receptor by glycine as demonstrated by the similar concentration-response curve with WT. The results with ethanol suggest the presence of the α2 subunit of the GlyR in nAc. Neurons from nAc that were potentiated by ethanol in the α2 KI suggest the presence of a subunit other than α2, most likely the α1 subunit as indicated by the value of the EC_{50} for the concentration-response curve.
The results from behavior tests showed that α2 KI mice had normal neuromuscular/motor activity. These animals voluntarily drank more ethanol, and they had a faster recovery when injected with a sedative dose of ethanol as compared to WT mice.
These data suggest that the α2β subunit of the GlyR is important in some ethanol-induced behaviors.

Disclosures: S.S. Gallegos: None. **R. Viveros:** None. **L. San Martin:** None. **A. Araya:** None. **G.E. Homanics:** None. **D.M. Lovinger:** None. **L.G. Aguayo:** None.

Poster

159. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms I

Location: SDCC Halls B-H

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH R01AA025718
MRC G0500833

Title: Effects of ethanol on accumbal neurons in glycine receptor alpha2 subunit knockout mice

Authors: *L. S. SAN MARTIN[1], S. GALLEGOS[1], R. VIVEROS[1], R. J. HARVEY[2], J.-M. RIGO[3], B. BRONE[3], L. AGUAYO[1]
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Abstract: The glycine receptor (GlyR), a ligand-gated ion channel, is known to be critical for inhibitory neurotransmission in brainstem and spinal cord. Interestingly, in recent years much more attention has been focused on the presence of GlyRs in supraspinal regions. Previous studies have shown that GlyRs are potentiated by low and clinically-relevant concentrations of ethanol. Recent data from several laboratories have shown that GlyRs are important in the brain reward system and that α_1 and α_2 are the predominant subunits expressed in the nucleus accumbens (nAc). In the present study, we characterized the effects of ethanol in nAc and behavior in GlyR α_2 subunit knockout (Gly$R\alpha_2$KO) mice. Because the Gly$R\alpha_2$ subunit gene is located on the X chromosome, all adult males used in the study were hemizygous (-/Y) for the *Glra2* gene. Gly$R\alpha_2$KO mice exhibited normal brain weight and basal locomotor activity. However, in the accelerating rotarod assay, the latency to fall was significantly increased in Gly$R\alpha_2$KO mice compared to WT mice, indicating a difference in motor skill performance. Using electrophysiological recordings in isolated neurons (up to 8 weeks old), we found that accumbal neurons in Gly$R\alpha_2$KO mice exhibited smaller glycine-evoked currents (~100 pA at 1 mM of glycine) compared to C57BL/6J mice (WT) (~500 pA at 1 mM of glycine). The glycine concentration-response curve showed an EC_{50} of 55 ± 8 μM in these neurons. Compared with WT mice, the effect of ethanol on accumbal neurons was smaller, showing only a small potentiation with 50 and 100 mM ethanol. We also examined the effect of ethanol on sedation and drinking behaviors. When we assayed Gly$R\alpha_2$KO mice for loss of the righting reflex (LORR) in presence of 3.5 g/kg of ethanol, we found a decrease in the onset (1.6 ± 0.1 min) and duration (27± 4 min) of LORR compared to WT mice (2.4 ± 0.2 min and 37 ± 2 min, respectively). Finally, using the drinking in the dark (DID) paradigm, we found that Gly$R\alpha_2$KO

mice have a larger ethanol consumption compared to WT mice. These results support the existence of the α_2 subunit GlyRs in accumbal neurons, since the glycine-evoked currents in GlyRα_2KO mice were markedly smaller. The small potentiation of the glycine current in presence of ethanol also suggests the presence of the GlyRα_1 subunit in nAc neurons. Regarding ethanol effects, we found differences in glycine-activated currents in these neurons as well as changes in behavioral studies, indicating the importance of the GlyRα_2 subunit as a target for alcohol in supraspinal regions. Thus, GlyRs containing the α_2 subunit are a biologically relevant target for the regulation of the reward system and the rewarding properties of ethanol.

Disclosures: L.S. San Martin: None. **S. Gallegos:** None. **R. Viveros:** None. **R.J. Harvey:** None. **J. Rigo:** None. **B. Brone:** None. **L. Aguayo:** None.

Poster

159. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 159.20/ZZ7

Topic: G.08. Drugs of Abuse and Addiction

Support: R37AA019455
R01AA026186

Title: Endocannabinoid control of the insular-BNST circuit regulates negative affective behaviors associated with alcohol abstinence

Authors: *S. CENTANNI[1], T. L. FETTERLY[1], S. PATEL[2], D. G. WINDER[1]
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Abstract: Alcohol use disorders (AUDs) are highly comorbid with psychiatric maladies such as generalized anxiety disorder and major depression. Abstinent alcoholics commonly report stress and negative affect as potent triggers of cravings and relapse, and the severity of negative affect is strongly correlated with relapse susceptibility. Treating these symptoms with traditional antidepressants has yielded inconsistent results, and in some cases these drugs can *increase* alcohol drinking, underscoring the need to identify novel alternative therapeutic beyond traditional antidepressants and anxiolytics through a greater understanding of the complex neurocircuitry regulating the disease state. To this end, we used a mouse model of contingent, continuous access chronic ethanol drinking followed by forced abstinence (CDFA) to study abstinence-induced negative affect. CDFA mice consume significant amounts of ethanol and exhibit abstinence-induced negative affective behaviors. We hypothesized that the bed nucleus of the stria terminalis (BNST), a region heavily implicated in negative affect in both humans and rodents, is critically involved in CDFA-induced negative affect. Indeed, protracted abstinence increased neuronal activity in the BNST, evidenced by increases in c-fos expression, sEPSC

frequency, and *in vivo* calcium transients during affective behavioral tests. The endogenous cannabinoid system is intimately involved in mediating stress responses, therefore this system was explored as a potential pharmacological target for affective disorders. Enhancing endogenous levels of the CB_1R ligand 2-AG with JZL184 blocks CDFA-induced depressive-like symptoms, highlighting a role for eCB-based compounds in CDFA-induced negative affect. Furthermore, we determined that JZL184 mitigates the CDFA-induced increase in sEPSC frequency. Next, we used a channelrhodopsin-assisted mapping strategy to identify excitatory inputs to the BNST that could contribute to abstinence-induced negative affect. We identified the insular cortex (insula), a region involved in regulating interoception as a dense, functional, endocannabinoid-sensitive input to the BNST. Using an inhibitory chemogenetic strategy, we demonstrate that activating insula hM4Di prevents a CDFA-induced increase in BNST c-fos, and moreover, prevents CDFA-induced negative affective behaviors. Collectively, we introduce the insula-BNST circuit as a key regulator of CDFA-induced negative affect and highlights the therapeutic potential of the endocannabinoid system for effectively treating negative affective disorders.

Disclosures: S. Centanni: None. **T.L. Fetterly:** None. **S. Patel:** None. **D.G. Winder:** None.

Poster

159. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms I

Location: SDCC Halls B-H

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Program #/Poster #: 159.21/ZZ8

Topic: G.08. Drugs of Abuse and Addiction

Support: T32GM007347
NIAAA (AA019455)

Title: Identification and characterization of a control network for BNST-projecting insular neurons

Authors: *J. LUCHSINGER[1], S. CENTANNI[2], T. L. FETTERLY[1], D. G. WINDER[1]
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Abstract: While substance abuse is a chronic, relapsing disease with significant economic and personal costs, the biological basis remains elusive. Anxiety disorders and alcohol use disorders are often comorbid conditions, and stress can exacerbate the propensity for relapse. The insula and bed nucleus of the stria terminalis (BNST) have garnered attention for their suspected roles in stress responses and addiction. Our lab has recently obtained data suggesting a key role for insular inputs to the BNST in alcohol abstinence induced negative affective disturbances. To develop a better understanding of the control of this circuit, we here utilized a combination of viral-based anatomical and functional studies in mice to assess the insula-BNST control network.

Using the rabies-based method, tracing the relationships of inputs and outputs (TRIO), which transsynaptically labels cells synapsing on a target population, we have identified several afferent populations upstream of the insula→BNST cells. Populations were identified in both ipsilateral and contralateral cortices while subcortical regions were only found ipsilaterally. Specific thalamic nuclei (i.e. parafascicular and paraventricular) known to project to the insula were also labeled, increasing our confidence in the technique. Likewise, somatosensory cortex is known to send significant projections to the insula and was found to be the region with the highest number of afferent cells, again with ipsilateral outnumbering contralateral inputs. AAV1 anterograde transsynaptic tracing was then combined with channelrhodopsin assisted mapping and electrophysiology as a convergent strategy to both confirm monosynaptic connectivity and initiate functional studies of the circuit. AAV1-Cre (anterogradely crosses the synapse) and AAV5-ChR2-eYFP were injected into a TRIO-identified region while AAVRetro-DIO-TdTomato was injected into the BNST. Cells that receive afferents from the TRIO-identified region and project to the BNST are subsequently fluorescently tagged. TdTomato positive cell bodies were readily observed in the insula using this approach. Excitatory connectivity was confirmed using this strategy in 15 whole cell patch clamped cells in *ex vivo* slices of the insula from 5 mice using optogenetics. Identified cells were then electrophysiologically characterized. This work demonstrates a useful method for efficiently confirming rabies tracing results and provides new insight into potential mechanisms for control of a circuit involved in alcohol abstinence and negative affect.

Disclosures: J. Luchsinger: None. **S. Centanni:** None. **T.L. Fetterly:** None. **D.G. Winder:** None.

Poster

159. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 159.22/ZZ9

Topic: G.08. Drugs of Abuse and Addiction

Support: Pennsylvania State University College of Health and Human Development
Social Science Research Institute (Pennsylvania State University)

Title: The role of somatostatin positive interneurons in the prelimbic cortex in alcohol consumption

Authors: *N. A. CROWLEY[1], S. N. MAGEE[2], A. J. BOURCIER[2]
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Abstract: Alcohol Use Disorders (AUDs) and major depressive disorder (MDD) are highly comorbid disorders, both of which are major health and social concerns—each costing the US an

estimated $200 billion per year (e.g., increased burden on healthcare, social service and criminal justice systems) (Sacks 2015; Greenberg et al 2015). Somatostatin (SST) expressing GABAergic interneurons in the forebrain have long been implicated in MDD (Fee, Banasr, & Sibille, 2017), though their role in AUDs is currently under investigated. Previous work has shown that artificially increasing the function of SST neurons globally in the brain has antidepressant drug treatment-like consequences (Fuchs, Jefferson et al. 2016), and our data demonstrates this mutation also protects against unpredictable chronic mild stress-induced deficits in synaptic transmission (Crowley & Luscher, unpublished). Experiments with these mice showed that these same genetic-induced increases in SST neuronal function (SST-Cre x $\gamma^{2f/f}$ mice) reduce binge drinking in male, but not female, mice utilizing the Drinking in the Dark (DID) model of binge alcohol exposure (Crowley & Luscher, unpublished). Therefore, we further investigated how SST neurons in the prelimbic cortex are altered following DID-binge like ethanol exposure. Utilizing whole-cell patch clamp electrophysiology, we show that ethanol exposure alters GABAergic synaptic drive onto pyramidal neurons in the prelimbic cortex in a sex-dependent manner of C57Bl/6J mice, replicating work published using vaporized alcohol and highlighting the potential role for SST neurons in binge drinking. We further demonstrate how SST neurons in the prelimbic cortex are altered following binge drinking, and by bath application of ethanol in SST-Ai9 mice. Future experiments will investigate whether drugable targets on SST neurons are capable of altering binge drinking behavior in male and female mice.

Disclosures: N.A. Crowley: None. **S.N. Magee:** None. **A.J. Bourcier:** None.

Poster

159. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms I

Location: SDCC Halls B-H

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Program #/Poster #: 159.23/ZZ10

Topic: G.08. Drugs of Abuse and Addiction

Support: CIHR Grant
NIH Grant

Title: The α5 nicotinic acetylcholine receptor subunit may mediate anxiety and alcohol reinforcement through progesterone signaling in the interpeduncular nucleus in a sex specific manner

Authors: *S. CALIGIURI, V. P. MATHIS, M. V. MICIONI DI BONAVENTURA, P. J. KENNY
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Abstract: Introduction: Single nucleotide polymorphisms for CHRNA5, the gene encoding the α5*nicotinic acetylcholine receptor (nAchR) subunit, increase the risk for tobacco and alcohol

co-dependence. To enhance our understanding of this subunit in alcohol reinforcement, we utilized α5*nAchR knockout models in a sex and brain region specific manner.
Methods and Results: Male and female global CHRNA5 KO and WT littermates were trained on a fixed ratio-3 and a progressive ratio schedule for varying doses of ethanol (2.5, 5, 10, 20%) or 5% sucrose (control) (n=100). Female WT mice earned on average 63% more self-administered ethanol rewards and exhibited higher blood alcohol levels versus global α5*nAchR KO females ($p<0.05$). In contrast, WT and KO males were indistinguishable. The elevated ethanol reinforcement may be related to the anxiety-like behavior of the WT females; WT females exhibited indices of higher anxiety-like behavior versus the female CHRNA5 KOs as evidenced by open field, light/dark box, and plasma corticosterone. The mechanism for the sex-specific phenotype may be due to progesterone, as it induces anxiety-like behavior, and is a negative allosteric modulator of α4β2 nAchRs but a positive transcription factor for α5*nAchRs. The ethanol reinforcing properties of progesterone were evidenced by pre-treatment with a progesterone receptor antagonist which reduced ethanol rewards earned in female WT mice but not KOs, and vice versa, progesterone pre-treatment significantly increased ethanol reinforcement in female WTs but not KOs. The effects of progesterone on ethanol reinforcement may be localized to the interpeduncular nucleus (IPN); progesterone pre-treatment resulted in robust c-fos labelling in WT female mice in the IPN, an inhibitory brain region thought to play an important role in social hierarchy, familiarity, and nicotine withdrawal. By contrast, there was little to no c-fos labelling in the IPN of CHRNA5 KO females. CRISPR technology was developed to locally KO α5*nAChRs in the IPN with a 15% *in vivo* mutation rate. Local KO of CHRNA5 in the IPN re-capitulated the differences in anxiety-like behavior between the female WTs and KOs. Ethanol self-administration is currently underway in order to observe the specific role of α5*nAChRs in the IPN of female mice in regard to ethanol reinforcement.
Conclusion: Female binge drinking is an arising issue that can further perpetuate life stressors and end organ damage. We identified that progesterone may act on α5*nAchRs in the IPN to induce anxiety-like behavior and alcohol reinforcement in female rodents.

Disclosures: S. Caligiuri: None. **V.P. Mathis:** None. **M.V. Micioni Di Bonaventura:** None. **P.J. Kenny:** None.

Poster

159. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms I

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Topic: G.08. Drugs of Abuse and Addiction

Support: Finnish Foundation for Alcohol Studies
Finnish Cultural Foundation

Title: Accumbal kappa-opioidergic mechanisms in relapse-like ethanol intake in rats: Effect of kappa-opioid receptor antagonist JDTic

Authors: *J. K. UHARI-VÄÄNÄNEN[1], T. ETELÄINEN[1], P. BÄCKSTRÖM[2], F. I. CARROLL[3], V. OINIO[1,2], A. RAASMAJA[1], K. KIIANMAA[2], P. PIEPPONEN[1]
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Abstract: Relapse to ethanol drinking is a major challenge when treating patients with ethanol use disorders. The currently available drugs for ethanol dependence are not efficient for relapse prevention in all patients. The mechanisms behind relapse to ethanol drinking are also still unclear.
The mesolimbic reward pathway and opioidergic systems that interact with it have been shown to be central in mediating the reinforcing effects of ethanol, in addition to participating in relapse behavior. Especially, the kappa-opioidergic system seems to have a key role in mediating relapse. Therefore, the aim of this study was to examine the role of kappa-opioid receptors in relapse-like drinking in rats in a central brain area of the reward tract, the nucleus accumbens shell. The effect of intra-accumbally administered JDTic, a selective kappa-opioid receptor antagonist, on relapse-like drinking was evaluated using the alcohol deprivation effect (ADE) model. The ADE is defined as a transient increase in ethanol intake over baseline ethanol intake levels after a forced period of abstinence. JDTic was also administered systemically to evaluate the overall effect of kappa-antagonism on the ADE.
Male Long-Evans rats were trained to voluntarily consume 10% (V/V) ethanol solution. After stable ethanol intake baselines had been reached and bilateral guide cannulas had been implanted above the nucleus accumbens shell for intracranial infusions, ADE cycles were initiated. One cycle consisted of 10 days of 90 min access to ethanol followed by 6 days of ethanol deprivation. On re-access to ethanol, the ADE was evaluated. Following a stable ADE, rats received JDTic either intra-accumbally (15 microg/site) or subcutaneously (10 mg/kg, sc) 24h prior to re-access to ethanol, and the effects on the ADE were measured. The effect of the non-selective opioid antagonist naltrexone (0.3 mg/kg, sc, 20 min prior) on the ADE was also evaluated.
According to the results, both intra-accumbally and systemically administered JDTic significantly prevented the ADE on the first day on re-access to ethanol. Systemically administered naltrexone also significantly prevented the occurrence of the ADE on the first day after the deprivation period, in addition to decreasing the consumed amount of ethanol below baseline ethanol intake levels.
These results suggest that kappa-opioidergic mechanisms participate in mediating relapse to ethanol drinking and that accumbal shell kappa-opioid receptors have an important role in ethanol relapse behavior. The results also propose that selective kappa-opioid receptor antagonism might be beneficial for drugs aimed at preventing ethanol relapse.

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Poster

159. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms I

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Program #/Poster #: 159.25/ZZ12

Topic: G.08. Drugs of Abuse and Addiction

Support: T32AA007474
P50AA010761
R37AA009986

Title: Activity in the lateral orbitofrontal cortex during operant self administration of sucrose and ethanol

Authors: *D. A. GIOIA, J. J. WOODWARD
Inst. of Psychiatry, Med. Univ. of South Carolina, Charleston, SC

Abstract: Consumption of alcohol is well-known to alter judgement and impair decision making, partially through disruption of activity within areas of the prefrontal cortex. Additionally, chronic abuse of alcohol has been shown to impair behavioral flexibility leading to the persistent consumption of alcohol despite the emergence of negative physiological and social consequences. Preclinical and clinical studies have demonstrated that this maladaptive behavior is, in part, regulated by the lateral orbitofrontal cortex (lOFC). Our lab has previously shown that neurons in the lOFC are inhibited by acute ethanol exposure and that chronic ethanol exposure impairs plasticity of lOFC neurons. Additionally, we showed that inhibition of the lOFC alters ethanol consumption in alcohol-dependent animals. However, it is unclear how this brain region communicates *in-vivo* during alcohol self-administration. To address this question, we utilized fiber photometry to measure calcium-dependent signaling in lOFC neurons during operant sucrose and ethanol self-administration. Adult male C57BL/6J mice were injected with an adeno associated virus into the lOFC to express the genetically encoded calcium indicator GCaMP6f (Penn Vector Core) and a custom made fiber optic probe was implanted in the same location. This allowed us to record changes in calcium-dependent fluorescence during operant self-administration of 20% sucrose or 10% ethanol on an FR1 schedule of reinforcement. Mice were trained to self-administer solutions using a postprandial drinking protocol. In this protocol, mice are food deprived overnight and then given access to food pellets 1 hour before the operant session, at this time water bottles are removed from cages. In this protocol, mice eat right before the drinking session and become thirsty thus motivating them to drink during the operant session. Using lickometer circuitry, we evaluated GCaMP-dependent signals at discrete time points using TTL pulses time-locked to active lever presses and drinking bouts. We analyzed lOFC activity after lever pressing, during a drinking bout and immediately after consumption. We found that there was no significant change in lOFC activity related to lever pressing. However, the GCaMP

signal decreased during consumption of both solutions followed by a rebound in activity immediately after the drinking bouts. This post-drinking spike in activity likely encodes the value of the reward, as this brain region is known to track changes in reward value. Future studies will determine if the magnitude of this post-drinking signal changes following chronic intermittent ethanol exposure.

Disclosures: D.A. Gioia: None. **J.J. Woodward:** None.

Poster

159. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms I

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The Pearson Center for Alcoholism and Addiction Research
The Sigrid Juselius Foundation

Title: Activation of orphan G protein-coupled receptor GPR139 specifically in the habenula decreases compulsive-like alcohol drinking and hyperalgesia in alcohol-dependent rats

Authors: *D. E. CONLISK, J. KONONOFF, M. KALLUPI, A. J. KIMBROUGH, G. DE GUGLIELMO, O. GEORGE
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Abstract: The orphan G protein-coupled receptor (GPCR) GPR139 is expressed mainly in the brain, with the highest expression in the medial habenula. It has been hypothesized that modulation of GPR139 receptor function may be beneficial in the treatment of some mental disorders, however, behavioral studies have not yet provided causal evidence of the role of GPR139 in brain dysfunction. Because of the high expression of GPR139 in the habenula, a critical brain region in addiction, we hypothesized that GPR139 may play role in alcohol dependence. Thus, a selective, brain penetrant GPR139 agonist (JNJ-63533054) was used and its effect on addiction-like behaviors in alcohol dependent and alcohol intake in nondependent rats was investigated. Systemic administration of JNJ-63533054 (30 mg/kg but not 10 mg/kg, p.o.) decreased the escalation of alcohol self-administration in alcohol-dependent rats, without affecting water or saccharin intake. Moreover, systemic JNJ-63533054 administration decreased withdrawal-induced hyperalgesia, without affecting somatic signs of alcohol withdrawal. Further

analysis demonstrated that JNJ-63533054 was effective only in a subgroup of dependent rats that exhibited compulsive-like drinking. Finally, site-specific microinjection of JNJ-63533054 (0.25 µg/0.5µl) in the habenula but not interpeduncular nucleus reduced both alcohol self-administration and withdrawal-induced hyperalgesia in dependent rats. These results provide robust preclinical evidence that GPR139 may be a novel target for the treatment of alcohol use disorder. Additionally, these findings are a major step forward in our understanding of the function of the orphan receptor GPR139.

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Poster

159. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms I

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NIH Grant U24 AA020929
NIH Grant P50 AA010761
NIH Grant T32 AA007474
VA Medical Research BX000813

Title: Chemogenetic inhibition of dynorphinergic projections from the central amygdala to the BNST attenuates binge-like ethanol consumption male mice

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Abstract: Binge alcohol (ethanol) consumption is a risk factor for the development of alcohol use disorder and is the most common form of excessive drinking. This pattern of voluntary binge drinking can be modeled in rodents using the Drinking in the Dark (DID) paradigm where ethanol consumption during a 4 hr period results in blood ethanol concentrations well above the legal limit of intoxication. We have recently demonstrated a role for the dynorphin/kappa opioid receptor (DYN/KOR) system in binge drinking but the specific circuitry mediating this effect remains unknown. The central amygdala (CeA) has been implicated in excessive ethanol intake and is rich in dynorphinergic neurons (DYN+). Furthermore, a dense population of these neurons project to the bed nucleus of the stria terminalis (BNST), which is rich in KOR and also modulates ethanol intake. This study examined whether dynorphinergic-neurons projecting from the CeA to BNST (CeA-BNST-DYN+) drives binge ethanol drinking behavior. Male prodynorphin-IRES-Cre mice were used to selectively target the CeA-BNST-DYN+ circuit for

chemogenetic inhibition. A Cre-dependent retro-AAV (AAVrg-hSyn-DIO-hM4Di-mCherry) was infused into the BNST allowing for bilateral expression of an inhibitory designer receptor exclusively activated by designer drugs (DREADD) in BNST-DYN+ afferents. Bilateral microinjector guide cannula were then positioned above the CeA for infusion of clozapine-*N*-oxide (CNO) to activate the inhibitory DREADD and silence the CeA-BNST-DYN+ circuit. Upon recovery, mice were introduced to the DID procedure consisting of 2 hr access to a single bottle of 20% ethanol (v/v), 3 hr into the dark cycle, for 3 consecutive days. On the 4th day, mice were given extended access to ethanol in a 4 hr binge session and intake was assessed at both 2 hr and 4 hr. At 30 min prior to this binge session, vehicle (VEH; 1xPBS) or CNO (1 μM/side) were microinjected into the CeA. Chemogenetic inhibition of the CeA-BNST-DYN+ circuit significantly decreased binge ethanol intake at both 2 hr (VEH = 1.42 g/kg, CNO = 0.56 g/kg; $p< 0.01$) and 4 hr (VEH = 3.48 g/kg, CNO = 1.54 g/kg; $p<0.01$) time points. In a complementary study using male C57BL/6J mice, bilateral microinjector guide cannula were positioned over the BNST for infusion of the KOR antagonist, norBNI (2.5μg/side), prior to the binge drinking session. Intra-BNST norBNI infusion significantly decreased ethanol intake at both 2 hr (VEH = 2.36 g/kg, norBNI = 0.51 g/kg; $p<0.001$) and 4 hr (VEH = 4.25 g/kg, norBNI = 1.66 g/kg; $p<0.001$) time points. Together, these data demonstrate that KOR signaling in the BNST via the CeA-BNST-DYN+ pathway contributes to binge-like ethanol drinking.

Disclosures: H.L. Haun: None. **W.C. Griffin:** None. **M.F. Lopez:** None. **H.C. Becker:** None.

Poster

159. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 159.28/ZZ15

Topic: G.02. Motivation

Support: NIH Grant AA017072

Title: Nucleus accumbens shell ox1rs are critical mediators of the excessive alcohol drinking in excessive-binging individuals

Authors: *K. LEI, C. KWOK, V. G. PEDROZO, S. GHOTRA, M. FOUAD, MARY, L. ANDERSON, J. YU, L. NAKAYAMA, F. W. HOPF
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Abstract: Binge alcohol drinking (with excessive levels of intake) and compulsive alcohol intake (where drinking persists despite negative consequences) are both potent and pernicious obstacles to treating Alcohol Use Disorder (AUD). Heavy drinking individuals are responsible for much of the substantial costs of AUD. Thus, by identifying key mechanisms that drive intake in higher-drinkers, we may provide novel and translationally useful therapeutic interventions.

Orexin-1-receptors (Ox1rs) drive states of high motivation, and systemic Ox1r inhibition disrupts binge alcohol intake and compulsion-like alcohol drinking. However, little has been known about the neural circuits in which Ox1rs promote pathological intake. We measure alcohol binging using a two-bottle-choice variant of alcohol (15%) drinking in the dark (2bc-DID) in adult male C57BL/6 mice. We discovered that binge drinking requires Ox1rs in medial nucleus accumbens (NAc) shell (Shell). Our additional results now demonstrate that there are individual differences in Ox1r regulation of binge alcohol consumption, where Shell Ox1rs promoted intake preferentially in higher-drinking individuals, with little impact in moderate bingers. Identifying different populations of drinking individuals has required large data sets to have some certainty that there were more general patterns. Together, we hypothesize that Shell Ox1rs are critical mediators of the excessive drinking in excessive-binging individuals. This was observed using both the 2bc-DID, and with a three overnight per week, intermittent two-bottle choice alcohol (20%) intake paradigm. More general inhibition of Shell with muscimol/baclofen gave similar effects as intra-Shell Ox1r inhibition during 2bc-DID. Data from quinine-resistant alcohol intake suggests that Ox1r regulation of intake (tested systemically) might also vary with basal drinking tendencies. Also, drinking in “moderate bingers” would be predicted to on average have binge levels of intake, and thus the lack of impact of Shell Ox1r inhibition on moderate binging is unlikely to reflect a floor effect. Ox1r inhibition in NAc core did not reduce binge drinking, suggesting a subregion-specific impact of Ox1rs on intake. Taken together, our studies support the conclusion that excessive alcohol intake during binging is dependent on a Shell Ox1r activation. We are presently working to understand the mechanism(s) that mediates this differential Shell Ox1r contribution across different levels of basal drinking.

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Poster

159. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms I

Location: SDCC Halls B-H

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Topic: G.02. Motivation

Support: NIH AA021445
NIH AA024109

Title: Anterior insula-locus coeruleus area inputs promote compulsive alcohol drinking via a1-adrenergic signaling

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Abstract: Compulsive alcohol intake, where drinking continues despite negative consequences, is a major obstacle to treating alcohol use disorders (AUDs) in humans. Our previous work identified an anterior insular (aINS) circuit that is specifically required for promoting compulsive alcohol intake, but with no role in the regular, unpunished alcohol drinking. This was observed when inhibiting aINS-NAcCore or mPFC-NAcCore inputs with optogenetics, and inhibiting non-canonical NMDARs in NAcCore with pharmacology and shRNA. Interestingly, the idea that compulsive drinking would be driven by cortical-subcortical circuits that process conflict has been proposed based on clinical findings (Tiffany and Conklin, 2000; Bechara and Naqvi, 2010). In particular, it is the conflict, where intake persists despite negative consequences that requires the use of specific cortical circuits to successfully maintain compulsive behavior such as drinking despite punishment. For regular drinking, there is no conflict and no need for any of this circuitry, and habit-related circuits are likely involved. In fact, ours is the first and only evidence for this model. In addition, we found that both aINS-NAcCore and mPFC-NAcCore projections are critical for driving compulsive intake regardless of whether the aversive consequence is shock or bad tasting quinine. This is important because the quinine-resistant compulsive drinking model is widely used and technically simple, but the insula is also related to primary taste, but our results implicate these circuits for compulsive drinking independent from the actual type of punishment. Since compulsion-like drinking also involves aversion, we examined here whether the adaptive stress system, especially the Locus Coeruleus (LC) and its noradrenergic projections, also play a specific and potent role in compulsion-like intake. Inhibition of the α1-adrenergic receptor with prazosin systemically or in the mPFC significantly and selectively reduced compulsive alcohol intake. Prazosin inhibition of compulsive drinking was not related to basal intake levels, suggesting compulsion and self-administration are separate factors, similar to compulsion for cocaine. In addition, aINS-LC projections were also strongly required to allow compulsive drinking, with no role in regular alcohol intake. Thus, adaptive stress responding may be a central part of the mechanism that allows the anterior insula to drive compulsive responding for alcohol, in particular through a feed-forward mechanism whereby aINS drives the LC to activated the mPFC, which is essential for allowing compulsive drinking patterns.

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Poster

159. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms I

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Title: Early-life stress augments kappa opioid receptor function selectively on glutamate and dopamine terminals in caudal nucleus accumbens of rats

Authors: *S. EWIN, S. ALBERTSON, S. JONES, J. WEINER, A. KARKHANIS
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Abstract: Adolescent social isolation (aSI) in rats engenders robust and enduring increases in behaviors linked to alcohol use disorder (AUD) vulnerability. Acute stress elevates levels of the endogenous kappa opioid receptor (KOR) agonist dynorphin. Activation of KORs inhibits dopamine (DA) and glutamate release in the nucleus accumbens (NAc), a region central to the neurobiology of stress, anxiety, and reward-seeking behavior. To investigate aSI-induced changes in KORs, we housed rats individually or in groups (aGH) throughout adolescence. We recently showed an aSI-induced increase in KOR function at DA synapses in the NAc of male rats. In the current study we expand upon these findings and measured KOR-mediated changes in DA release and glutamatergic transmission using ex vivo voltammetry and electrophysiology, respectively, in rostral and caudal NAc of male and female rats. Bath application of U50488, a KOR agonist, revealed augmented KOR-mediated inhibition of DA release selectively in caudal NAc of male and female aSI rats. A sex comparison showed that KOR function was enhanced in male aSI rats. Electrophysiological studies revealed increased glutamatergic synaptic transmission in male aSI NAc slices. In ongoing experiments, we are examining KOR function at glutamatergic synapses in rostral and caudal NAc in female rats. To test whether augmented KOR function plays a causal role in the increased ethanol (EtOH) intake observed in aSI rats, a Cre-dependent KOR overexpressing virus was injected into the VTA of male aGH TH:Cre positive rats (aGH-KOR), to mimic the increase in KOR function observed in aSI rats. A two-bottle choice intermittent EtOH access paradigm revealed greater EtOH intake in aGH-KOR compared to control aGH rats and similar intake compared to aSI rats. In females, activation of KORs with U50488 (2.4mg/kg) enhanced EtOH intake in aSI, but not aGH rats. Together, these data indicate that NAc KOR-function is enhanced primarily in the caudal NAc and contributes to the escalation in EtOH intake promoted by aSI. Overall, these studies provide insight into how KORs mediate stress-induced vulnerability to develop AUD, demonstrate the therapeutic potential of KORs, and provide a foundation to develop evidence-based therapeutic interventions for AUD.

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Poster

160. Neural Mechanisms of Amphetamine Addiction

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Topic: G.08. Drugs of Abuse and Addiction

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Title: Phosphodiesterase inhibitor roflumilast attenuates relapse to methamphetamine self-administration after forced abstinence

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Abstract: Methamphetamine (Meth) is a highly addictive drug of abuse with a lifetime use prevalence of 6.5% in the United States. While it is unclear how Meth produces drug-dependency, Meth causes protracted inflammation peripherally and in the brains of humans, and in rodents that self-administer Meth or receive investigator-administered Meth. However, it is unknown how inflammation may contribute to Meth abuse relapse. To address the role of peripheral inflammation in Meth abuse relapse, rats were trained to self-administer Meth such that each bar press gave an intravenous infusion of 0.1 mg/kg Meth. Rats had access to Meth for 6 hours daily over 14 days. After 14 days, rats underwent 7 days of forced abstinence in their home cages after which pro-inflammatory mediators were measured in the brain. To assess the role of inflammation in Meth relapse, COX-2 and GFAP were measured and found to be increased in the striatum. In addition, rats were dosed with the anti-inflammatory drug and selective phosphodiesterase 4 inhibitor, roflumilast, during the forced abstinence period. Roflumilast is approximately 99% protein bound and thought to be restricted to the periphery. To verify this, we gavaged roflumilast into rats and measured roflumilast in the serum and brain. Although roflumilast and its active metabolite were detected in the serum collected from trunk blood, neither were detectable with microdialysis of the striatum, suggesting that roflumilast was restricted to the periphery at the doses used in this study. After 14 days of Meth self-administration followed by 7 days of forced abstinence, rats were reintroduced to the self-administration chambers and allowed to bar press for Meth after an intraperitoneal 1 mg/kg Meth drug prime. Roflumilast reduced the self-administration of Meth after 7 days of forced abstinence compared to the 14th (last) day of self-administration. The effect was dose-dependent, as 3.0 and 1.5 mg/kg roflumilast, but not 0.75 mg/kg roflumilast significantly attenuated this relapse behavior. These data suggest that peripheral inflammation can mediate relapse to Meth self-administration behavior and may be a novel therapeutic target for the treatment of substance abuse disorders.

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Poster

160. Neural Mechanisms of Amphetamine Addiction

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 160.02/ZZ19

Topic: G.08. Drugs of Abuse and Addiction

Title: A novel psychoactive substance, alpha-PVT, produces behavioral sensitization in rat with implications for GSK3beta signaling in the nucleus accumbens

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Abstract: Alpha-pyrrolidinopentiothiophenone (α-PVT), a novel psychoactive substance, is a structural analog to amphetamine and functions as a potent blocker at the dopamine and norepinephrine transporters. Previously it has been shown that α-PVT stimulates locomotor activity and produces conditioned place preference in mice. However, no study has been performed yet to determine whether α-PVT develops behavioral sensitization similar to those arising from other psychomotor stimulants. In this study, we administered either saline or α-PVT (20 mg/kg, i.p.) to rats with a total of four injections once every 2 to 3 days. After two weeks of withdrawal periods, each pre-exposure group of rats was sub-divided into two additional groups and challenged with either saline or low dose of α-PVT (10 mg/kg, i.p.). Rats, when challenged with α-PVT, showed sensitized locomotor activity in α-PVT compared to saline pre-exposed groups. Next, we analyzed the phosphorylation levels of GSK3β in the NAcc obtained from the rats after sensitization challenge. Interestingly, while acute administration of α-PVT increased the phosphorylation levels of GSK3β in this site, these effects were disappeared in sensitized rats. Correlation analysis between the locomotor activity and phospho-GSK3β shows that there exists a strong negative correlation only in α-PVT chronic injection groups of rats, but not in acute injection. Collectively, these findings demonstrate that α-PVT induces locomotor sensitization similar to classic psychomotor stimulants and further suggest that GSK3β signaling in the NAcc may have an important role in this process.

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Poster

160. Neural Mechanisms of Amphetamine Addiction

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Topic: G.08. Drugs of Abuse and Addiction

Support: Valley Research Partnership Award, Phoenix, AZ

Title: Inactivation of AKT in ventral tegmental area prevents social stress-induced psychostimulant cross-sensitization and GABA-A receptor expression in rats

Authors: Z. T. MORRISSON[1], M. L. RUDOLPH[1,2], Z. YELLOWMAN[1], T. C. WILLIAMS[1], R. L. NEVE[3], R. P. HAMMER, Jr[1,2], *E. M. NIKULINA[1]
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Abstract: Intermittent social defeat stress in rats augments locomotor response to psychostimulant drugs such as amphetamine (AMPH), a phenomenon called cross-sensitization; in humans, cross-sensitization may manifest as increased substance abuse potential. Social stress causes upregulation of mu-opioid receptors in the ventral tegmental area (VTA), whose effect is mediated in part by increased AKT signaling in VTA GABA neurons. The objective of this study was to selectively target AKT signaling in the VTA by HSV-mediated gene transfer of dominant-negative AKT (DN-AKT-HSV), then measure the effect on amphetamine cross-sensitization and GABA-A receptors. Experimental male Sprague Dawley rats were exposed to aggressive Long-Evans rats or control handling procedures four times over a period of 10 days, and then received bilateral stereotaxic injections of the DN-AKT-HSV construct or sham surgeries. Three days later upon maximal viral overexpression, locomotor activity was quantified following saline (1.0 mL/kg), then low-dose amphetamine (1.0 mg/kg, i.p) treatment. The next day, brains were removed and processed by quantitative immunohistochemistry for GABA-A receptor expression. Stressed rats exhibited amphetamine cross-sensitization after sham surgery, while locomotor activity in stressed rats that received DN-AKT-HSV was significantly reduced to the level exhibited by handled rats. Baseline of locomotor activity was similar after saline treatment in all groups, suggesting that manipulation of VTA AKT alone did not alter locomotion. Furthermore, DN-AKT-HSV reduced GABA-A receptor labeling in the VTA. These data show that intra-VTA DN-AKT-HSV prevented cross-sensitization, suggesting that AKT signaling in the VTA is necessary for the expression of stress-induced cross-sensitization. The reduction of VTA GABA-A receptor expression by DN-AKT-HSV treatment suggests that social stress-induced VTA GABA-A upregulation is dependent on AKT signaling. We propose a mechanism whereby social stress enhances AKT signaling in VTA GABA neurons leading to increased GABA-A receptor expression which inhibits VTA GABA activity and disinhibits

dopamine neurons following social stress. Further studies using VTA cell-type specific inhibition of AKT signaling may clarify this complex process, promoting the development of targeted therapeutic agents to treat stress-induced substance abuse susceptibility.

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Poster

160. Neural Mechanisms of Amphetamine Addiction

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 160.04/ZZ21

Topic: G.08. Drugs of Abuse and Addiction

Title: Expression and functionality of adenylate cyclase V in rats under chronic treatment with amphetamine

Authors: *D. G. MELCHOR[1], J. AVALOS-FUENTES[2], C. RANGEL BARAJAS[3], F. PAZ-BERMÚDEZ[2], D. ERLIJ[4], G. B. FLORAN[5]
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Abstract: Chronic administration of L-dopa in patients with PD induces abnormal involuntary movements (dyskinesias) associated with hyperactivity of adenylyl cyclase and increased expression of FosB and ΔFosB. Administration of amphetamine also increases the expression of these genes, suggesting similar mechanisms mediated by both drugs. In this work we study if chronic amphetamine treatment also produces these changes in adenylyl cyclase in the nucleus acummbens, where the motivational reward processes and addiction are integrated and in striatum the sensorimotor integration center.
We conducted experiments with chronic administration of amphetamine (1 mg/kg) in male rats Wistar 200-250 g. for 5 days, a pharmacological challenge with the same dose of amphetamine three days after treatment was also evaluated. After sacrifice, we studied the expression of adenylyl cyclase V by western blot in homogenates, also perform studies of cAMP formation and release of radioactive GABA in slices, stimulating adenylyl cyclase with forskolin and D1 receptors with SKF 38393. We found a significant increase in the expression of adenylate cyclase V after 5 days of administration of amphetamine, compared with the group that received only saline. Pharmacological challenge showed a further increase in this expression. Interestingly there is a correlation between the protein increase with its activity. The stimulation of adenylate cyclase with forskolin in rats treated with amphetamine increased the formation of cAMP by approximately 15% compared to the saline group and this increase was greater in the

pharmacological challenge, approximately 30% in the striatum and in the nucleus accumbens. These results are related with an increase in the release of GABA with the same treatment. When stimulation of D1 receptors was blocked with the selective D1 antagonist SCH 23390, the effects of sensitization were completely reversed.
These data suggest that administration of amphetamine in normal animals as well as the L-DOPA in animal models of disease Parkinson hypersensitize adenylyl cyclase V also explain why the use of drugs that activate receptors metabotropic inhibiting adenylyl cyclase activity are useful for the treatment of addiction as well as for dyskinesia induced by L-DOPA.

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Poster

160. Neural Mechanisms of Amphetamine Addiction

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NIGMS P30 GM110702

Title: Chromatin immunoprecipitation (ChIP) analysis of histone H3/4 acetylation at HDAC promoters: Differential effects of modafinil and methamphetamine on the mouse prefrontal cortex

Authors: *V. BISAGNO[1], B. GONZÁLEZ[1], A. BERNARDI[1], O. V. TORRES[2], S. JAYANTHI[3], N. GOMEZ[1], M. SOSA[1], F. J. URBANO[4], E. E. GARCIA-RILL[5], J. L. CADET[6]
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Abstract: Methamphetamine (METH) and modafinil are psychostimulants with different addictive and cognitive profiles: METH causes neuroplastic changes that negatively impact the prefrontal cortex (PFC) leading to cognitive deficits and addiction. In contrast, modafinil has little abuse liability and is a cognitive enhancer that improves PFC function. Epigenetic mechanisms are contributory factors in drug-induced neuroadaptations. Specifically, acetylation of histone tails appears to be involved in long-term plasticity associated with cognition and

addiction. Histone deacetylases (HDACs) are key players that regulate chromatin functions, thus generating short-term and long-term metaplastic cellular changes responsible for mnemonic adaptations. In the brain, these mechanisms may be pivotal for drug-induced alterations in gene expression and behavioral manifestations. HDACs are divided into zinc-dependent (classical HDACs) or NAD-dependent (sirtuins 1-7) enzymes. Classical HDACs are clustered in class I (HDAC1-3 and 8), class II (HDAC4-7, 9, and 10), or class IV (HDAC11). Class III HDACs are the sirtuins (Sirt1-Sirt7). In our continuing efforts to identify epigenetic effects of METH and modafinil, we tested the possibility that a single dose or chronic administration of these drugs might be followed by changes in histone acetylation (H3ac and H4ac) at the promoters of various HDACs using mouse mPFC. METH (1 mg/kg) or modafinil (90 mg/kg) were administered as a single-dose or repeatedly (daily for 7 days and 4 days washout) and the PFC was used in chromatin immunoprecipitation (ChIP)-PCR studies. We found that, after a single injection, i) METH increased H4Ac abundance at Hdac1 and Hdac10 promoters, but decreased H4Ac acetylation at Hdac4 and Hdac5 promoters; ii) modafinil increased H3Ac but decreased H4Ac at the Hdac7 promoter; iii) both drugs decreased H4Ac at Hdac2 and Hdac8 promoters. Repeated injections were associated with different epigenetic modifications. Specifically, i) METH increased H3Ac at the Sirt3 promoter and H4Ac at Hdac4, Hdac5, Hdac11 and Sirt2 promoters, but promoted decreased H3Ac at Hdac1 and Hdac8 promoters and decreased H4Ac at the Sirt7 promoter; METH also decreased both H3 and H4 acetylation at the Hdac2 promoter; ii) both drugs increased H3 acetylation at Hdac3, Hdac4 and Sirt6 promoters but decreased H4 acetylation at the Hdac8 promoter. These results show different patterns of epigenetic modifications after single-dose in contrast to repeated injections. Specific METH- and modafinil-induced epigenetic alterations may play important roles in the differential effects of the drugs on cognition, addiction, and mPFC functions.

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Poster

160. Neural Mechanisms of Amphetamine Addiction

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Title: Role of histone deacetylase inhibition and environmental condition in altering phases of amphetamine self-administration

Authors: *T. J. WUKITSCH, D. L. ARNDT, E. J. GARCIA, M. E. CAIN
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Abstract: Gene-environment interactions play a significant role in drug abuse and addiction. Epigenetics (the study of how environmental stimuli alter gene expression) has gained attention in recent years as a significant contributor to many behavioral phenotypes of drug addiction. The current study sought to determine if differential rearing conditions can alter a specific epigenetic mechanism, histone deacetylase (HDAC), and how HDAC inhibition can affect drug-taking and drug-seeking behaviors differently among enriched, isolated, or standard-housed rats. Ninety male Sprague-Dawley rats were reared for 30 days in enriched (EC), isolated (IC), or standard (SC) conditions prior to amphetamine (0.1 mg/kg/infusion, i.v.) self-administration, extinction, and reinstatement sessions. Trichostatin A (TsA; 0.3 mg/kg, i.v.), an HDAC inhibitor, was injected 30 min prior to extinction and reinstatement sessions. Brains were then extracted and prepared for western blot analysis of acetylated histone K9 (acH3K9) in the dorsal striatum and nucleus accumbens. EC, IC, and SC rats had similar rates of amphetamine self-administration. While enrichment facilitated the extinction of active lever pressing, there was no effect of TsA on lever pressing behavior during extinction. TsA administration decreased cue-, but not drug-induced reinstatement, with IC-TsA rats exhibiting significantly attenuated cue-induced reinstatement compared to IC-vehicle rats. Western blot analysis revealed that IC rats had significantly lower levels of acH3K9 in the dorsal striatum compared to both SC and EC rats. However, there were no differences in the nucleus accumbens and no overall TsA effect on acH3K9 expression in either brain area. These findings suggest that differential rearing can alter HDAC mechanisms within the striatum that may contribute to cue-seeking behaviors, particularly in rats reared in isolated conditions.

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Poster

160. Neural Mechanisms of Amphetamine Addiction

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Topic: G.08. Drugs of Abuse and Addiction

Support: AA020098
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BX003671

Title: Overexpression of membrane lipid raft protein caveolin-1 in D1R expressing neurons in the dorsal striatum promotes meth addiction-like behavior

Authors: *S. S. SOMKUWAR, M. FANNON, 92161, S. WANG, M. TERRANOVA, B. HEAD, C. MANDYAM, 92121
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Abstract: Evidence from previous studies indicates that methamphetamine (Meth) self-administration upregulates dopamine D1 receptors (D1Rs) in the dorsal striatum. It is therefore hypothesized that D1R expressing medium-sized spiny neurons in the dorsal striatum may contribute to reinforcing effects of Meth and produce dependence-like behavior. New evidence demonstrates that D1R signaling occurs in discrete plasmalemmal microdomains termed membrane lipid rafts (MLRs). MLRs are enriched in cholesterol- and sphingolipid and the cholesterol binding and scaffolding protein caveolin-1 (Cav-1). Cav-1 is important in regulating D1R subcellular localization, signaling and function. Furthermore, Cav-1 overexpression in neurons enhances expression of the signaling molecule ERK1/2 in response to agonist mediated D1R receptor signaling. Therefore, here we seek to determine if overexpressing Cav-1 in D1R neurons in the dorsal striatum alters Meth self-administration via ERK1/2 activation. A viral vector-mediated approach was used to overexpress Cav-1. Preliminary findings from behavior data reveal that Cav-1 injected animals increased responding for Meth in an extended access schedule and exhibited a vertical and rightward shift in the self-administration dose-response function, indicating that Cav-1 overexpression enhanced the reinforcing actions of Meth. Postmortem tissue analysis will determine the cellular mechanisms in the dorsal striatum that correlate with enhanced Meth addiction-like behavior. Parallel studies conducted in human IPSC cells demonstrates that Meth treatment enhanced expression of D1R and activation of ERK1/2 in concert with phosphorylation of Cav-1 at tyrosine residue 14 (Y14). These cellular studies demonstrate that Meth leads to alterations in activation of Cav-1 to regulate intracellular signaling molecules associated with reinforcing effects of Meth. These data suggest that enhanced functioning and activity of D1R expressing neurons via enhanced expression of Cav-1 in the dorsal striatum is necessary for promoting Meth addiction-like behavior and enhancing reinforcing effects of Meth.

Disclosures: S.S. Somkuwar: None. **M. Fannon:** None. **S. Wang:** None. **M. Terranova:** None. **B. Head:** None. **C. Mandyam:** None.

Poster

160. Neural Mechanisms of Amphetamine Addiction

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 160.08/ZZ25

Topic: G.08. Drugs of Abuse and Addiction

Title: Chronic methamphetamine alters expression and activation of corticotropin-releasing factor receptor 1 cells in the hypothalamus and extended amygdala

Authors: *J. JACOBSKIND[1], R. M. DE GUZMAN[1], Z. J. ROSINGER[1], D. N. FICO[1], B. SAGLIMBENI[1], K. SZAFRANSKA[1], N. J. JUSTICE[2], D. G. ZULOAGA[1]
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Abstract: Corticotropin-releasing factor (CRF) binding to the CRFR1 receptor subtype has repeatedly been shown to play a key role in drug-related behaviors. Specifically, CRFR1 signaling increases drug seeking during abstinence and promotes stress-induced relapse to drug use. Furthermore, CRFR1 gene expression is upregulated in select brain regions following repeated exposure to drugs and alcohol, although effects of methamphetamine (MA) on CRFR1 are largely unknown. In this study, C57BL/6J CRFR1-GFP reporter mice were i.p. injected with MA (5 mg/kg) or saline once daily for 10 consecutive days. Following a 10-day withdrawal period, all animals were given an i.p. challenge injection of MA (5 mg/kg) and sacrificed 120 minutes later. CRFR1 cell number and co-localization of CRFR1 with neural activation and transcription markers c-Fos and pCREB were assessed in the hypothalamus, extended amygdala, and nucleus accumbens (NAc). Chronic MA exposure significantly increased CRFR1 cell number in the central amygdala (CeA), arcuate hypothalamus (Arc), and ventral basolateral amygdala (vBLA). Chronic MA treatment also increased the number of CRFR1/c-Fos co-localized cells within the Arc and vBLA, but attenuated co-localization in the paraventricular hypothalamus (PVH) following MA challenge. No differences in CRFR1 cell number or co-localization with c-Fos were observed in anteroventral or dorsolateral nuclei of the bed nucleus of the stria terminalis, the anteroventral periventricular hypothalamus, dorsomedial hypothalamus, or NAc. Interesting, sex differences were found in CRFR1/pCREB co-localization. Acute exposure to MA following prior chronic exposure increased CRFR1/pCREB in the PVH and nucleus accumbens shell (NAcSh) in males, but decreased co-localization in the female NAcSh. CRFR1/pCREB was increased in the Arc of both males and females. Alterations in CRFR1 expression and activation of CRFR1 cells may reflect a neuroadaptive response to repeated MA exposure. Furthermore, sex differences in CRFR1/pCREB co-localization may underlie observed sex differences in patterns of MA abuse.

Disclosures: J. Jacobskind: None. **R.M. De Guzman:** None. **Z.J. Rosinger:** None. **D.N. Fico:** None. **B. Saglimbeni:** None. **K. Szafranska:** None. **N.J. Justice:** None. **D.G. Zuloaga:** None.

Poster

160. Neural Mechanisms of Amphetamine Addiction

Location: SDCC Halls B-H

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Program #/Poster #: 160.09/ZZ26

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant R33DA041876
NIH Grant R21DA041876
NIH Grant K01NS073700

Title: Cell-specific spinophilin function following psychostimulant-induced behavioral sensitization regimens

Authors: *D. S. WATKINS[1], A. J. BAUCUM II[2]
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Abstract: Proper synaptic transmission is critical for maintaining neuronal communication. There is increasing evidence that in many neurological disease-states such as: Obsessive Compulsive Disorder (OCD), Attention Deficit Disorder (ADD/ADHD), drug addiction, and countless others, synaptic transmission is perturbed. Reversible protein phosphorylation regulates many of the neuronal signal transduction pathways that reside in the post-synaptic density (PSD) and this regulation occurs via tight and transient events. While serine/threonine kinases obtain substrate specificity, in part, by phosphorylating specific consensus sites, serine/threonine phosphatases such as protein phosphatase 1 (PP1), are much more promiscuous. To obtain substrate selectivity PP1 associates with targeting proteins. The major targeting protein in the PSD of dendritic spines is spinophilin. Spinophilin binds PP1 and F-actin as well as multiple other synaptic proteins. Our lab has found that dopamine depletion, an animal model of PD, modulates spinophilin protein-protein interactions in the striatum. However, spinophilin function under hyperdopaminergic signaling following psychostimulant-induced behavioral sensitization is less well characterized. Here, we begin to report the functional role of spinophilin in mediating behavioral changes associated with amphetamine-induced locomotor sensitization as well as beginning to define changes in spinophilin interactions following psychostimulants. To more specifically elucidate spinophilin function we have generated transgenic spinophilin animals that Cre-dependently express an epitope-tagged human form of the protein. Our data suggest that spinophilin interactions are modulated in specific cell types following psychostimulant administration. The implications for regulating spinophilin interactions in specific cell types following psychostimulant administration will be discussed. In addition sex differences will be discussed.

Disclosures: D.S. Watkins: None. **A.J. Baucum II:** None.

Poster

160. Neural Mechanisms of Amphetamine Addiction

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 160.10/AAA1

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH/NIDA DA034738

Title: The role of Parkin in mediating the rewarding and reinforcing effects of methamphetamine in young adult rats

Authors: *A. SHARMA, A. HARUTYUNYAN, A. MOSZCZYNSKA
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Abstract: Methamphetamine (METH) use disorder (MUD) is a significant problem in the USA. Chronic METH abuse, particularly at high doses, has severe physical and psychological consequences, including cognitive impairments and psychosis. As no FDA-approved pharmacotherapy is available to treat MUD, identification of new drug targets is essential. Parkin is an E3 ubiquitin ligase which regulates trafficking and turnover of multiple proteins and a potential drug target in MUD. Parkin has neuroprotective properties and plays a role in modulating dopaminergic (DAergic) neurotransmission, which, in the nucleus accumbens (NA), and dorsolateral striatum (DLS), mediates the rewarding and reinforcing effects of METH. METH can oxidatively damage Parkin, resulting in Parkin loss of function, which can cause an impairment of DAergic neurotransmission leading to MUD. No studies have yet evaluated the role of Parkin in MUD. The specific aims of the present study were: (Aim 1) to assess rewarding and reinforcing properties of METH in Parkin knockout (PKO) vs. wild-type (WT) rats and (Aim 2) to attenuate METH-induced reward and reinforcement by Parkin overexpression (PO) in different striatal regions in WT rats. We hypothesized that METH would induce a stronger reward and reinforcement in PKO than WT rats during condition place preference (CPP) and 15h-long METH self-administration (LA METH SA) paradigms. Additionally, we hypothesized that overexpression of Parkin in the NA or DLS of WT rats would attenuate rewarding and reinforcing properties of METH. Methods: Young adult male Long Evans WT rats, PKO rats (Sage Labs), and WT rats with bilateral PO in the NA (ML 3.2mm, AP 1.8mm, DV 7.6mm at 16-degree angle from cranium) or in the DLS (ML 3.4mm, AP 0.6mm, DV 4.8mm from cranium) (generated in our lab) (N = 6-8 rats per group) were subjected to METH-CPP (to measure METH rewarding effects) and LA METH SA for 10 days (to measure METH rewarding and reinforcing effects). Results: We found a significant increase in lever pressing in PKO rats as compared to WT rats at fixed ratio five during LA METH SA ($p < 0.05$, RM ANOVA). In contrast to PKO rats, the PO rats (PO in NA or DLS) did not differ from the WT rats during LA METH SA. Neither the PKO nor the PO rats developed a preference for METH-associated context during the CPP as did the WT rats. Conclusions: The results suggest that the observed augmentation of LA METH SA and a paradoxical decrease in METH CPP in PKO rats as compared to WT controls are a consequence of adaptive changes induced by brain-wide Parkin knockout. Importantly, our results also suggest that parkin is a potential drug target in METH abuse as it can decrease rewarding properties of METH.

Disclosures: A. Sharma: None. **A. Harutyunyan:** None. **A. Moszczynska:** None.

Poster

160. Neural Mechanisms of Amphetamine Addiction

Location: SDCC Halls B-H

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Program #/Poster #: 160.11/AAA2

Topic: G.08. Drugs of Abuse and Addiction

Title: Overexpression of radixin in the nucleus accumbens blocks the expression of conditioned locomotor activity induced by amphetamine

Authors: *W. CAI, W. KIM, M. KWAK, J.-H. KIM
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Abstract: The ezrin-radixin-moesin (ERM) proteins have been implicated not only in cell-shape determination but also in cellular signaling pathway. We have previously shown that amphetamine (AMPH) decreases phosphorylation levels of ERM proteins in the nucleus accumbens (NAcc), an important brain area mediating addictive behaviors. However, it has not been determined yet what functional role ERM proteins play in this site in relation with drugs of abuse. In the present study, we bilaterally microinjected lenti viral vectors, which contains either GFP only control, wild-type or mutant radixin gene, into the NAcc core and measured conditioned locomotor activity induced by repeated AMPH injections. Two weeks after virus injection surgery, rats were randomly assigned to three groups: Paired, Unpaired and Control. Rats received AMPH (1 mg/kg, IP) in locomotor activity boxes on day 1 and saline in their home cages on day 2 (Paired), saline in the activity boxes on day 1 and AMPH in their home cages on day 2 (Unpaired), or saline in both environments (Control), with a total of five 2-day blocks. On the test, one week after the last conditioning block, all rats were tested for their conditioned locomotor response in the activity boxes for one hour following an IP saline injection. As expected, with control GFP in the NAcc, rats in Paired group showed increased locomotor activity compared to those in Unpaired or Control groups. Interestingly, however, overexpression of wild-type radixin in this site inhibited the expression of conditioned hyper-locomotor activity, while mutant gene had no effects, compared with control GFP. These results first time indicated that radixin in the NAcc core plays a role in controlling the expression of conditioned locomotor activity induced by AMPH.

Disclosures: W. Cai: None. **W. Kim:** None. **M. Kwak:** None. **J. Kim:** None.

Poster

160. Neural Mechanisms of Amphetamine Addiction

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 160.12/AAA3

Topic: G.08. Drugs of Abuse and Addiction

Support: Valley Research Partnership Award, Phoenix, AZ

Title: Social stress-induced amphetamine cross-sensitization: Essential role of GluA1 AMPA receptors in ventral tegmental area dopamine neurons of male and female rats

Authors: *M. L. RUDOLPH[1,2], A. F. AZUMA[2], T. J. ZAFAR[1], R. L. NEVE[3], R. P. HAMMER, Jr[1,2], E. M. NIKULINA[1]
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Abstract: Converging evidence from human and rodent studies have revealed sex differences in drug use; females progress more rapidly to drug dependence that is worsened by stress. Our recent data suggest that intermittent social stress induces AMPA subunit GluA1 expression primarily in dopamine cells of male rats, which plays a critical role in the induction of stress-induced amphetamine (AMPH) cross-sensitization. The objective of this study was to compare behavioral and neurochemical effects of social stress-induced cross-sensitization across sexes. Male and female TH-Cre rats were exposed to aggressive attacks by male or lactating female Long-Evans rats, respectively, four times over a 10-day period; control rats were handled on the same schedule. Vaginal smears of females were taken daily to determine their estrous stages. Social stress increased GluA1 expression in VTA dopamine neurons, as evidenced by a greater density of GluA1/TH double-labeling in VTA neurons of stressed compared to handling rats. Stressed females were more sensitive to AMPH (0.5 mg/kg i.p.) which significantly augmented locomotion during proestrus; this drug effect was markedly greater than AMPH (1.0 mg/kg i.p.) challenge in stressed male rats, despite the lower dose. Additionally, AMPH treatment induced greater c-Fos expression in the nucleus accumbens (NAc) of proestrous, compared to metestrous or saline-treated control female rats. Furthermore, social stress induced ΔFosB expression in the NAc, which occurred concomitantly with social stress-induced AMPH cross-sensitization. To determine whether GluA1 is necessary for the effects of social stress, a Cre-dependent wildtype GluA1 construct packaged in AAV (AAV-wt-GluA1) was used to selectively overexpress GluA1 in TH neurons. AAV-wt-GluA1 or control AAV-GFP viral constructs were bilaterally infused into the VTA three weeks prior to behavioral testing. AMPH significantly augmented locomotion in female and male rats with GluA1 overexpression compared to control rats, even in the absence of social stress. Taken together, these results suggest that females are more sensitive to social stress-induced AMPH cross-sensitization than male rats, and that GluA1 in VTA

dopamine neurons plays an essential role in the development of this sensitized response. Elucidating sex-dependent differences in the mechanisms of stress-induced drug cross-sensitization is critical to targeting potential therapeutic agents for the treatment of substance abuse susceptibility in males and females.

Disclosures: M.L. Rudolph: None. **A.F. Azuma:** None. **T.J. Zafar:** None. **R.L. Neve:** None. **R.P. Hammer:** None. **E.M. Nikulina:** None.

Poster

160. Neural Mechanisms of Amphetamine Addiction

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 160.13/AAA4

Topic: G.08. Drugs of Abuse and Addiction

Support: Whitehall Foundaton

Title: Methamphetamine-induced heterogeneous catecholamine regulation in limbic brain areas

Authors: *J. PARK, R. BHIMANI, A. FIMMEL
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Abstract: Methamphetamine (METH), one of the most powerful and highly addictive psychostimulant drugs, exerts many of its behavioral and reinforcing effects by increasing extracellular concentrations of the two major catecholamines, norepinephrine (NE) and dopamine (DA), in the brain. Interestingly, accumulating evidence shows that METH has stronger, more potent effects on NE than DA. However, how METH heterogeneously modulates NE and DA regulation (release and clearance) is unclear. In the present study, we characterized how METH dose-dependently modulates catecholamine regulation in two relatively understudied limbic brain areas critically involved in drug withdrawal and addiction, the bed nucleus of the stria terminalis (BNST) and olfactory tubercle (OT). For these studies, fast-scan cyclic voltammetry with carbon-fiber microelectrodes was employed to monitor subsecond changes of BNST-NE and OT-DA signaling in real time in anesthetized and behaving rats. We demonstrate how low to high doses of METH (0.2 – 10 mg/kg, i.p) distinctly modulate phasic and tonic NE and DA regulation through action potential dependent and/or independent mechanisms. These results will extend our understanding of distinct METH-induced dysfunction of local NE and DA regulatory mechanisms and their contribution to METH abuse and addiction.

Disclosures: J. Park: None. **R. Bhimani:** None. **A. Fimmel:** None.

Poster

160. Neural Mechanisms of Amphetamine Addiction

Location: SDCC Halls B-H

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Program #/Poster #: 160.14/AAA5

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA DA 033049

Title: METH self-administration selectively increases phasic glutamate in female rats

Authors: *A. LAVIN, J. I. PENA-BRAVO, C. M. REICHEL
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Abstract: Pre-clinical and clinical research has show that females are more vulnerable to the rewarding effects of stimulants, and it has been proposed that estrogens may play a role in the enhanced sensitivity however differences in METH -induced neuroplasticity have not been explored. To address this gap in knowledge we recorded from the prelimbic PFC of male and females rats following long access METH SA and investigated the resulting long-term synaptic neuroadaptations. We report that males and females took similar amounts of METH during self-administration; however, female rats exhibit significant synaptic baseline differences when compared to males. Furthermore METH SA elicited a significant increase in phasic glutamate only in females. This increase in phasic glutamate was correlated with increases in NMDA currents and was significantly reduced by application of an Nr2B selective blocker. We propose that female rats have a higher baseline contribution of Nr2B NMDAR in the PFC, and this constitutive difference may underlie the differential effects of METH. Our results may provide a mechanistic explanation for the increased susceptibility to METH addiction in females.

Disclosures: A. Lavin: None. **J.I. Pena-Bravo:** None. **C.M. Reichel:** None.

Poster

160. Neural Mechanisms of Amphetamine Addiction

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 160.15/AAA6

Topic: G.08. Drugs of Abuse and Addiction

Support: DA033049
GM08716

Title: Inhibition of the prelimbic to nucleus accumbens core pathway decreases methamphetamine cued reinstatement

Authors: A. M. KEARNS, R. A. WEBER, J. S. CARTER, S. S. COX, J. PETERS, *C. M. REICHEL
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Abstract: Methamphetamine (meth) causes enduring changes within the medial prefrontal cortex (mPFC) to nucleus accumbens (NAc) circuitry. Projections from the mPFC to the NAc have a distinct dorsal-ventral distribution; neurons originating in the prelimbic (PL) mPFC project to the NAc core (c), whereas, those originating in the infralimbic (IL) mPFC project to the NAc shell. Inhibition of these parallel pathways has opposing effects on relapse. Inhibition of PL-NAcc reduces cued reinstatement of cocaine-seeking following extinction and IL-NAc shell inhibition reinstates previously extinguished rats. Meth, however, exhibits a different profile, as pharmacological inhibition of both the PL and IL decrease cued reinstatement of meth-seeking. Given these contrasting findings, the opposing roles of the PL-NAcc and IL-NAc shell found with cocaine remains unclear in regard to meth-seeking. To begin to address this issue, we used a combinational viral approach that employs a Cre-dependent retrograde traveling AAV (AAVrg) virus injected in the NAcc and a Cre-dependent inhibitory DREADD (DIO-hM4Di) in the PL. Rats had viral infusions before going through meth or sucrose self-administration (8 hr daily, 15 days) followed by object recognition tests during abstinence and reinstatement tests after extinction. Meth intake escalated over time and resulted in pronounced deficits in recognition memory; whereas sucrose intake remained stable and did not impact object recognition. Meth and sucrose associated cues reinstated extinguished responding. However, inhibition of the PL-NAcc circuit with clozapine-N-oxide (CNO) decreased meth but not sucrose reinstated lever pressing. In a follow-up experiment rats received the aforementioned combinatorial viral approach (AAVrg + DIO-hM4Di) or were infused with AAV-DIO mCherry as a control (AAVrg + DIO-mCherry). Male and female rats self-administered meth (2 hr daily, 15 days) then went through extinction and reinstatement testing. Tests were conducted with ip injections of the following: 1) DMSO (veh control), 2) 3 mg/kg CNO, 3) 10 mg/kg CNO, or 4) 0.1 mg/kg clozapine. DIO-hM4Di rats robustly reinstated in the presence of meth cues following vehicle and this was significantly decreased by inhibition of the PL-NAcc circuit with 10 mg/kg CNO. Reinstatement of meth seeking was not interrupted by 3 mg/kg CNO or 0.1 mg/kg clozapine. DIO-mCherry rats robustly reinstated during all test conditions. Combined, these studies show that inhibition of the PL-NAcc circuit can inhibit reinstated meth seeking in a manner similar to cocaine. The next series of study will determine the role of the IL-NAc shell circuit on reinstated meth seeking.

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Poster

160. Neural Mechanisms of Amphetamine Addiction

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Program #/Poster #: 160.16/AAA7

Topic: G.08. Drugs of Abuse and Addiction

Support: AA0074560
DA036651
DA043799
27IR-0047

Title: Identification of neural circuits that are recruited during psychostimulant withdrawal using whole-brain imaging

Authors: *A. J. KIMBROUGH[1], A. COLLAZO[3], M. KALLUPI[2], O. GEORGE[4]
[1]Neurosci., The Scripps Res. Inst., La Jolla, CA; [2]Neurosci., The Scripps Res. Inst., San Diego, CA; [3]Caltech, Pasadena, CA; [4]Dept. of Neurosci., Scripps Resch Inst., La Jolla, CA

Abstract: A central theory in the addiction field is that drug withdrawal is mediated by the specific recruitment of a small brain stress network and inactivation of a small brain reward network. However, this theory has been difficult to test because imaging and quantifying large numbers of brain regions in an unbiased way has been historically difficult. The goal of this study was to identify neural circuitry that is recruited during withdrawal from nicotine, methamphetamine, and cocaine using immunolabeling-enabled three-dimensional imaging of solvent-cleared organs (iDISCO) whole-brain imaging using Fos immunostaining as a proxy for neural reactivity. C57BL/6J mice were surgically implanted with osmotic minipumps that were filled with saline (n = 4), nicotine (n = 5; 24 mg/kg/day), methamphetamine (n = 5; 4 mg/kg/day), or cocaine (n = 5; 60 mg/kg/day). The mice were then returned to their home cages with the minipumps for 1 week. After 1 week, the minipumps were removed. The mice were then transcardially perfused 8 h (for saline, nicotine, and cocaine) or 12 h (for methamphetamine) into withdrawal, and brains were collected for iDISCO brain clearing and Fos immunostaining. The brains were imaged using a light-sheet microscope. The data were analyzed using the ClearMap pipeline to obtain counts of Fos-positive neurons in ~200 brain regions. We identified five major networks in saline rats that were significantly altered during withdrawal from all of the drugs. Withdrawal from all of the drugs was associated with functional reorganization of the entire brain and the emergence of novel networks that were specific for each drug. Hierarchical clustering and graph theory analysis identified novel networks beyond the classic stress/reward classification that may mediate drug withdrawal. These results demonstrate that drug withdrawal has a much greater impact on the brain than previously thought and suggest that drug withdrawal is mediated by the coordinated recruitment

of a large and distributed network of brain regions that extend beyond the classic definition of stress and reward systems.

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Poster

160. Neural Mechanisms of Amphetamine Addiction

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Program #/Poster #: 160.17/AAA8

Topic: G.08. Drugs of Abuse and Addiction

Support: Stanford Neuroscience Institute NeuroChoice Initiative

Title: Neural predictors of relapse to stimulant use

Authors: *K. H. MACNIVEN[1], E. L. S. JENSEN[1], S. I. HUDSON[1], K. HUMPHREYS[2], B. KNUTSON[1]
[1]Psychology, Stanford Univ., Stanford, CA; [2]Psychiatry, VA Palo Alto Hlth. Care Syst., Palo Alto, CA

Abstract: Addiction to stimulants (e.g., cocaine, methamphetamine) has been hypothesized to involve enhanced neural responses in the mesolimbic circuit to drug cues, as well as diminished responses to conventional reward cues. Such a reallocation of neural evaluative responses to drug versus conventional reward cues may encourage relapse. We combined a drug cue task with functional magnetic resonance imaging (FMRI) in a longitudinal study of detoxified patients with a stimulant use disorder (n=36) and healthy control subjects (n=40) to test whether stimulant use disorder is characterized by distorted mesolimbic responses to drug versus conventional reward cues, and whether these responses would predict relapse. Our analyses focused on three predicted regions which colocalize with mesolimbic dopamine projections: medial prefrontal cortex (mPFC), nucleus accumbens (NAcc), and ventral tegmental area (VTA). Relative to controls, patients showed evidence of sensitized neural reward responses to drug cues in all three regions. Patients also exhibited blunted responses to conventional reward (food) cues in NAcc, but not mPFC or VTA. Further, patients' increased NAcc responses to drug cues specifically predicted relapse months later, above and beyond predictions afforded by self-report and clinical measures. Relapse classification based on NAcc responses to drug cues performed as well as classification based on whole-brain activity, further suggesting that activity in this region uniquely supports neural processing that perpetuates stimulant dependence. If neural responses to drug cues predict and promote relapse, neuroimaging methods may confer added value in helping clinicians to identify individuals at greatest risk for relapse to stimulant use.

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Poster

160. Neural Mechanisms of Amphetamine Addiction

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Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

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Topic: G.08. Drugs of Abuse and Addiction

Support: Department of Veterans Affairs Clinical Sciences Research and Development Merit Review Program, I0CX001558
DOJ 2010-DD-BX-0517
NIDA P50DA018165
NIAAA R21AA020039

Title: Neuroimaging classification of substance use disorder

Authors: *W. F. HOFFMAN[1], M. KOHNO[2], H. H. MCCREADY, 97239[3], L. H. DENNIS, 97239[3]
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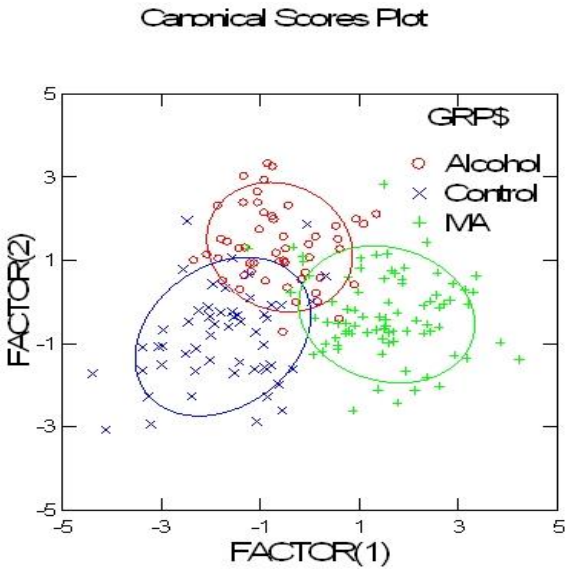
Abstract: Elucidation of patterns of neural features that differentiate substance using groups can aid in the understanding of differential drug effects, mechanisms of addiction and response to treatment. We examined 66 subjects with Alcohol Use Disorder (AUD), 72 with Methamphetamine (MA) Use Disorder and 49 Control subjects (CS). Substance using groups met DSM-IV or V criteria and CS had never met criteria for any substance use.
We used FreeSurfer to extract cortical and sub-cortical volumes according to a standard atlas. Additionally, we calculated resting state functional connectivity with FSL using seeds in insula, anterior cingulate cortex, amygdala, middle frontal gyrus, hippocampus, putamen, caudate, parietal cortex, precuneus and inferior frontal gyrus. These 80 volumes (40 right hemisphere and 40 left hemisphere) and the functional connectivity correlations were entered into a linear discriminant analysis to examine features that differentiated the three diagnostic groups. We used SYSTAT DISCRIM with bootstrap resampling (to account for the large number of predictors) with 100 samples to calculate two linear discriminant functions. The analysis correctly classified 82% of AUD, 78% of CS and 73% of MA users and an overall correct classification rate of 77% (Figure).
Inspection of the linear discriminant functions revealed that AUD individuals had greater volume loss in frontal and parietal regions, while MA users had greater loss in subcortical, as well as less loss in frontal and parietal regions. These findings suggest that classification schemes based on

anatomical and functional neuroimaging data may be used to identify differential effects of drugs and alcohol on the neural substrate.



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Poster

160. Neural Mechanisms of Amphetamine Addiction

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant R01NS079774

Title: Cell assembly logic underlying increased risk-taking behavior associated with drug abuse

Authors: *G. FOX, K. XIE, J. Z. TSIEN
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Abstract: Illicit psychostimulant drugs such as methamphetamine are not only addictive, but are also widely known to increase risk-taking behavior, which can lead to unsafe sexual behavior, fatal accidents, violence, and crime. In comparison to the tremendous progress in the study of addiction and reward mechanisms, very little is known about why these drugs exert such powerful effects on the increase of risk-taking behavior. We propose that the prefrontal cortex (PFC) has a conserved cell-assembly logic for the evaluation of risk-taking behavior, and that this computational logic is highly sensitive to disruption by methamphetamine and is regulated

by the activity patterns of dopamine (DA) neurons. Here, we tested these hypotheses by using large-scale *in vivo* recording techniques to evaluate functional connectivity patterns of cell assemblies in the PFC while mice were subjected to aversive events. We found that the PFC utilizes the specific-to-general cell assembly logic for processing dangerous events, and that the organized cell assembly patterns became disrupted after methamphetamine administration. Interestingly, we found that methamphetamine administration dramatically suppressed the basal tonic firing of DA neurons in the ventral tegmental area while having only small or no effect on temporal dynamic responses to aversive stimuli. Thus, reduced firing of DA neurons after methamphetamine administration decreased DA output to the PFC, leading to the degradation of the specific-to-general assembly code and temporal coordination in the PFC. These data validate not only the existence of the basic cell-assembly logic in the PFC, but also its vulnerability to methamphetamine intake as our hypotheses have predicted. These results provide novel insights into how dynamic PFC-DA interactions underlie the inhibitory control of risk-taking behavior in the normal brain and how it is impaired by drug use.

Disclosures: G. Fox: None. **K. Xie:** None. **J.Z. Tsien:** None.

Poster

160. Neural Mechanisms of Amphetamine Addiction

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 160.20/AAA11

Topic: G.08. Drugs of Abuse and Addiction

Support: Stanford NeuroChoice Institute
NIMH T32 MH020006

Title: White-matter tract supporting incentivized inhibition links trait impulsivity to stimulant use disorder

Authors: *J. K. LEONG, S. I. HUDSON, K. H. MACNIVEN, B. KNUTSON
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Abstract: Poor impulse control can compromise life outcomes. While high impulsivity is a trait of people with stimulant use disorders, it is unclear which neural circuits mediate this relation. Circuits that can support people's ability to control incentivized impulses may be specifically compromised in people with stimulant use disorders. Prior research implicates nucleus accumbens (NAcc) and anterior insula (AIns) activity during anticipation of incentive gain and loss, respectively. Further, AIns and ventrolateral prefrontal cortex (VLPFC) activity have been associated with inhibiting motor responses. Tract tracing studies in animals suggest that white-matter tracts directly connect these regions, which can now be identified in humans with diffusion weighted imaging techniques (DWI). Here, we examined whether trait impulsivity is

associated with the coherence of these white-matter tracts supporting incentivized inhibition (or the ability to control impulses in the face of high stakes), which might then relate to a diagnosis of stimulant use disorder. Confirming previous findings, people with a stimulant use disorder (n=32) reported higher impulsivity than healthy controls (n=40; Barratt Impusiveness Scale: $t=4.20, p<0.01$). Subjects then underwent diffusion weighted imaging, and tractography was performed to measure the coherence of white-matter tracts connecting the AIns to the NAcc, as well as the AIns with the VLPFC. Coherence of both AIns-NAcc and AIns-VLPFC tracts was less in patients than controls (AIns-NAcc: $t=-2.98, p<0.01$; AIns-VLPFC: $t=-2.40, p<0.05$). To test the spatial specificity of the structural findings, control tracts were also traced with deterministic tractography. Coherence of these control tracts, however, did not differ between patients and controls. In addition, AIns-VLPFC tract coherence statistically mediated the association between trait impulsivity and stimulant use disorder diagnosis, such that higher impulsivity was associated with reduced coherence of the AIns-VLPFC tract ($b=-0.48, p<0.05$), and less tract coherence was characteristic of patients versus controls ($b=-0.23, p=0.05$). Together, these findings suggest the contribution of high impulsivity to stimulant use disorders may depend upon diminished white-matter coherence in tracts that support incentivized inhibition, and so highlight specific behaviors and neural paths for intervention.

Disclosures: J.K. Leong: None. **S.I. Hudson:** None. **K.H. MacNiven:** None. **B. Knutson:** None.

Poster

161. Molecular and Pharmacological Effects of Cocaine

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 161.01/AAA12

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH, NIDA Grant DA033935

Title: Candidate gene targets identified in the rat medial prefrontal cortex (mPFC) associated with impulsivity

Authors: *C. R. MERRITT[1], D. J. SHOLLER[1], K. P. PAZRAK[1], V. D. BREHM[1], K. T. DINELEY[1,2], N. C. ANASTASIO[1,3], K. A. CUNNINGHAM[1,3]
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Abstract: The limbic-corticostriatal circuit plays a complex and important role in impulsive action, or the inability to withhold a prepotent motor response, as demonstrated by lesion, reversible inactivation, and genetic manipulations. We employed next-generation sequencing as a discovery-based approach to investigate the unique transcriptomic profile associated with

levels of impulsive action in male Sprague-Dawley rats. Low impulsive (LI) and high impulsive rats (HI) were defined by lower and upper quartiles of premature responses in the 1-choice serial reaction time (1-CSRT) task (n = 4-7/phenotype), respectively. Following behavioral phenotyping, we isolated the medial prefrontal cortex (mPFC) and processed mRNA for transcriptomic profiling. Interpretation of transcriptomic data was achieved using statistical gene-set enrichment methods in which differentially expressed genes are intersected with sets of genes that are associated with a particular biological function or pathway. Comparison analyses revealed expression of 612 transcripts in the mPFC of HI rats that were significantly higher or lower relative to LI rats. Employment of Panther Pathway and Protein Class Analysis identified several signaling networks that were significantly higher (e.g., heterotrimeric G-protein signaling pathways) and lower (e.g., apoptosis signaling pathway) in the mPFC of HI, relative to LI, rats. We then employed Ingenuity Pathway Analysis to identify upstream transcriptional regulators. Predicted upstream regulators (Z-score > 2 and overlap $p < 0.01$) included growth factors and cytokines [e.g., tumor growth factor β1 (TGFβ1), Z = 4.060, p = $4.97e^{-13}$; brain-derived neurotrophic factor (BDNF), Z = 2.693, p = $2.6e^{-11}$] as well as transcription regulators [Specificity protein 1 (Sp1), Z = 3.305, p = $8.64e^{-7}$; catenin β1 (CTNNB1), Z = 3.662, p = $9.08e^{-6}$]. Interestingly, there were genes in our dataset previously reported to be regulated by cocaine (Z = 2.254, p = $2.40e^{-7}$) (e.g., Fos, Arc, Egr2), although these subjects were not previously exposed to cocaine. We verified the expression profile of Egr2 in HI and LI rats through quantitative real-time polymerase chain reaction (RT-PCR) analyses, and observed a positive correlation of Egr2 mRNA expression with premature responses ($R^2 = 0.29$; $p < 0.05$). Thus, our tandem utilization of next-generation sequencing and pathway analyses identified a registry of candidate gene targets in the mPFC associated with impulsivity, including Egr2.

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Poster

161. Molecular and Pharmacological Effects of Cocaine

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Topic: G.08. Drugs of Abuse and Addiction

Support: DA042100-01A1 (DMW)
P30 DK079637 (AW)
P50MH096890 (EJN)
R01051399 (EJN)
P01DA008227 (EJN)

Title: Adolescent stress reprograms the medial amygdala transcriptome and sex differences in reward

Authors: *D. M. WALKER[1], X. ZHOU[6], A. RAMAKRISHNAN[1], M. E. CAHILL[2], C. K. LARDNER[3], C. J. PENA[1], H. M. CATES[2], O. ISSLER[2], R. C. BAGOT[7], E. S. CALIPARI[8], G. E. HODES[9], M. A. DOYLE[10], E. A. RIBEIRO[4], S. J. RUSSO[2], P. J. KENNEDY[11], A. WOLFE[12], B. ZHANG, 10128[5], E. J. NESTLER[13]
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Abstract: Adolescence, a time of heightened sensitivity to rewarding stimuli, is associated with vulnerability to psychiatric disorders. Male rodents that experience adolescent social isolation stress (SI) form stronger preferences for drugs of abuse in adulthood. However, little is known about how females respond to SI. The medial amygdala (meAMY) is sexually dimorphic, develops during adolescence and is sensitive to SI. Our preliminary data suggest that SI reverses sex differences in adult reward behaviors and permanently reduces baseline sex differences (M>F) in neuronal projections from meAMY to ventral tegmental area (VTA). Across adolescent development (postnatal day (P)22, P32, P42 & P72), SI females show a male-typical developmental pattern in corticosterone, and progesterone is reduced in SI adults (M & F). Given these peripheral and behavioral alterations, we tested the hypothesis that SI alters the meAMY transcriptome in a persistent and sex-specific manner. Mice were isolated or group housed (GH) from P22 - P42, then GH until ~P90. Transcriptome-wide changes in meAMY were investigated by RNA-seq after cocaine (acute or chronic) or saline. Sexually dimorphic genes were disproportionately affected by SI (Sex X SI: 869 genes). Gene co-expression analysis revealed that SI results in the loss of sexually dimorphic gene co-expression in the meAMY and identified key drivers of sexually dimorphic gene expression. We are now manipulating levels of key driver genes in meAMY to determine if their overexpression recapitulates the effects of SI. Together, these data suggest that the meAMY plays an important role in sex differences in cocaine reward and that SI disrupts sex-specific adolescent development of brain connectivity, gene transcription and endocrine measures.

Disclosures: D.M. Walker: None. **X. Zhou:** None. **A. Ramakrishnan:** None. **M.E. Cahill:** None. **C.K. Lardner:** None. **C.J. Pena:** None. **H.M. Cates:** None. **O. Issler:** None. **R.C. Bagot:** None. **E.S. Calipari:** None. **G.E. Hodes:** None. **M.A. Doyle:** None. **E.A. Ribeiro:** None. **S.J. Russo:** None. **P.J. Kennedy:** None. **A. Wolfe:** None. **B. Zhang:** None. **E.J. Nestler:** None.

Poster

161. Molecular and Pharmacological Effects of Cocaine

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Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 161.03/AAA14

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA

Title: Elucidating the cell type-specific transcriptional patterns differentiating stimulant versus opiate addiction

Authors: *C. K. LARDNER[1], P. J. HAMILTON[1], H. G. KRONMAN[1], H. M. CATES[1], D. M. WALKER[1], J. FENG[2], E. J. NESTLER[1]
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Abstract: The onset and persistence of drug addiction is mediated, in part, via cell-type specific mechanisms of transcription in the brain. However, between classes of abused drugs, little is known about how common transcriptional mechanisms converge and diverge to cause an addictive phenotype. Within the nucleus accumbens (NAc), ΔFosB, a Fos family transcription factor, accumulates solely in D1-type medium spiny neurons (MSNs) following repeated exposure to cocaine and most other classes of drugs of abuse, but in both D1- and D2-type MSNs uniquely following repeated exposure to morphine or other opiates. This phenomenon makes ΔFosB and its downstream effectors promising therapeutic targets, but it is unknown how ΔFosB coordinates the distinct effects of stimulants versus opiates via actions in these two cell types. We are using two approaches to address this gap in knowledge. First, ΔFosB's transcriptional activity genome-wide has thus far been elusive due to the lack of antibodies suitable for ChIP-seq. To address this, we generated a knockin mouse in which ΔFosB is expressed with a hemagglutinin (HA) tag fused at its N-terminus, which allowed for anti-HA targeting of ΔFosB using ChIP-seq following chronic cocaine or morphine administration. Next, we investigated which of the targets identified by ChIP-seq are regulated in D1- or D2-MSNs. To do this, we adapted CRISPR/Cas9 technology to induce ΔFosB in a MSN subtype-specific manner, and performed RNA-seq. By inducing ΔFosB accumulation in the absence of drug, we recapitulated the whole-NAc transcriptional outcomes of ΔFosB's induction in D1- and/or D2-MSNs. Conversely, we also used CRISPR/Cas9 to suppress activity of the *FosB* gene in D1- and/or D2-MSNs in the context of drug exposure, in order to study the necessity of ΔFosB in these transcriptional outcomes. Understanding the cell type-specific genomic patterns of ΔFosB in the effects of cocaine and morphine will clarify their mechanisms of action and reveal new therapeutic opportunities. Moreover, our method for studying the genomic patterns coordinated by a single transcription factor more broadly serves as a novel mode of investigation into

understanding how cell type-specific transcriptional patterns interact with environmental input to guide behavior.

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Poster

161. Molecular and Pharmacological Effects of Cocaine

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Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 161.04/AAA15

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA

Title: Mechanisms of epigenetic priming in cocaine addiction

Authors: *P. MEWS[1], H. KRONMAN[1], A. RAMAKRISHNAN[1], S. SIDOLI[2], E. G. PECK[1], B. GARCIA[2], E. J. NESTLER[1]
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Abstract: The need for deep mechanistic insight into drug addiction is driven by sharp increases in drug abuse, and by the lack of efficacy of conventional pharmacotherapy. Growing evidence implicates altered gene expression in the brain's reward circuitry in mediating the lasting effects of cocaine, and more recent work supports a key role for epigenetic pathways in the molecular pathology of addiction. Permanent changes in chromatin structure are hypothesized to underlie the transcriptional dysregulation caused by cocaine, particularly in the nucleus accumbens (NAc), a key brain region of reward learning. However, such epigenetic priming is virtually unexplored in the context of psychiatric disease and the molecular mechanisms responsible remain unclear. Further, the NAc is composed (>90%) of two functionally distinct types of medium spiny neurons (MSNs), the D1 and D2 dopamine receptor expressing subtypes, therefore making the cell-type specific identification of epigenetic changes most critical. Here, we investigated cocaine-induced changes in chromatin accessibility genome-wide by ATAC-seq in pure populations of D1 and D2 MSNs through which we distinguished immediate versus persistent alterations in chromatin in a cell-type specific manner. Combining these data with unbiased histone modification profiling by mass spectrometry, we found that chronic cocaine persistently alters the chromatin structure especially in D1 MSNs. Specifically, genome accessibility is markedly increased at key neuronal genes in D1 MSNs, linked to dysregulated gene expression even after prolonged periods of withdrawal. Our investigations provide novel insight into epigenetic priming as an important mechanism whereby drugs of abuse induce long-lasting transcriptional dysregulation. Since epigenetic aberrations may be reversible, this

mechanistic understanding of such chromatin 'scarring' by drugs of abuse will pave the way to novel epigenetic interventions to treat drug addiction.

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Poster

161. Molecular and Pharmacological Effects of Cocaine

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Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 161.05/AAA16

Topic: G.08. Drugs of Abuse and Addiction

Support: PUT1686

Title: The changes in the levels of DNA methyltransferases and demethylases in cocaine-induced behavioral sensitization model in rats with different exploratory activity

Authors: *K. ANIER[1], K. VAHER[2], K. LAUGUS[2], M. JÜRGENSON[1], J. HARRO[2], A. KALDA[1]
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Abstract: Epigenetic modifications, such as DNA methylation and demethylation, are involved in the cocaine-induced behavioral sensitization (BS) mechanisms. The aims of study were to investigate a) the development and expression of cocaine-induced BS in rats with high and low exploratory activity (HE- and LE-rats, respectively); b) to evaluate whether the epigenetic DNA modifications in reward-related brain structures, such as nucleus accumbens (NAc) and lateral habenula (LHb), may be the underlying mechanism of the behavioral differences in LE- and HE-rats. We found that only LE-animals had increased locomotor activity over repeated test sessions in response to repeated cocaine (RC, 12 mg/kg, i.p.) administration. The challenge exposure to cocaine was carried out 14 days of withdrawal period after 7 day induction period. The RC groups of both LE- and HE-rats demonstrated increased locomotor activity compared to the control groups. However, the BS to RC administration was only expressed in the LE-rats. Similar changes with LE-rats were described after repeated amphetamine treatment (Alttoa et al., 2005). Cocaine-induced changes in mRNA and enzyme activity levels of DNA methyltransferases (Dnmts) and demethylases (ten-eleven translocases enzymes, Tet1-3) were specific for NAc and LHb, but missed from cerebellum. However, NAc and LHb did not significantly differ in the expression of the Dnmts and Tets. In RC group, HE-rats showed statistically significant increases in *Dnmt3b* and *Tet3* mRNA levels both in NAc and LHb. Similarly, in the acute cocaine (AC) group, HE-rats showed statistically significant differences in *Tet1* and *Tet2* mRNA levels in LHb. These changes coincided with results of DNMT and TET enzyme activity levels, as we found that in RC group, DNMT activity was higher in HE-rats

compared to LE-rats both in NAc and LHb. Also, in the RC group, TET activity was higher in NAc of HE-rats compared to LE-rats. Thus, our results suggest that animals with active or passive style of adaptation with environment differ in the development and expression of cocaine-induced BS and DNMT/TET enzyme activity levels both in NAc and LHb. The observed changes in DNMT and TET activities could explain the behavioral differences between HE- and LE-animals in response to cocaine.

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Poster

161. Molecular and Pharmacological Effects of Cocaine

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Program #/Poster #: 161.06/AAA17

Topic: G.08. Drugs of Abuse and Addiction

Support: T32GM008076
DP1- DA044250

Title: Cocaine-specific induction of Nr4a regulates dopaminergic target gene expression during abstinence

Authors: *M. CARPENTER[1], Q. HU[1], S. I. LOMBROSO[1], M. E. WIMMER[2], C. PIERCE[3], E. A. HELLER[4]
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Abstract: The overall goal of this study is to examine a novel mechanism of cocaine-mediated gene regulation via upregulation of the transcription factor, Nuclear Receptor Subfamily 4 1 and 3(*Nr4a*), which was identified in a comprehensive, next-generation RNA-sequencing study. Transcriptome analysis, of the mouse nucleus accumbens (NAc) following cocaine (or saline) self-administration revealed upregulation of the transcription factors *Nr4a1 and Nr4a3,* as well as several of their target genes relevant to cocaine-mediated behavior, including tyrosine hydroxylase (*TH*), brain derived neurotropic factor (*Bdnf),* period circadian clock 2 (*Per2*), cocaine and amphetamine-regulated transcript peptide (*CARTp*), and vesicular monoamine transporter (*VMAT*). Interestingly, regulation of these Nr4a-target genes were found at both 1 day and 28 days of abstinence. Based on these preliminary data and that of the neurobiological literature, the central hypothesis of this study is that cocaine-induced enrichment of *Nr4a* and Nr4a-mediated histone modifications at dopamine-related target genes in NAc play a functional role in gene expression changes and drug reward behaviors that persist during abstinence. Given

that Nr4a induces the expression of *Th, Per2, Bdnf, Cartp, VMAT*, via the recruitment of histone modifying enzymes, we predicted that cocaine activation of *Nr4a* expression leads to stable enrichment of H3K27/K4me3 at target gene promoters that persists during abstinence. Thus, we have measured the enrichment of Nr4a and related histone modifications at putative target genes at both early and late abstinence. We found that cocaine regulated the enrichment of Nr4a1 and methylation of histone H3 lysines 4 and 27 at promoter regions of target genes. We then used viral-mediated expression of CRISPR-activation (dCas9-VP64) or inhibition (dCas9-KRAB) targeted to *Nr4a* to determine the causal role of *Nr4a1* gene expression on down-stream target gene activation. We found that CRISPR dCas9-VP64 or -KRAB, increased and decreased NR4a1 expression, respectively, both in vitro (N2A cells) and in vivo (intra-NAc). Additionally, we found that viral-mediated expression of these constructs significantly regulated conditioned cocaine preference. In conclusion, we utilized the CRISPR/dCas9 technology to regulate *Nr4a1* and target-gene expression, as well as cocaine reward, providing direct causal evidence that *Nr4a* gene activation leads to downstream epigenetic target gene regulation that underlies cocaine reward.

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Poster

161. Molecular and Pharmacological Effects of Cocaine

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Program #/Poster #: 161.07/AAA18

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA P01 DA08227
NIDA R01 DA07359

Title: Microtubule-associated proteins control dendritic spine morphology in the nucleus accumbens and promote cocaine seeking

Authors: *A. GODINO[1], E. S. CALIPARI[2], M. SALERY[1], E. G. PECK[1], D. DAMEZ-WERNO[1], J. A. LANDRY[1], M. E. CAHILL[3], D. M. DIETZ[4], E. J. NESTLER[1]
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Abstract: Addictive behaviors, including drug-seeking and relapse, are thought to depend in part on long-lasting drug-induced adaptations in dendritic spine signaling and morphology in the

nucleus accumbens. Therefore, it is critical to characterize the molecular mechanisms through which newly-formed spines are stabilized and maintained over longer periods of time to ultimately drive drug seeking. While activity-dependent actin remodeling has been extensively documented, the role of microtubules and associated proteins or signaling pathways remains poorly understood. Here we investigate the roles of microtubule-End-Binding Protein 3 (EB3) and Src kinase phosphorylation in this process. We used biochemistry and confocal imaging to measure synaptic protein expression and dendritic spine morphology after short *versus* long withdrawal periods from cocaine self-administration. We then examined the effects of viral-mediated over-expression of EB3 or Srcin1, a negative regulator of Src phosphorylation, on the reinforcing efficacy of cocaine, cocaine seeking, and dendritic spine morphology. Phosphorylation of Src at an activating site was induced after one day of withdrawal while EB3 levels were increased only after 30 days. Further, EB3 or Srcin1 overexpression recapitulated the spine morphology changes observed at the different withdrawal time points. Additionally, EB3 overexpression increased the motivation to consume cocaine and increased cocaine-seeking, while Srcin1 overexpression had the opposite effects. Finally, blocking Src phosphorylation during early withdrawal abolished incubation of cocaine craving. This study suggests that cocaine self-administration leads to the formation of immature dendritic spines, which serve as a scaffold for the stabilization of cocaine-associated memories via a process that involves Src-dependent invasion of EB3-bound microtubules at later stages.

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Poster

161. Molecular and Pharmacological Effects of Cocaine

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Title: Role of IL-1 receptor-associated kinases in cocaine addiction

Authors: *R. WU[1], J. LIU[2], B. JOHNSON[1], J. VU[1], J. LI[1]
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Abstract: Drug addiction has been suggested to interact with neuroimmune system, which in turn modulates several stages of drug addiction. Recent studies demonstrated that Toll-like receptor 4 (TLR-4), a prototypic pattern-recognition receptor which plays a crucial role in the innate immune system, regulated cocaine reward and reinforcement. However, the role of TLR-4 dependent signaling pathway in drug addiction is still elusive. Among the downstream signaling pathways, IL-1 receptor-associated kinases (IRAKs), such as IRAK-1 and IRAK-4, are important mediators in TLR4 signaling. Recent studies showed that ethanol could activate IRAK, suggesting that IRAKs might participate in drug addiction. In this study, we investigated the effects of cocaine using on the expression of IRAKs in drug reward system. Rats were trained with two different cocaine self-administration paradigms, short access (2h/day, 10 days) and long-access (6h/day, 10 days). Tissues from the ventral tegmental area (VTA) and the Nucleus Accumbens (NAc) were collected 1 day after short-access cocaine training, or immediately after cocaine-seeking tests on day 1 and day 45 (WD1 and WD45) after cocaine withdrawal in the long-access training paradigm. We found that in the short-access cocaine paradigm, there was no difference in the expression levels of phosphorylated and total IRAK-1 neither in the VTA or the NAc in cocaine rats compared with control rats. Cocaine long-access training resulted in an incubation in cocaine-seeking tests after abstinent from cocaine (WD45 vs. WD1). However, there was no change in the expression levels of IRAK-1 in the VTA and NAc on WD1 or WD45 compared with control group. Taken together, these results suggested that IRAK-1 may not play a role in cocaine addiction. Our future study will further investigate the role of other IRAKs, such as IRAK-4, in cocaine addiction.

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Poster

161. Molecular and Pharmacological Effects of Cocaine

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Title: Effects of cocaine self-administration on melanocortin-4 receptors in selected rat brain structures

Authors: *D. GAWLINSKI, K. MUDLAFF, M. FRANKOWSKA, K. WYDRA, M. FILIP
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Abstract: Background: Substance use disorder is a disease of the central nervous system related to the physiological, behavioral and cognitive disorders, among which the intake of a substance

(e.g. cocaine) dominates over other behaviors that were previously valuable for patient. Numerous chronic and acute health problems are associated with drug use and what is equally important, an enormous social and economic burden. Previous research suggests that the melanocortin system may play an important role in cocaine and alcohol abuse. The system including melanocortin-4 (MC-4) receptor, which are largely expressed brain areas associated with feelings of pleasantness and drug self-administration.
Aim: The aim of this study was to investigate the effects of the cocaine self-administration on on the level of MC-4 receptors in the selected brain structures in male rats.
Methods: At 63 postnatal day male Wistar rats was introduced three weeks of cocaine self-administration protocol (daily 2 h sessions) - stable cocaine dose (0.5 mg/kg/inf.) with increasing schedule of reinforcement fixed ratio (1- 5). To generate a proper control group, a yoked control procedure was applied. The yoked animals received an infusion of saline every time when a self-administered rat received cocaine. The animals were sacrificed through decapitation immediately following the last 18. experimental cocaine session and the prefrontal and frontal cortex, hippocampus, nucleus accumbens, dorsal striatum, ventral tegmental area, amygdala and hypothalamus were dissected for biochemical experiments. In the isolated synaptosomal fraction changes in MC-4 receptors levels were evaluated with using an enzyme-linked immunosorbent assay (ELISA).
Results: We observed that in rats self-administered cocaine MC-4 receptor levels significantly increased as compared to yoked saline control in brain regions important in reward pathway. We found increased MC-4 receptors levels in the prefrontal cortex ($p<0.01$), frontal cortex ($p<0.001$), hippocampus ($p<0.05$), nucleus accumbens ($p<0.05$) and dorsal striatum ($p<0.001$).
Conclusions: Our data suggest that self-administered cocaine induces adaptive changes in MC-4 receptors levels in brain. Thus, it seems that MC-4 receptors may play important role in rewarding properties of cocaine and they may be considered as a new potential target for the treatment of cocaine use disorder.

Disclosures: D. Gawlinski: None. **K. Mudlaff:** None. **M. Frankowska:** None. **K. Wydra:** None. **M. Filip:** None.

Poster

161. Molecular and Pharmacological Effects of Cocaine

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 161.10/AAA21

Topic: G.08. Drugs of Abuse and Addiction

Support: DA007287
DA033935

Title: Growth hormone secretagogoue receptor 1α (GHSR1α) antagonism differentially impacts cocaine intake and cue reactivity in male rats

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Abstract: Ghrelin is the endogenous ligand for the growth hormone secretagogue receptor 1 alpha (GHSR1α), a G-protein-coupled-receptor (GPCR) distributed within nodes of the mesolimbic reward circuit. Ghrelin administration increased cocaine-induced hyperactivity and plasma ghrelin correlated with the magnitude of cocaine-seeking, while blockade of GHSR1α suppressed cocaine-evoked behaviors in rodents. Here, we tested the hypothesis that the GHSR1α antagonist JMV2959 (JMV) will suppress cocaine intake during self-administration (SA) or lever presses for cocaine-associated cues during abstinence (cue reactivity). Male Sprague-Dawley rats were trained to stability on cocaine self-administration (0.25 mg/kg/0.1 ml/infusion) prior to assessment of intraperitoneal (i.p) injection of 0.5, 1 or 2 mg/kg of JMV or saline (0.9%) administered 20 min before the SA session in a within-subjects design. A second cohort of rats was trained to stability on an FR5 schedule of cocaine SA (0.75 mg/kg/0.1 ml/infusion) in daily 180-min sessions. Twenty-four hours after the last cocaine SA session, rats were injected with JMV (1 or 2 mg/kg; i.p.) or saline 20 min before the cue reactivity test during which presses on the previously active lever resulted in delivery of cocaine-associated cues, but not cocaine. Pretreatment with JMV did not suppress cocaine intake nor alter inactive lever presses at any dose tested. Pretreatment with JMV suppressed cocaine cue reactivity at 2 mg/kg; no JMV effects were observed on inactive lever responses. Time course evaluations of the cue reactivity test session revealed a significant suppression of lever presses for cocaine-associated cues during the last 40 min of the 60 min session following pretreatment with 2 mg/kg of JMV ($p < 0.05$). These results indicate that the GHSR1α antagonist JMV differentially impacts the drive to seek, but not take, cocaine. Given that cue reactivity (attentional bias toward cocaine-associated cues) is a key phenotype that sets up vulnerability to relapse during recovery, future studies will investigate the neurocircuitry through which GHSR1α systems regulate this facet of relapse vulnerability in cocaine use disorder.

Disclosures: E.J. Garcia: None. **V.D. Brehm:** None. **R.G. Fox:** None. **N.C. Anastasio:** None. **K.A. Cunningham:** None.

Poster

161. Molecular and Pharmacological Effects of Cocaine

Location: SDCC Halls B-H

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Program #/Poster #: 161.11/AAA22

Topic: G.08. Drugs of Abuse and Addiction

Support: P30 DA029925
R33 DA038019
F32 DA043931

Title: Allosteric neurotensin receptor 1 modulator confers β-arrestin bias and selectively attenuates addiction-associated behaviors

Authors: *L. M. SLOSKY[1], K. TOTH[1], Y. BAI[1], L. ROCHELLE[1], V. POGORELOV[1], C. RAY[1], R. CHANDRASEKHAR[1], H. YUAN[2], A. BADEA[1], W. WETSEL[1], A. PINKERTON[3], M. G. CARON[1], L. S. BARAK[1]
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Abstract: Psychostimulant addiction is a large and growing public health concern for which there are no effective therapeutics. Psychostimulants, including methamphetamine (meth) and cocaine, incite aberrant activation of the mesolimbic dopamine system. Restoration of dopamine signaling homeostasis may be achieved by targeting the G protein-coupled receptor (GPCR) neurotensin receptor 1 (NTR1). Characteristic of GPCRs, NTR1 signals not only through the canonical activation of G proteins, but also through β-arrestins to mediate distinct cellular and physiological effects. Drug-like, small molecule NTR1 agonists have remained elusive, despite decades of screening using G protein-based approaches. Employing a β-arrestin-based screening platform we have identified a promising preclinical lead: a compound known as SBI-553. Here, we report that SBI-553 exhibits novel pharmacodynamic properties, acting as both a β-arrestin biased ligand and a positive allosteric modulator that biases neurotensin-NTR1 signaling towards the β-arrestin pathway. This β-arrestin-biased modulator attenuates physiologic and behavioral evidence of psychostimulant exposure in animal models of drug taking and abuse without the dose-limiting side effects characteristic of unbiased NTR1 agonism. Using state-of-the-art, small animal ^{18}F-flurodeoxyglucose micro positron emission tomography (PET)/computed tomography (CT) imaging, we found that systemic SBI-553 pre-treatment reduced stimulant-like, dopamine-induced changes in murine regional brain glucose metabolism. Critically, SBI-553 attenuated meth- and cocaine-induced hyperlocomotion and reduced intravenous cocaine self-administration in C57BL/6J mice. In line with its biochemical mechanism of action, studies in whole body and neuron-specific β-arrestin2 knockout mice revealed that SBI-553 locomotor modulation requires β-arrestin2 to be present in striatal dopamine D2 receptor-expressing neurons. In contrast to the action of unbiased NTR1 agonists, the suppression of psychostimulant-associated behaviors by SBI-553 was not concurrent with hypothermia or sedation. These data demonstrate the ability of a small molecule to confer complete functional selectivity to an endogenous ligand and, with it, more directed pharmacological action.

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Poster

161. Molecular and Pharmacological Effects of Cocaine

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 161.12/AAA23

Topic: G.08. Drugs of Abuse and Addiction

Support: IRP/NIDA/NIH/DHHS

Title: Cocaine-induced deficit in orbitofrontal function is prevented by systemic administration of a sigma-1 receptor antagonist

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Abstract: The orbitofrontal cortex (OFC) is necessary for inferring expected outcomes to guide appropriate responding. This function can be shown in a sensory preconditioning task, in which behavior to the preconditioned but not the directly conditioned cue is sensitive to inactivation of the OFC after learning. Self-administration of cocaine causes similar deficits in preconditioning, suggesting drug-induced problems in model-based inference that might complicate treatment. The sigma-1 receptor (Sig-1R), is a chaperone protein predominantly expressed on the ER membrane. It has been reported that the receptor modulates the function of other proteins by physical interaction. Many synthetic compounds, including psychiatric drugs and psychostimulants bind to the Sig-1R, and cocaine is known to act as an agonist. We previously reported that a Sig-1R antagonist, BD1063, given prior to administration of cocaine, attenuated behavioral sensitization caused by cocaine, and reversed the cocaine-induced hypoexcitability of medium spiny neurons in nucleus accumbens. Here, we investigated the potential role of Sig-1R in cocaine-induced functional alterations of OFC. Sig-1Rs were localized in the rat OFC by immunohistochemistry and western blot. Rats were given the opportunity to spontaneously (without cue light) acquire self-administration of cocaine or sucrose, with a prior injection of either BD1063 or saline for 12 days. After four weeks of cocaine withdrawal, rats were trained in a sensory preconditioning task. As reported previously, rats withdrawn from cocaine self-administration exhibited a deficit in sensory preconditioning performance, failing to respond appropriately to the preconditioned cue. This deficit was not present in rats that had received the Sig-1R antagonist BD1063 prior to each cocaine self-administration session. The potential mechanisms underlying this result were further examined, focusing on the gamma-aminobutyric acid receptor (GABAR) and serotonin 2A receptor (5HT-2AR) neuronal responses in the OFC. An association between GABAR or 5HT-2AR with Sig-1R was found using

coimmunoprecipitation in HEK cells overexpressing these receptors. Ongoing electrophysiological experiments are examining the activity of native 5-HT-2ARs in the OFC of rats from these treatment groups. Thus far, our results are consistent with involvement of the Sig-1R in the effects of cocaine on OFC function. Although more experiments are needed including the use of Sig-1R antagonist during cocaine withdrawal, our data suggest the Sig-1R as a potential therapeutic target in the treatment of cocaine addiction.

Disclosures: Y. Kimura: None. **M. Sharpe:** None. **A. Zapata:** None. **A. Wright:** None. **L. Mueller:** None. **C. Lupica:** None. **G. Schoenbaum:** None. **T. Su:** None.

Poster

161. Molecular and Pharmacological Effects of Cocaine

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 161.13/AAA24

Topic: G.08. Drugs of Abuse and Addiction

Support: R00 DA033374

Title: Role of medial prefrontal cortex NMDA receptors in inherent impulsivity

Authors: *B. D. DAVIS-REYES[1], V. M. CAMPBELL[1], H. L. CHAPMAN[1], S. STAFFORD[1], N. C. ANASTASIO[2]
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Abstract: Impulsivity, broadly defined as behavior without sufficient forethought, is a multifaceted behavioral manifestation that has implications in several disorders including addiction, schizophrenia, and obesity. Glutamate neurotransmission in the medial prefrontal cortex (mPFC), an important brain region in decision-making and goal-oriented behaviors, has implications in inherent impulsivity. The N-methyl-D-aspartate receptors (NMDARs) are glutamate receptors that are localized throughout the brain, including the mPFC. Functional receptors are heterotetramers composed of at least two NMDAR1 (GluN1) subunits and two NMDAR2 (GluN2A-D). Localization of these receptors within the synapse plays an important functional role within the mPFC. GluN2A-containing NMDAR are predominantly found in the synapse, while GluN2B-containing NMDAR are primarily localized extrasynaptically. Here, we tested the hypothesis that individual differences in impulsivity are driven by the composition of the NMDAR complex, specifically the expression and localization of the NMDAR subunits, within the mPFC. Outbred male Sprague Dawley rats were identified as high (HI) or low (LI) impulsive using the one-choice serial reaction time (1-CSRT) task; the upper and lower quartile of animals were identified as HI or LI rats, respectively. Following phenotypic identification, mPFC synaptosomal protein was extracted from HI and LI rats to assess the composition of the

NMDAR complex via immunoblot and/or immunoprecipitation techniques. Synaptic localization was investigated by immunoprecipitation for postsynaptic density 95 (PSD95) with subsequent western blotting for GluN2A and GluN2B. Performance on the 1-CSRT task was rapidly acquired and the HI/LI phenotype was stably expressed across training. HI rats had lower mPFC GluN1 and GluN2A, but higher GluN2B synaptosomal protein expression ($p<0.05$) vs LI rats. Further, levels of pGluN2B were also higher in HI vs LI rats ($p<0.05$). Co-immunoprecipitation analyses indicate a higher GluN2B:PSD95 synaptosomal protein complex in HI vs LI rats ($p<0.05$). Thus, there is a possible transformation of the mPFC NMDAR complex composition and/or synaptic localization that may underlie high inherent motor impulsivity. Increased understanding of the complex regulation of NMDAR balance within the mPFC as it relates to individual differences in impulsivity may lead to a better understanding of risk factors and treatments for several neurological disorders, including addiction.

Disclosures: B.D. Davis-Reyes: None. **V.M. Campbell:** None. **H.L. Chapman:** None. **S. Stafford:** None. **N.C. Anastasio:** None.

Poster

161. Molecular and Pharmacological Effects of Cocaine

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Program #/Poster #: 161.14/AAA25

Topic: G.08. Drugs of Abuse and Addiction

Support: NSF1456818
NIH NS104705
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Nebraska Department of Health & Human Services

Title: Glutamate delta-1 subunit regulates cocaine-induced plasticity in the nucleus accumbens

Authors: *J. LIU, P. GANDHI, R. PAVULURI, G. SHELKAR, S. DRAVID
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Abstract: Cocaine exposure induces plasticity of glutamatergic synapses of medium spiny neurons (MSNs) in the nucleus accumbens (NAc) which contributes to addictive behavior. The orphan glutamate delta-1 (GluD1) receptor is a member of the ionotropic glutamate receptor family with an unusual synaptogenic function. GluD1 is highly enriched in the NAc but its role in reward behavior, MSN function and drug-induced plasticity remains unknown. Using a constitutive GluD1 KO model we evaluated the effect of GluD1 ablation on cocaine conditioned place preference (CPP). Morphological analysis of dendritic spines and brain slice electrophysiology were used to assess the contribution of GluD1 in cocaine-induced structural and functional plasticity and basal neurotransmission. A pharmacological approach was used to

address mechanism of cocaine preference in GluD1 KO. GluD1 KO mice showed higher cocaine CPP. Higher cocaine preference in GluD1 KO correlated with an increase in spine density, greater maturation of dendritic spines and basally upregulated spine-regulating active cofilin. GluD1 loss did not affect basal excitatory neurotransmission or plasticity, but masked the generation of cocaine-induced silent synapses. Finally, loss of GluD1 increased the GluN2B subunit contribution to NMDA receptor currents in MSNs and a GluN2B partial agonist normalized the higher active cofilin and cocaine preference in GluD1 KO mice. These findings demonstrate a critical role of GluD1 in controlling susceptibility to cocaine preference and cocaine-induced plasticity by modulating NMDA receptor subunit contribution.

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Poster

161. Molecular and Pharmacological Effects of Cocaine

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Center for Addiction Research at the University of Texas Medical Branch

Title: The 5-HT_{2A} receptor (5-HT_{2A}R) antagonist/inverse agonist pimavanserin suppresses impulsive action and cocaine cue reactivity in rats

Authors: *D. J. SHOLLER[1], S. J. STUTZ[1], R. G. FOX[1], N. C. ANASTASIO[1], F. G. MOELLER[2], K. A. CUNNINGHAM[1]
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Abstract: Background: Impulsivity and the responsivity to cocaine-linked cues ("cue reactivity") are interlocked contributors to relapse in cocaine use disorder (**CUD**), and new pharmacotherapeutic strategies that effectively diminish both are likely to promote abstinence in cocaine-dependent individuals. The underlying neurobiology of impulsivity and cue reactivity includes a regulatory role for serotonin (**5-HT**) neurotransmission, particularly through the 5-HT_{2A} receptor (**5-HT_{2A}R**). Systemically administered preferential 5-HT_{2A}R agonists increase while selective 5-HT_{2A}R antagonists/inverse agonists decrease impulsive action, or the inability to withhold a prepotent motor response. Further, selective 5-HT_{2A}R antagonists/inverse agonists

consistently reduce both cue- and cocaine-evoked reinstatement of cocaine self-administration (**SA**) in rodents. The emergence of the clinically-available, FDA-approved 5-HT_{2A}R antagonist/inverse agonist pimavanserin (Nuplazid®) presents a novel pharmacotherapeutic prospect for the management of relapse vulnerability in CUD.
Aims: We hypothesized that pimavanserin would dose-dependently suppress impulsive action and cocaine cue reactivity during forced abstinence (**FA**) from cocaine SA in male rats, and we evaluated whether baseline levels of impulsive action would influence the efficacy of pimavanserin to suppress cocaine cue reactivity.
Methods: The 1-CSRT task was employed to assess premature responses as a measure of impulsive action. A cohort of rats was assessed in the 1-CSRT task prior to acquisition of cocaine SA (0.75 mg/kg/inf). Cue reactivity was assessed as previously-active lever presses reinforced by cocaine-associated cues (e.g., lights, pump sound) at 1 or 30 days of FA from cocaine SA.
Results: Pimavanserin (0.3-3.0 mg/kg) dose-dependently decreased premature responses in the 1-CSRT task ($p<0.05$ vs. vehicle) and previously-active lever presses (1.0-10.0 mg/kg) on FA Day 30, but not FA Day 1, from cocaine SA ($p<0.05$ vs. vehicle). A one-way analysis of covariance revealed that baseline levels of impulsive action predicted the efficacy of pimavanserin to suppress cocaine cue reactivity on FA Day 30 ($p<0.05$).
Conclusions: The efficacy of pimavanserin to suppress impulsive action and cocaine cue reactivity associated with relapse highlights the therapeutic potential for pimavanserin in the treatment of CUD.

Disclosures: D.J. Sholler: None. **S.J. Stutz:** None. **R.G. Fox:** None. **N.C. Anastasio:** None. **F.G. Moeller:** Other; Dr. Moeller is an uncompensated consultant for INDIVIOR and receives research support from Boehringer-Ingelheim.. **K.A. Cunningham:** None.

Poster

161. Molecular and Pharmacological Effects of Cocaine

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 161.16/BBB1

Topic: G.08. Drugs of Abuse and Addiction

Title: On the role of trpv1 receptors within the brain in anxiety elicited by cocaine cues

Authors: *W. NORZE, A. LOYOLA, E. TORRES, C. MALDONADO VLAAR
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Abstract: Transient Receptor Potential Vanilloid receptor 1 (TRPV1) is activated in peripheral terminals of nociceptive fibers by noxious heat, low pH, and natural product such as capsaicin, and is expressed in the brain where it seems to be involved in antinociception, locomotor control and regulation of affective behaviors. Capsazepine is a competitive antagonist of this vanilloid

receptor. We investigated the effects of blockade of TRPV1 receptors in (a) eliciting anxiolytic responses following exposure to cocaine related cues (b) changes in TRPV1 receptor expression within the mesocorticolimbic structures following cocaine exposure. Male Sprague Dawley rats received daily intraperitoneal injections of cocaine (10 mg/kg) for five consecutive days prior to being placed in activity chambers. During the daily 90 min sessions, rats paired visual and olfactory cues with the cocaine treatment. Following one day of abstinence, animals were divided into two groups which received either vehicle or capsazepine (10 μg/kg, ip) and returned to the activity chambers for 30 min followed by Elevated plus Maze (EPM) to test anxiety response to cocaine related cues. Results showed that cocaine significantly increased locomotor activity and produced behavioral sensitization within the first five days. Animals treated with capsazepine showed a significant decrease in locomotor behavior on Day 7 when compared to vehicle treated group ($p<0.05$, T-test). In the EPM, the capsazepine elicited an anxiolytic response in the cocaine treated animals vs. controls. Moreover, we found a tendency to decrease TRPV1 receptor expression in the Nucleus Accumbens (NAc) and medial Prefrontal Cortex (mPFC) regions following cocaine exposure and blockade of TRPV1 receptors. The present thesis presents for the first-time new evidence that implicates TRPV1 receptors within the dopamine mesocorticolimbic regions in regulating behaviors triggered by cocaine abuse.

Disclosures: W. Norze: None. **A. Loyola:** None. **E. Torres:** None. **C. Maldonado Vlaar:** None.

Poster

161. Molecular and Pharmacological Effects of Cocaine

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Program #/Poster #: 161.17/BBB2

Topic: G.08. Drugs of Abuse and Addiction

Support: P50 DA0333935

Title: Novel serotonin (5-HT) 5-HT_2 receptor neuroprobes exhibit unique pharmacological properties

Authors: *E. HOLLIDAY[1,2], Y. CHEN[4], Y. YANG[4], R. G. FOX[2], D. J. SHOLLER[2], C. SOTO[2], F. G. MOELLER[5], S. R. GILBERTSON[4], N. C. ANASTASIO[3], K. A. CUNNINGHAM[2]
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Abstract: The 5-HT_{2A} receptor (5-HT_{2A}R) and 5-HT_{2C}R are G protein-coupled receptors (GPCRs) implicated in the regulation of normal function (e.g., satiety, sleep) and in facets of

neuropsychiatric disorders (e.g., addiction, schizophrenia). The main functional GPCR unit for the 5-HT_{2A}R and 5-HT_{2C}R is proposed to be their homomeric form, and recent evidence supports the formation of 5-HT_{2A}R:5-HT_{2C}R heteromeric complexes, presenting prospects for novel ligands for these receptor assemblies. Given that the selective 5-HT_{2A}R antagonist M100907 (M) and the 5-HT_{2C}R agonist WAY163909 (W) each suppress motor impulsivity and cocaine-evoked behaviors, and low doses of M100907 plus WAY163909 synergize, we synthesized homomeric (M-M) and heteromeric (M-W) ligands through a tether linkage using click chemistry methods. As the length of the tether attached to parent pharmacophores increased (6-24 atoms), the molecules exhibited novel pharmacological profiles *in vitro*. We templated a bivalent compound by tethering M100907 to M100907 with the 12 atom tether (M-12-M) and this bivalent retained efficacy to suppress cocaine-induced hyperactivity, without alteration of spontaneous activity. Heterobivalent ligands with desired pharmacological properties were synthesized (14 atom linker, M-14-$W_{\pm}$; 17 atom linker, M-17-$W_{\pm}$ or M-17-$W_{S,S}$, 20 atom linker, M-20-$W_{\pm}$) from corresponding monovalent derivatives. M-17-$W_{\pm}$ (1 mg/kg, ip) did not suppress spontaneous or cocaine-evoked (15 mg/kg, ip) locomotor activity. M-17-$W_{S,S}$ (5 mg/kg,ip) suppressed total horizontal ambulation; however, the heterobivalent did not alter cocaine-evoked locomotor activity. Excitingly, M-17-$W_{S,S}$ (2 mg/kg) significantly suppressed motor impulsivity in the one-choice serial reaction time task. Novel M100907:M100907 homobivalent and M100907:WAY163909 heterobivalent molecules have distinct activities *in vitro* and *in vivo*. Given our new knowledge of a 5-HT_{2A}R:5-HT_{2C}R heterocomplex, we propose to optimize the tether length of molecules and explore the bivalents to elucidate the structural and functional source of activity.

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Poster

161. Molecular and Pharmacological Effects of Cocaine

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Program #/Poster #: 161.18/BBB3

Topic: G.08. Drugs of Abuse and Addiction

Support: R00 DA033734
P50 DA033935
T32 DA07287

Title: Cys23ser single nucleotide polymorphism alters function and localization of the serotonin 2C receptor (5-ht_{2C}r) *in vitro*

Authors: *M. LAND[1], H. L. CHAPMAN[1], K. A. CUNNINGHAM[3], G. F. MOELLER[4], L. A. ELFERINK[2], N. C. ANASTASIO[3]
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Abstract: The application of pharmacogenetics provides an opportunity to greatly improve treatment outcome by identifying potential biomarkers to facilitate the development of personalized pharmacotherapies for cocaine use disorder. A non-synonymous SNP of the human 5-HT_{2C}R gene that converts a cysteine (Cys) to a serine (Ser) at amino acid codon 23 (Cys23Ser) appears to impact 5-HT_{2C}R pharmacology at a cellular and systems level. The Cys23Ser SNP has been linked to changes in efficacy of psychiatric therapeutics and clinically to several psychiatric disorders and related behaviors, including impulsivity and cocaine cue reactivity, and thus may serve as a biomarker for cocaine use disorder-related behaviors. While the functional impact of this SNP is not well understood, overall the Ser23 variant could impact behavioral and pharmacological responses, possibly due to reduced function and a distinct subcellular localization profile. The ultimate level of 5-HT_{2C}R functionality is determined by a culmination of factors, such as effective coupling to/activation of intracellular signaling components and trafficking/endosomal recycling. We hypothesized that the Cys23Ser SNP alters 5-HT_{2C}R intracellular signaling via changes in receptor subcellular localization *in vitro*. We generated CHOp38 cell lines *stably* expressing the Cys23 or Ser23 variant. 5-HT evoked a concentration-related Ca_i^{++} release in the Cys23 (EC_{50}=0.58 nM) and the Ser23 (EC_{50} 2.29 nM) cell lines. The Ser23 variant demonstrated 43% lower maximum 5-HT-induced Ca_i^{++} release and a rightward shift in potency vs the Cys23 variant ($p<0.05$). Western blot and immunocytochemistry results show lower 5-HT_{2C}R plasma membrane expression in the Ser23 vs the Cys23 cell lines ($p<0.05$); no differences in total protein expression between the Cys23 or Ser23 variant was detected. Subcellular localization studies show that both the Cys23 and Ser23 variants can enter the recycling pathway essential for receptor resensitization. Interestingly, receptor distribution within this pathway is altered, with the Ser23 variant having decreased colocalization with the early endosomal maker (EEA1, $p<0.05$). Thus, the Ser23 variant exhibits a distinct pharmacological and subcellular localization profile vs the Cys23 variant, which could impact aspects of receptor pharmacology like dosing and tolerance to 5-HT_{2C}R ligands in individuals expressing the Cys23Ser SNP.

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Poster

161. Molecular and Pharmacological Effects of Cocaine

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Program #/Poster #: 161.19/BBB4

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH RO1DA040965
P30GM103398-05

Title: Perineuronal nets alter intrinsic excitability and synaptic transmission following cocaine conditioned place preference

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Abstract: Persistent drug-associated memories facilitate drug craving, which prompts relapse in drug addicts. Our laboratory is interested in the molecular underpinnings that are responsible for the formation of pervasive drug memories. Perineuronal nets (PNNs) are specialized extracellular matrix (ECM) structures that primarily surround inhibitory parvalbumin-containing fast-spiking interneurons, which play a role in the formation and stabilization of drug memories. Previous work by us and our colleagues showed that degradation of PNNs within the medial prefrontal cortex (mPFC) attenuates cocaine-induced reinstatement and alters firing properties of pyramidal cells. This study expands upon our previous findings and defines the changes in synaptic transmission and intrinsic excitability of PNN-expressing interneurons following cocaine-induced conditioned place preference (CPP). To characterize these changes within the prelimbic mPFC, brain slices were prepared following cocaine-induced CPP and whole-cell electrophysiological recordings were performed. We found that there was a time-dependent attenuation in the number of current-induced action potentials after re-exposure to the CPP chamber in PNN-expressing interneurons, which returned to control levels 24hrs after CPP testing. In addition, we found significant changes in both the frequency and amplitude of miniature events following re-exposure to the CPP chamber. Through this work, we aim to identify the functional consequences of cocaine-induced PNN alterations, which may impact memory formation/stability and contribute to persistent drug craving.

Disclosures: E.T. Jorgensen: None. **D.J. Burchi:** None. **B.A. Sorg:** None. **T.E. Brown:** None.

Poster

161. Molecular and Pharmacological Effects of Cocaine

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 161.20/BBB5

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant R01-DA034696 to KDW
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The University of Minnesota Viral Vector and Cloning Core

Title: Selective manipulation of inhibitory signaling in dopamine neurons of the ventral tegmental area alters behavioral response to cocaine

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Abstract: Dopamine (DA) neurons of the ventral tegmental area (VTA) are an important component of reward circuitry, and have been widely implicated in the cellular and behavioral responses to drugs of abuse. Inhibitory G protein signaling in DA neurons, including that mediated by $GABA_B$ receptors ($GABA_B$Rs) and dopamine 2 receptors (D_2Rs), can regulate the excitability of VTA DA neurons, DA neurotransmission, and behaviors modulated by DA. Inhibitory G protein signaling in DA neurons is weakened by exposure to cocaine. Furthermore, genetic ablation of G protein-gated inwardly rectifying K+ (GIRK) channels, a major downstream effector of $GABA_B$Rs and D_2Rs, from DA neurons increases behavioral responses to cocaine in locomotor and self administration assays. Thus, the objective of this exploratory research is to test the hypothesis that the strength of inhibitory G protein signaling in VTA DA neurons is inversely related to the magnitude of the behavioral response to cocaine. A key prediction of this hypothesis is enhancing GIRK channel signaling in VTA DA neurons will decrease behavioral sensitivity to cocaine, and vice versa. To increase GIRK channel-dependent signaling in VTA DA neurons, we employed a Cre-dependent viral approach to overexpress a GIRK channel subunit important for channel function (*i.e.*, GIRK2), or to express $G_{i/o}$-linked designer receptors exclusively activated by designer drugs (DREADDs) in the VTA of DATCre(+) mice. To decrease GIRK channel-dependent signaling in VTA DA neurons, we overexpressed a GIRK channel subunit with a negative regulatory role (*i.e.*, GIRK3) in the VTA of DATCre(+) mice. Following overexpression, cocaine-induced locomotor activity and $GABA_B$R and D_2R signaling were assessed. This study uses balanced numbers of male and female mice, aged at least 45 d at the time of surgery and approximately 90 d at the end of behavior and time of electrophysiology. Sample sizes our lab has previously found appropriate for behavior and electrophysiology (~ 12 mice or cells/treatment) are the goal for this on-going study. Control viruses of the same volume, serotype, promotor, and fluorophore were used in

DATCre(+) mice. We find overexpression of GIRK2 increases $GABA_BR$ and D_2R signaling and decreases cocaine-induced locomotion. Similarly, inhibition of VTA DA neurons with $G_{i/o}$ DREADD decreases cocaine-induced locomotion. Conversely, overexpression of GIRK3 decreases GABABR and D2R signaling and increases cocaine-induced locomotion. Together, our data suggest VTA DA neuron GIRK channels contribute to behavioral sensitivity to drugs of abuse, and could be a promising therapeutic target for addiction.

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Poster

161. Molecular and Pharmacological Effects of Cocaine

Location: SDCC Halls B-H

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Program #/Poster #: 161.21/BBB6

Topic: G.08. Drugs of Abuse and Addiction

Support: W.M. Keck Foundation
NIH Grant R01EB022015

Title: A new tool for high-precision in-situ pharmacokinetic and pharmacodynamic measurements within the brain

Authors: *K. PLOENSE[1], N. ARROYO-CURRAS[2], J. GERSON[3], K. W. PLAXCO[4], T. E. KIPPIN[5]
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Abstract: It has long been recognized that cocaine evokes complex neurochemical responses that mediate their influence of neural circuits underlying behavioral control, such as the dopamine pathways that we now know drive reward anticipation. However, the time resolution with which existing methods can measure cocaine is *orders of magnitude* poorer than the resolution with which they can measure dopamine, and thus our understanding of the relationship between cocaine delivery/metabolism and the dopamine response is limited. In response to this problem, our group has recently developed a new technology, electrochemical aptamer-based sensors (E-AB sensors), which are the size of a human hair and can detect physiologically relevant concentrations of specific drugs *in-situ*, in awake, freely behaving animals. Here, we tested this new technology on rats that have been administered 5 mg/kg of cocaine intravenously, then measured concentrations of cocaine within the lateral ventricle concurrent with monitoring locomotor activity. We successfully measured the concentration of cocaine every 3 s for 20 minutes and generated a pharmacokinetic profile for cocaine within the lateral ventricle. As cocaine concentrations increased (from 0 to 25 μM), they directly corresponded with behavioral

stereotypy, which is typically associated with increased dopamine, in rats. In conclusion, we have developed a novel, potentially revolutionary, technology that can measure the pharmacokinetics and pharmacodynamics of psychoactive drugs within the brain.

Disclosures: N. Arroyo-Curras: None. **J. Gerson:** None. **K.W. Plaxco:** None. **T.E. Kippin:** None.

Poster

161. Molecular and Pharmacological Effects of Cocaine

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 161.22/BBB7

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

Support: NS021229
DA036572
DA009411

Title: Down-regulation of K^+, Cl^- cotransporter KCC2 in the ventral tegmental area contributes to the motivational properties of cocaine

Authors: *A. OSTROUMOV, Y. ZHANG, A. MCHUGH, E. REGO, J. DANI
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Abstract: Drugs of abuse trigger multiple neuroadaptations within the mesocorticolimbic dopamine system leading to the emergence of various drug-related behaviors. In rats, acute exposure to cocaine alters inhibitory signaling in the ventral tegmental area (VTA) due to post-translational down-regulation of the neuron-specific anion transporter, KCC2. KCC2 extrudes chloride out of the neuron to maintain hyperpolarizing $GABA_A$ receptor function, and in the VTA this transporter is expressed exclusively in non-dopaminergic neurons. In VTA GABA neurons, cocaine-induced down-regulation of KCC2 leads to intracellular chloride accumulation, which produces a depolarizing shift in the $GABA_A$ reversal potential (E_{GABA}). Cocaine-induced depolarization of E_{GABA} requires D1 receptor activation and can be observed in male and female rats. Analogous to acute exposure, cocaine self-administration down-regulates KCC2 and depolarizes E_{GABA} in VTA GABA neurons. Restoring normal KCC2 function in the VTA, normalizes GABA signaling and decreases motivation to self-administer cocaine under a progressive-ratio schedule. These results suggest that cocaine exposure alters inhibitory circuitry within the VTA, and KCC2 is a potential therapeutic target to decrease pathological motivation for cocaine.

Disclosures: A. Ostroumov: None. **Y. Zhang:** None. **A. McHugh:** None. **E. Rego:** None. **J. Dani:** None.

Poster

162. Animal Cognition and Behavior: Decision Making: Corticolimbic Circuits

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 162.01/BBB8

Topic: H.01. Animal Cognition and Behavior

Title: Dynamics of neuronal signals in primate midbrain dopamine neurons and orbitofrontal cortex neurons during value-to-decision transformation

Authors: *M. YUN[1], T. KAWAI[2], M. NEJIME[2], H. YAMADA[2], M. MATSUMOTO[2]
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Abstract: In economic choice behavior, animals evaluate the value of an option and then decide to choose or not to choose that option. Although several cortical and subcortical areas are known to signal value information and contribute to decision-making, the dynamics of their neuronal signals during the value-to-decision transformation remains unclear. To explore and compare the neural dynamics between cortical and subcortical areas, we recorded single-unit activity from midbrain dopamine (DA) neurons and neurons in the orbitofrontal cortex (OFC) in monkeys performing a value-based decision-making task. In this task, only one option appeared and the monkey needed to immediately decide to choose or not to choose it. Briefly, six visual stimuli were associated with different amounts of a liquid reward, and one of them was presented as an option. The monkey was required to decide to choose or not to choose that option within a limited time. After the decision, another stimulus was presented. If the monkey had chosen the former option, the animal obtained the reward associated with that option at the end of the trial. If the monkey had not chosen the former option, the animal obtained the reward associated with the latter stimulus. We recorded the activity of 91 DA neurons and 266 OFC neurons from two monkeys, and found three groups of neurons that represented different signals related to animals' choice behavior. The first group represented the value of the option. The second group represented the value of the option but only when the monkey chose that option, and did not represent the value when the option was not chosen. Thus, the activity of these neurons was influenced not only by the value but also by the decision. The third group represented the decision itself, i.e., whether the monkey decided to choose or not to choose that option. Such three groups were observed not only in OFC neurons but also in DA neurons. To examine the dynamics of these signals, we next performed a regression analysis with the three variables (i.e., value, decision-dependent value, and decision) for each neuron using a sliding window, and explored the proportions of neurons signaling the three variables as a function of time. We found that the dynamics in both DA and OFC neurons resembled the time course of value-to-decision transformation. Shortly after the onset of the option, the proportion of neurons signaling the value rapidly arose. Then, the proportion of neurons signaling the decision-dependent value

increased. Lastly, neurons signaling the decision emerged. These results suggest that not only OFC neurons, even DA neurons are involved in the neural mechanism underlying value-to-decision transformation.

Disclosures: M. Yun: None. **T. Kawai:** None. **M. Nejime:** None. **H. Yamada:** None. **M. Matsumoto:** None.

Poster

162. Animal Cognition and Behavior: Decision Making: Corticolimbic Circuits

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 162.02/BBB9

Topic: H.01. Animal Cognition and Behavior

Support: TAKEDA SCIENCE FOUNDATION

Title: Stable and unstable signals for encoding expected values in ventral and dorsal striatum of monkeys

Authors: *H. YAMADA, M. MATSUMOTO
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Abstract: Encoding of the expected values are widely observed phenomena in the brain, indicating that many brain regions are somehow involved in value-based decision makings. However, the nature of these signals is poorly understood in terms of i) integration of probability and magnitudes and ii) its temporal dynamics. To this end, we examined the following two questions: i) whether activity of neurons encode the expected values as a combination of probabilities and magnitudes of rewards, and ii) whether the evolved signals appeared stable or instantaneous during the presentation of the cue stimuli. We used simple classical conditioning task, in which the expected values of stimulus was indicated by pie-chart visual stimuli to the monkeys with a great precision; 0.1 to 1.0 probability of fluid rewards by 0.1 increment and 0.1 ml to 1.0 ml rewards by 0.1 ml increment. This enable us to map out the neuronal activity in the space of probability and magnitude of rewards (i.e., expected values) from the onset to offset of the cue. During the presentation of pie-chart stimuli, we recorded 197 and 137 presumed projection neurons (PANs) from the dorsal and ventral striatum of two monkeys, both of which are candidates for encoding the expected values. First, PANs in both ventral and dorsal striatum showed a phasic activity after the cue presentation with a wide variety of the response latency. These phasic activities cover the whole time period of cue presentation. A conventional analysis to detect the expected value modulation, a linear regression using the expected values as a regressor, revealed that more than 60% of neurons in both ventral and dorsal striatum showed the significant modulations, but this would be erroneously detected. A more elaborated analysis, one using both the probability and magnitude as regressors separately, indicated that the ventral

striatum carry the signals of expected values and of non-expected values as a mixture in its population, while dorsal striatum carry the signal of probability or magnitude predominantly. By using state-space analysis (Monte et al., 2013), we found that the signals maintained in ventral striatum population were very stable through cue presentation, but in contrast, the probability and magnitude signals in dorsal striatum population were unstable and fluctuated. These results indicated dynamic relaying activity enable the ventral striatum to represent rigid expected value signals at the level of population.

Disclosures: H. Yamada: None. **M. Matsumoto:** None.

Poster

162. Animal Cognition and Behavior: Decision Making: Corticolimbic Circuits

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 162.03/BBB10

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant MH002928-01

Title: The amygdala is implicated in stimulus-based, but not action-based, reinforcement learning in rhesus macaques

Authors: *K. SCARIM[1], V. D. COSTA[3], C. A. TASWELL[2], E. A. MURRAY[5], B. B. AVERBECK[4]
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Abstract: Survival requires learning to make choices that deliver rewards and avoid punishments, a behavioral process known as reinforcement learning. Organisms learn to make advantageous decisions by updating the value of the state of the environment, as well as the value of actions performed within that state, based on rewarding and punishing outcomes. Previous work from our lab found that monkeys with lesions of the ventral striatum (VS) exhibit deficiencies in stimulus-based, but not action-based reinforcement learning. We decided to test this finding in monkeys with amygdala lesions because the basolateral portion of the amygdala has strong projections to the VS, and evidence exists that it is involved in RL. We presented a probabilistic two-arm bandit reversal learning task to unoperated control monkeys (n = 6) and monkeys with bilateral excitotoxic amygdala lesions (n=2). The task had two conditions, which were run in randomly interleaved blocks of 80 trials. In the What condition the monkeys had to learn which of two images was more frequently rewarded, and in the Where condition they had to learn which of two saccade directions was most frequently rewarded. At the beginning of each block of 80 trials we introduced two new images. If it was a What block we assigned a high

reward probability to one of the images, and a low reward probability to the other. If it was a Where block we assigned a high reward probability to one of the saccade directions (left or right), and a low reward probability to the other. In each trial the animals made a saccade to one of the images to indicate their choice. Following their choice, they were stochastically rewarded. In addition, on a randomly chosen trial between 30 and 50 the choice-outcome mappings were reversed, so the opposite choice within the same condition became more frequently rewarded. Compared to unoperated controls, monkeys with amygdala lesions displayed significant deficits in choice consistency in the What condition but remained unimpaired in the Where condition. Therefore, like the VS, the amygdala is implicated in assigning values to objects but not actions within the environment, suggesting a different neural circuitry exists for action-based RL. Data collection is pending for an additional two monkeys with amygdala lesions.

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Poster

162. Animal Cognition and Behavior: Decision Making: Corticolimbic Circuits

Location: SDCC Halls B-H

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Topic: H.01. Animal Cognition and Behavior

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Title: Dissociable roles of anterior cingulate cortex and basolateral amygdala in learning and choice under perceptual uncertainty

Authors: *A. STOLYAROVA[1], M. RAKHSHAN[2], M. A. PETERS[4], H. LAU[5], A. SOLTANI[3], A. IZQUIERDO[6]
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Abstract: Most tasks that assess reward learning use clearly discriminable and unambiguous cues, leaving open the question as to how the brain copes with perceptual uncertainty when learning from positive and negative feedback. The subjective sense of certainty, or confidence, that accompanies perceptual decisions may modulate reward prediction errors to also support learning. We trained rats to discriminate between horizontal (H)- and vertical (V)-oriented sinusoidal gratings of variable contrast levels embedded in noise to make a response (using a touchscreen) according to a certain rule: e.g. H→left and V→right. Crucially, following discrimination, rats could wait a self-timed delay in anticipation of reward or initiate a new trial.

We found that this waiting time, as a proxy for confidence, increased with accuracy and was negatively correlated with reaction time, consistent with what has been reported in humans. We identified pairs of stimuli for each animal that produced matched decision accuracy, but different confidence levels. Rats were assigned to a low- (LC) or high-confidence (HC) group in a subsequent reversal learning task. Interestingly, reversal learning was improved by confidence; learning was faster in the HC group, despite matched discrimination capacity across the LC and HC groups prior to reversal. We next studied the roles of rat anterior cingulate cortex (ACC) and basolateral amygdala (BLA) in this task by chemogenetically silencing projection neurons in these regions. Prior to the reversal, inhibition of ACC, but not BLA, decreased metacognitive sensitivity (i.e., the trial-by-trial correspondence between accuracy and confidence), rendering confidence read-out invariant to the strength of the evidence, and decreased the effects of confidence on reversal learning. BLA silencing similarly slowed reversal learning in the HC group. Furthermore, ACC and BLA inhibition had dissociable effects on trial-by-trial response to reward feedback during reversal. Inhibition of BLA decreased the tendency to switch following unrewarded trials (lose-switch), demonstrating reduced sensitivity to negative feedback. In contrast, ACC inhibition attenuated response to positive feedback and decreased the tendency to persist with the correct strategy across consecutive trials. Collectively our results point to dissociable roles for ACC and BLA in behavior: the ACC may aid in estimating the reliability of perceptual information to guide action selection and to support a persistence with the correct strategy, whereas the BLA may aid in modifying behavior in response to negative feedback.

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Poster

162. Animal Cognition and Behavior: Decision Making: Corticolimbic Circuits

Location: SDCC Halls B-H

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Program #/Poster #: 162.05/BBB12

Topic: H.01. Animal Cognition and Behavior

Support: KAKENHI 17022052
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Title: The effect of 5-$HT_{1A, 2A, 4}$ receptor antagonists on reward-based decision-making

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Abstract: When we choose an action from some alternatives to acquire reward, we compare each reward value that depends on the balance between reward amount and the effort to obtain it. Previous studies have shown that animals and humans tend to choose smaller immediate reward than larger delayed reward when the 5-HT level in the brain decreases, suggesting that 5-HT modulates the temporal discounting of the reward value. Additionally, 5-HT might also modulate the function of some of the loop structures such as a basal ganglia-thalamocortical loop which is thought to be involved in the reward-based decision-making and the goal-directed behavior because 5-HT neurons project to these structures.
Here, to investigate the role of 5-HT_{1A}, 5-HT_{2A} receptors (rich in cortical regions) and 5-HT_4 receptors (rich in basal ganglia) on choices depending on reward value, we used a decision-making task varying reward amount and effort to obtain it. In the task, two choice targets presented on the monitor in front of a monkey were randomly picked up from 16 different combinations of reward amount and schedule, that is, 1, 2, 3 or 4 drops of water (reward) by 1, 2, 3 or 4 repeats of a bar-release trial (effort). After the monkey chose one target by touching either left or right bar, the chosen reward schedule task consisted of the bar-release trial was started. We administrated 5-HT_{1A}R antagonist WAY100635 (0.3 mg/kg), 5-HT_{2A}R antagonist MDL100907 (0.002 mg/kg), 5-HT_4R antagonist GR125487 (1 mg/kg), or vehicle to two or three monkeys systemically, and compared the choice behavior and subsequent schedule task performance.
All 5-HT receptor antagonists had no effect on choice probability and the value of discount factor estimated from fitting the choice data by using exponential discounting model of reward value. On the other hand, in the reward schedule task, the error rates increased by WAY100635 and MDL100907, and decreased by GR125487. Furthermore, by fitting the error rates of the schedule task using modified temporal-difference learning model (Setogawa et al., 2014), we found that the discount rate of future reward decreased while the coefficient of the sunk cost parameter increased by GR125487 in three monkeys. Since 5-HT_4 receptor is rich in the striatum of monkeys (Tavares et al., 2014), this suggests that 5-HT_4 receptor in the basal ganglia have a role in persisting with on-going task, while 5-HT_{1A} and 5-HT_{2A} receptors in cortical regions maintain the motivation to perform it.

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Poster

162. Animal Cognition and Behavior: Decision Making: Corticolimbic Circuits

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Program #/Poster #: 162.06/BBB13

Topic: H.01. Animal Cognition and Behavior

Support: ERC/ BIOMOTIV
ANR/ NEUROEFFORT
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Title: Comparing the role of noradrenaline and serotonin in the effort-reward trade off: Pharmacological approach in monkeys

Authors: *S. BOURET[1], J. MATTIONI[1], N. BORDERIES[1], S. BALTASSIS[2], C. I. JAHN[1]
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Abstract: Ascending neuromodulatory systems have a strong impact on decision making, which consists in selecting actions based on their expected costs and benefits. Given the strong implication of neuromodulatory systems in mental health, it is critical to identify their specific functional role in decision making. To that end, we compare the relation between several neuromodulatory systems and specific components of decision making such sensitivity to effort costs, sensitivity to reward benefits and intrinsic motivation, in monkeys performing tasks that manipulate these variables. We test 3 monkeys in a computerized binary choice task manipulating physical effort cost and reward magnitude independently. To complete a trial, monkeys must squeeze one of 2 grips (left or right) in order to obtain the associated reward. Each trial starts with 2 visual cues indicating the difficulty and reward level for each of the 2 options (left vs right). The difficulty is defined by the force to exert on the grip, and the reward level by the amount of juice the monkey would obtain upon trial completion. Monkeys readily use the information about reward and effort costs to select the option that maximizes rewards and minimizes effort. We manipulate noradrenaline (NA) levels with systemic injection of clonidine, which decreases NA levels. Clonidine (0.5 mg/kg, IM) or placebo is injected 30 minutes before each daily session, and we alternate treatment every week, with a 2 days washout on week ends. Clonidine specifically affects effort processing, with a significant increases in the weight of effort costs on choices (*clonidine* = -2.55 +/- 0.23, *placebo* = -1.65 +/- 0.28, $F_{(1,145)} = 14.81$, $p = 1.78\times10^{-4}$). By contrast, clonidine does not affect the weight of reward on choices (*clonidine* = 2.29 +/- 0.21, *placebo* = 2.45 +/- 0.30, $F_{(1,145)} = 0.47$, $p = 0.49$). We manipulate serotonin using a chronic treatment with citalopram, to mimic its use in depressed patients. Monkeys receive a daily oral dose (0.5-1 mg/kg) for 4 consecutive weeks, and we compare performance during these 4 weeks with that of the preceding 4 weeks with placebo treatment. We are still collecting data but we expect the chronic treatment to affect not only the sensitivity to effort but also the intrinsic motivation, in line with its effect on mood in depressed patients. Thus, this comparative approach can readily identify the specific contribution of distinct neuromodulatory systems to specific components of decision making (e.g. effort for NA), which should contribute to better understand their role in psychiatric disorders such as depression.

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Poster

162. Animal Cognition and Behavior: Decision Making: Corticolimbic Circuits

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Topic: H.01. Animal Cognition and Behavior

Support: Wellcome Trust and Gatsby Charitable Foundation

Title: Network computation of threat from sensory evidence

Authors: *D. CAMPAGNER, R. VALE, P. IORDANIDOU, T. BRANCO
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Abstract: Detecting threats in the environment is critical for survival. Animals need to classify sensory stimuli into threatening and non-threatening in order to avoid harm from imminent danger through initiation of defensive behaviors, and also for building models of the world that predict threats and guide action selection accordingly. A fundamental behavioral computation is therefore to use sensory evidence and past experience to estimate the likelihood of threat and decide which action to take. While previous work in primates and rodents has elucidated mechanisms for computing decisions from sensory evidence in learned choice tasks, and recent work has identified circuits and mechanisms for computing instinctive escape decisions, it is not known how the brain integrates sensory evidence to compute threat.Here we present a behavioral assay where mice escape to a previously memorized shelter location from innately threatening auditory stimuli that are discrete and parametric. The novel discrete design of the stimulus allows precise control of the amount and rate of sensory evidence, and allows detailed probing of the sensory integration process leading to escape decisions. We find that the probability, reaction time and vigor of escape depend on the rate of sensory evidence, and are well described by a leaky integrator model. Chemogenetic experiments show that midbrain circuits involved in processing innately aversive visual looming stimuli are also necessary for integrating discrete auditory stimuli, suggesting common multisensory pathways and neural mechanisms for classifying stimuli as threatening. We are currently using high-density silicon Neuropixels probes to record neural activity in cortical and sub-cortical circuits of freely behaving mice to determine the neural network dynamics that support integration of sensory stimuli into threat.

Disclosures: D. Campagner: None. **R. Vale:** None. **P. Iordanidou:** None. **T. Branco:** None.

Poster

162. Animal Cognition and Behavior: Decision Making: Corticolimbic Circuits

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Topic: H.01. Animal Cognition and Behavior

Support: CIHR
NARSAD Young Investigator Grant

Title: Neurogenesis impacts delay-based decision making and affects neuronal activity in the ventral hippocampus

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Abstract: Depression is a complex disorder with disruptions in motivation, decision making and valuing future rewards. One brain region that has been implicated in the pathology of depression is the hippocampus. Interestingly, in humans it is the brain region that is capable of generating new neurons throughout life. Our aim was to identify the impact of adult hippocampal neurogenesis on the valuation of future rewards since previous research did mainly look at very basic motivational depressive-like behaviors. Here, we used a rat model and operant conditioning allowing us to investigate a complex behavior that is affected in depression. In detail, we used hGFAP-TK (TK) rats to deplete actively dividing neural progenitors by administering the antiviral drug Valganciclovir (VGCV) and consequently stop the production of new neurons. We tested TK rats on a delay discounting paradigm, where animals must choose between a low immediate reward and a larger delayed reward. Compared to WT rats, TK rats showed a decreased preference for the high reward with increasing delay times, indicating that neurogenesis increases the subjective value of future rewards. We were able to replicate this by ablating neurogenesis with irradiation in WT rats. On the contrary, increasing neurogenesis by running led to increased preference for the delayed high reward. On the cellular level, we found that the expression of the activity induced immediate early gene zif268 (erg-1) was reduced in VGCV treated TK rats in the general neuron population of the dentate gyrus specifically in the ventral hippocampus after performing the delay discounting task. Additionally, we labelled new-born neurons with a retrovirus before animals were trained on the delay discounting paradigm, and found that learning this task increased the dendritic complexity of 3-month-old neurons in both, the ventral and the dorsal dentate gyrus compared to cells from animals that were trained in a version of the task without delays. This project uses a novel rat model to study the impact of neurogenesis on future thinking. In summary, we find that neurogenesis increases the preference for future rewards and lack of neurogenesis leads to a reduction in neuronal activity in the ventral

dentate gyrus during task performance. Furthermore, learning this delay-based decision making task alters the integration of new born neurons into the dentate network by increasing their dendritic complexity. Our findings are important in understanding altered behaviors in neurological disorders, such as depression, addiction or Alzheimer's Disease, where value of future rewards is decreased and neurogenesis has been implicated in the disease pathology.

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Poster

162. Animal Cognition and Behavior: Decision Making: Corticolimbic Circuits

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 162.09/CCC2

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant 2R01N086104

Title: Primate insular cortex represents contextual information that modulates risk-attitude

Authors: *Y.-P. YANG[1], X. LI[2], V. STUPHORN[2]
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Abstract: The most successful descriptive economic theories of decisions under risk postulate that: (1) humans evaluate the outcomes of decisions as gains or losses relative to their current wealth level, and (2) humans show different risk-attitude when facing risky gains or losses. However, the neural mechanisms underlying these effects are not known. To investigate these questions, we designed a risk-based decision making task, in which the monkeys had to choose between a sure option with certain outcome and a gambling option with uncertain outcome with different explicitly indicated probabilities. Critically, the monkeys were trained to accept token as a secondary reinforcer. Across multiple trials, they had to accumulate six tokens to earn a standard fluid reward. This allowed us to test gamble options that resulted in a gain or a loss of token. In the token task, the monkeys could estimate the subjective value of the reward options based on the relative change in token number or on the resulting absolute token number.
Our behavioral results showed that the risk-attitude of the monkeys was both influenced by gain/loss domain and by currently accumulated token number. The monkeys showed an overall tendency of risk-seeking in both the gain and the loss domain. However, they displayed more preference for the gamble option when facing a risky gain than when facing a risky loss. In addition, we found the token asset effect that the token number a monkey had at the start of trial influenced its choice behavior both in the gain and the loss domain. With increasing token assets, monkeys were prone to choose the gamble option less often in the gain domain, but more or equally often in the loss domain.

To study the neuronal mechanisms underlying this results, we recorded from neurons in the anterior insular cortex (AIC). We found that many AIC neurons reflect the wealth level of the monkey, i.e. the token number at the start of trial. In addition, we found that many AIC neurons encode, whether the offers represented a gain or a loss. Some of them encoded the contextual difference between gain and loss in a binary manner. Other neurons represented a context-specific value signal. These neurons encoded the expected value of options in a parametrical manner, but asymmetrically, only in the gain or loss domain. These gain/loss context signals and wealth level signals are present before the decision is made.
In sum, our behavioral findings indicate that the monkey's choices depend heavily on their relative changes in wealth (gain or loss), as well as wealth level. Our neural data proposes an important role of AIC in representing these decision-related variables.

Disclosures: Y. Yang: None. **X. Li:** None. **V. Stuphorn:** None.

Poster

162. Animal Cognition and Behavior: Decision Making: Corticolimbic Circuits

Location: SDCC Halls B-H

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Topic: H.01. Animal Cognition and Behavior

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Title: Neuroligin-1 in complex cognitive behaviour

Authors: *J. LUO[1], N. BROSE[2], J. NITHIANANTHARAJAH[1]
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Abstract: The complex protein machinery at the synapse underlies information processing in the brain. Neuroligin-1 (*Nlgn1*) is a postsynaptic cell adhesion molecule required for normal NMDA receptor function and synaptic plasticity. Human mutations in *NLGN1* have been documented in autism spectrum disorder, in which cognitive dysfunction is a core symptom. To investigate the role of neuroligin-1 in complex cognitive behaviour, we assessed male and female neuroligin-1 null mutant mice (*Nlgn1*$^{-/-}$) on a comprehensive battery of cognitive tests using the rodent touchscreen system. Our data shows *Nlgn1*$^{-/-}$ mice show normal learning but specific alterations in distinct aspects of cognitive processing. Our findings provide a deeper understanding of the role of neuroligin-1 in regulating cognitive behaviour with relevance to disease.

Disclosures: J. Luo: None. **N. Brose:** None. **J. Nithianantharajah:** None.

Poster

162. Animal Cognition and Behavior: Decision Making: Corticolimbic Circuits

Location: SDCC Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Support: ANR-11—LABEX-0042
BLAN-SVSE4-023-01
BS4-0010-01

Title: Neurons in the primate amygdala differentially encode self and other's decisions

Authors: *R. CIRILLO, S. GILARDEAU, M. JAZAYERI, C. DUPUIS, S. C. WIRTH, J.-R. DUHAMEL
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Abstract: Social interactions can have favorable or unwelcome consequences for individuals. In a context of mutual choices that can affect either one's own or other's outcomes, it is essential to distinguish between two interacting agents. Previous findings suggest that during active decision-making basolateral amygdala (BLA) neurons "mirror" self and other's outcome experience (Chang et al., 2015). It remains unknown if neurons in this area could change their coding properties according to the agent's role as donor or recipient of a positive or negative outcome during forced decision-making. We explored the role of the centromedial portion of the amygdala (CM) and BLA in differentiating self and other's behavior while two male monkeys (*Macaca fascicularis*) performed an imperative social task. During each trial, the monkeys were required to choose a visual cue associated with a positive (reward) or negative (air puff) outcome for self, other, or nobody as recipients. Each monkey had to complete a block of trials to finally obtain a big amount of reward. In each task period, we found neurons that selectively encoded self or other as donor. The majority of neurons encoded self as outcome recipient, with the exception of the visual cue period in which we found a high proportion of cells modulated by not-self recipients. Moreover, not all the cells encoding the reward or the air puff for one interacting agent did so also for the other agent. Our results suggest that the amygdala contribution to social decisions is not merely based on value-mirroring activity, but neurons could adapt their activity according to the social context and the outcome valence.

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Poster

162. Animal Cognition and Behavior: Decision Making: Corticolimbic Circuits

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 162.12/CCC5

Topic: H.01. Animal Cognition and Behavior

Title: Hippocampal sequences and model-based planning in the rat

Authors: *S. C. VENDITTO[1], K. J. MILLER[2], N. D. DAW[2], M. M. BOTVINICK[3], C. D. BRODY[4]
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Abstract: Humans and animals construct internal models of the world around them and use these models to guide behavior. Such model-based cognition is often referred to as "planning", and its neural mechanisms remain poorly understood. Planning has been proposed to depend on hippocampal sequences, in which place cells "sweep out" trajectories through space while an animal is at rest (Foster & Knierim, 2012; Mattar & Daw, 2017). Research into the role of sequences in planning, and into the neural mechanisms of planning in general, has been hampered by a lack of tasks for animals which both demonstrably elicit planning behavior and are suitable for neural recordings. Recent work has lifted this limitation; advances from work with humans (Daw et al., 2011) has been adapted into a multi-step decision task for rats, showing that rats adopt a planning strategy which depends on neural activity in the dorsal hippocampus (Miller, Botvinick, & Brody, 2017). Here, we report the results of electrophysiological recordings made in dorsal hippocampus during planning behavior. We find that individual cells encode the states of the task, and that hippocampal sequences take place during sharp wave ripple events at the conclusion of most trials. The content of these sequences reflects knowledge of the structure of the task, consistent with a role in model-based planning. We used a traditional Bayesian decoding approach as well as a latent variable model to identify replay events during sharp wave ripples and characterize their relationship to the task on a trial-by-trial level. We seek to understand whether these replay events support planning by encoding action-outcome representations that evolve with changing reward contingencies.

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Poster

162. Animal Cognition and Behavior: Decision Making: Corticolimbic Circuits

Location: SDCC Halls B-H

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Program #/Poster #: 162.13/CCC6

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant MH109484

Title: Modulation of evoked spiking in nucleus accumbens by medial orbitofrontal cortex stimulation

Authors: *M. K. LOH, J. A. ROSENKRANZ
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Abstract: The nucleus accumbens (NAc) supports reward-directed actions, and its firing patterns are reflective of risky behavior and reward seeking. The NAc has the unique ability to integrate multiple inputs that modify its activity. Projections from the medial orbitofrontal cortex (mOFC) may influence the NAc's role in risky behavior as the mOFC contributes to the discrimination of probabilities and reward magnitudes. Additionally, basolateral amygdala (BLA)-NAc projections facilitate reward-seeking behaviors and have been shown to attenuate cortical interactions with the NAc. Due to the interactive nature of cortical and limbic inputs, it is likely that mOFC and BLA projections into the NAc interact and modify NAc output. We hypothesized that mOFC activity negatively regulates BLA's influence on the NAc. *In vivo* extracellular electrophysiology was employed to gauge the effects of mOFC activity on BLA-evoked NAc spiking. Single NAc neurons that spiked in response to BLA stimulation were recorded in urethane-anesthetized male rats. Single pulses were delivered at increasing interstimulus intervals (ISI; 1-, 6-, 11-, and 21-ms) and frequency-dependent trains (5, 10, 20, and 40 Hz) to the mOFC to assess effects of mOFC activity on BLA-evoked NAc spiking. Changes to spike probability as well as spike latency by mOFC stimulation were measured. Preliminary findings suggest that mOFC single-pulse stimulation enhances BLA-evoked NAc spiking in a time-dependent manner. Peak increases to BLA-evoked NAc spike probability by mOFC stimulation occurred during ≤6 ms ISIs. Shifts to BLA-evoked NAc spike probability by mOFC train stimulation were also observed to be frequency-dependent. Train stimulation of the mOFC delivered at ≤20 Hz enhanced BLA-evoked NAc spike probability but diminished spike probability when delivered at 40 Hz. Thus, the timing and frequency of mOFC activity are factors in modulating the activity of NAc neurons that are responsive to BLA inputs. Due to the role of the mOFC and BLA-NAc projections, these interactions may contribute to the neurobiological mechanism in the incorporation of risk information to influence reward-directed actions.

Disclosures: M.K. Loh: None. **J.A. Rosenkranz:** None.

Poster

162. Animal Cognition and Behavior: Decision Making: Corticolimbic Circuits

Location: SDCC Halls B-H

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Program #/Poster #: 162.14/CCC7

Topic: H.01. Animal Cognition and Behavior

Support: NIMH IRP

Title: Hippocampal-septal neural projections of male and female rats: An analysis of projection specific expression of androgen receptors

Authors: *G. NAGARAJAN, Y. CHUDASAMA
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Abstract: Gonadal hormones have many effects on the central nervous system that extend beyond their sex specific physical and physiological effects. Much of the work on gonadal steroid-induced cognitive function has focused on morphometric changes in cortical structures and neuromodulatory effects of hormones, but changes in specific brain structures, in males and females, contribute to variations in cognitive and emotional functions. These cognitive-emotional changes are thought to involve neuromodulatory actions of androgens on brain structures including the septum and hippocampus. Accordingly, androgen receptors are highly expressed in the lateral septum (LS) and hippocampus (HC). Although the afferent and efferent projections of LS and HC neurons are well documented (Risold and Swanson, 1997), whether these projecting neurons contain androgen receptors has not been systematically determined. In this study, we examine the presence of androgen receptors in ventral hippocampal-lateral septal (vHC-vLS) projecting neurons of male (n=3) and female (n=3) Long-Evans rats. Rats were injected with retrograde viral tracers [mRFP expressing PRV 152 and/or EGFP expressing 614] in rostro-caudal extent of the vLS. After 40-48 hr post inoculation and tissue processing, we observed PRV labelled neurons in the vHC of both male and females. Specifically, within the vHC, the more dorsally located CA1 neurons sent projections to the rostral and caudal portions of the vLS, while neurons in the ventral subiculum and adjacent CA1 neurons project primarily to the caudal vLS. In addition, a highly specific projection from the vHC CA3 neurons to the caudal vLS was also observed. To observe for the presence of androgen receptors in vHC and vLS neurons, alternate brain sections were processed with immunohistochemistry. Androgen receptors were clearly present in the ventral CA1, subiculum and CA3, as well as its target, the vLS in both males and females. These data suggest that although the vHC - vLSv projections has specific topological organization in both males and females, vHC and vLS neurons express androgen receptors in a non-specific manner.

Disclosures: G. Nagarajan: None. **Y. Chudasama:** None.

Poster

162. Animal Cognition and Behavior: Decision Making: Corticolimbic Circuits

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 162.15/CCC8

Topic: H.01. Animal Cognition and Behavior

Support: NIMH IRP

Title: Chemogenetic disconnection of the hippocampus and orbitofrontal cortex in adaptive decision making

Authors: *G. LARYEA[1], M. B. LEVENTHAL[2], Y. CHUDASAMA[2]
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Abstract: Converging anatomical and electrophysiological evidence has highlighted the significance of a direct hippocampal-orbitofrontal projection in influencing complex executive behavior. In rats, neurons in the ventral hippocampus (vHC) project directly to the ventral regions of the prefrontal cortex including the orbitofrontal cortex (OFC) (Jay and Witter, 1991, J Comp Neurol, 313:574; Verwer et al., 1997, Hippocampus, 7:397; Ishikawa and Nakamura, 2006, J Neurophysiol, 96:2134). In delay discounting tasks, rats with vHC lesions will opt more often for a small immediate reward, instead of a large delayed alternative (Abela and Chudasama, 2013, EJN, 37:640). These animals also act prematurely because they are unable to withhold the impulsive urge to respond (Abela et al., 2013). The orbitofrontal cortex (OFC) also contributes to some underlying aspects of decision-making, such as updating the value of an expected reward. Consequently, OFC lesions in rats can manifest as delay intolerance or disinhibition (for review, see Chudasama, 2011), just like the effects of vHC lesions. Although these studies implicate a role for both the vHC and OFC in adaptive decision-making, there has been little attempt to understand how these two structures are concurrently engaged in tasks that require flexible adaptation to changes in environmental contingency. Here we used DREADDs-mediated inhibition to disconnect the OFC and vHC to establish their conjoint importance in non-spatial delayed discounting and reversal learning paradigms using the touchscreen operant platform.
Rats received unilateral injections of the inhibitory DREADDs construct, AAV8-hSyn-hM4D(Gi)-mCherry in the same hemisphere (n=7, ipsilateral) or in opposite hemispheres (n=13, disconnection). The control group received AAV8-hSyn-GFP in opposite hemispheres (n=6, GFP control). During test, animals were given vehicle (20% DMSO in sterile water), 0.5mg/kg clozapine or 1mg/kg clozapine, counterbalanced across sessions.
In the delayed discounting task, rats chose between two different stimuli where one delivered a small immediate reward and the other a large reward. Our findings showed normal discounting behavior suggesting that vHC-OFC interaction is not necessary decision that involve a delayed

outcome. The same group of rats were subsequently tested in a modified simple discrimination task with two reversals. The data indicate that the OFC and vHC interaction is not necessary for response reversal. These preliminary data indicate that while the vHC-OFC interaction is important for the flexible control of inappropriate actions, it does not extend to other types of complex decision-making.

Disclosures: G. Laryea: None. **M.B. Leventhal:** None. **Y. Chudasama:** None.

Poster

162. Animal Cognition and Behavior: Decision Making: Corticolimbic Circuits

Location: SDCC Halls B-H

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Program #/Poster #: 162.16/CCC9

Topic: H.01. Animal Cognition and Behavior

Support: NIMH IRP

Title: Segregated cortical and subcortical relays connecting the dorsal hippocampus and prefrontal cortex: A transsynaptic tracing study

Authors: *K. MESSANVI, M. Q. PERKINS, Y. CHUDASAMA
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Abstract: In the rat, the dorsal and ventral subregions of the hippocampus serve different cognitive functions. Recent studies have shown that the cognitive functions of the ventral hippocampus (vHC) closely resemble those of the ventral prefrontal cortex (vPFC). Our own research has shown that interactions between these two structures may be necessary in controlling certain aspects of executive function (e.g., Abela et al., 2012, Cerebral Cortex; 23:1396; Chudasama et al., 2012, JN, 32(32):10915). In contrast, the dorsal hippocampus (dHC) and the dorsal prefrontal cortex (dPFC) is closely associated with spatial learning and memory (Bannerman et al., 2002, Behav Neurosci, 116:884), and certain aspects of attentional control (Chudasama and Robbins, 2004, Neurospsychopharm, 29:1628; Wilson et al., Cerebral Cortex, 2017; 27(2):1501). Consistent with their different functions, both regions show some level of topographical specificity in terms of their connections; the dPFC provide disynaptic input to the dHC, whereas the vPFC provides disynaptic input to the vHC (Prasad and Chudasama 2013, J.Neurosci. 33(19):8494).
In this study, we further explore the relationship between the PFC and HC with an emphasis on the inputs to the dorsal and ventral PFC. First, we injected an mRFP-expressing transsynaptic Pseudorabies virus (PRV-614) and a GFP-expressing Pseudorabies virus (PRV-152) directly into the dPFC and vPFC respectively, and discovered that the anterior portion of the dHC provides disynaptic input to both dorsal and ventral regions of the PFC via potential links in the vHC, septum and basal forebrain.

To confirm the relay or link between the dHC and the different prefrontal subdivisions, we injected the anterograde tracer Fluoro-Ruby (FR) into the dHC and the retrograde tracer Fluoro-Gold (FG) into the dPFC or the vPFC. We then examined the brain sections and looked for the presence of FR-labeled terminals and fibers and FG-labeled soma in the vicinity of each other. In those animals with injections into the dHC and vPFC, a high densitiy of FR-labeled fibers and FG-labeled neurons were observed within the CA1 region of the vHC suggesting that the vHC was the primary link between these two regions. In contrast, when FR and FG were injected into the dHC and dPFC respectively, labeled fibers and soma were found together in the horizontal limb of the diagonal band of Broca suggesting that the basal forebrain is the primary link. These preliminary results indicate a prominent dHC disynpatic influence on the prefrontal cortex via segregated cortical and subcortical links.

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Poster

162. Animal Cognition and Behavior: Decision Making: Corticolimbic Circuits

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Topic: H.01. Animal Cognition and Behavior

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Title: Comparisons between contingency encoding in prelimbic cortex and CA1 on a contingency-switching task for rats

Authors: *B. HASZ[1], A. D. REDISH[2]
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Abstract: Hippocampus (HPC) and prelimbic cortex (PL) are thought to play roles in a deliberative neural system which keeps track of environmental variables, and makes decisions which depend on those variables. To investigate how these brain areas represent such variables when they change, we designed a contingency-switching task for rats. This task consisted of a spatial maze with a single choice point between left or right. The contingency on each lap was either “left” (go left at the choice point to receive reward), “right”, or “alternate” (make the opposite choice as the previous lap to receive reward), and this contingency switched randomly every 30 +/- 5 laps. Neither the current contingency nor the switch were cued, other than by the delivery or absence of reward.
4 rats (3M, 1F) were run on the contingency switch task while recording simultaneously from hippocampal CA1 (with 24-tetrode drives) and prelimbic cortex (with 32-site silicon probes). Current contingency and spatial position were decoded using Bayesian decoding from PL or

HPC ensemble firing rates. We were able to decode the contingency more accurately, on average, from PL ensembles than from HPC ensembles. PL ensembles decoded contingency better than spike-time shuffled control datasets, but HPC ensembles were not significantly different from shuffled controls on average. Comparing Bayesian posterior probabilities over the course of an entire lap, we found that before a switch, both HPC and PL encoding of the pre-switch contingency was greater than that of the post-switch contingency. After a switch, encoding of the post-switch contingency became greater, with the average PL transition preceding the average HPC transition. CA1 ensemble firing rates were, on average, more highly correlated from lap to lap than PL ensemble firing rates. However, both PL and CA1 ensemble firing rates were more correlated between laps within a contingency than between contingencies. The most likely transition point in ensemble activity occurred within 2-5 laps after the switch for both PL and CA1. Consistent with previous studies, vicarious trial and error behaviors increased during this transition period.

Disclosures: B. Hasz: None. **A.D. Redish:** None.

Poster

162. Animal Cognition and Behavior: Decision Making: Corticolimbic Circuits

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Title: Non-local representation of future decisions in dorsal hippocampal CA1 during anxiety-like hesitation behavior in a robotic predator-inhabited foraging task

Authors: *C. J. WALTERS[1], M. ADKINS[1], A. D. REDISH[2]
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Abstract: Avoid-approach conflict tasks provide realistic but controlled environments to model natural conditions that typically elicit anxiety, by placing reward-seeking and threat-avoiding decision-making systems in conflict with one another. We trained rodents to forage for food provided alternately at the ends of a linear track and then presented them with a probabilistically attacking robotic predator (RoboScorpion, LEGO©) at one end of the track and an enclosed nest space at the other.
We recorded neural ensembles from dorsal CA1 of 4 food-deprived Brown Norway rats (2 M, 2 F) freely behaving in the predator-inhabited foraging arena. Anxious behavior was quantified through three measures: mid-track aborts on outbound journeys (toward the predator) compared to mid-track aborts on inbound journeys (toward the nest), outbound versus inbound lap

duration, and hesitation at the exit of the enclosed nest space.
Consistent with previous experiments (Choi and Kim, 2010, PNAS; Pare et al., 2015, J Neurosci; Walters et al., 2017, SFN), we found an increase in mid-track aborts on outbound journeys when compared to mid-track aborts on inbound journeys ($p = 0.0074$, matched pairs t-test). Similarly, we found an increase in outbound lap durations on completed outbound laps when compared to inbound lap durations on completed inbound laps ($p < 0.0001$, matched pairs t-test). In addition, after rats had been attacked by the robot there was a general increase in hesitation at the exit of the nest compared to predator-naive animals ($p = 0.0225$, two-sample t-test).
We found that dorsal hippocampal CA1 represented the future choice of the rats during anxiety-like hesitation behavior. Specifically, hippocampal activity represented the nest space prior to retreats and the middle track segment that led to the risky food source prior to approaches. Furthermore, we found that just prior to mid-track abort decisions, rats represented the zone immediately behind them before then turning around and aborting the foraging attempt. Just after mid-track abort decisions, CA1 representations swept reliably back to the nest and away from the robot.
Our results suggest rats are mentally representing planned trajectories before they are executed during avoid-approach conflict and that what might be underlying these commonly observed anxiety-like hesitation behaviors is an active consideration of potential future actions.

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Poster

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Title: Mice learn to avoid regret

Authors: A. E. MCLAUGHLIN[1], B. M. SWEIS[1], M. J. THOMAS[2], *A. D. REDISH[3]
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Abstract: Regret can be defined as the subjective experience of recognizing a mistake has been made and a better alternative could have been selected. The experience of regret is thought to

carry negative utility. This typically takes two distinct forms: augmenting immediate post-regret valuations to make up for losses; and, augmenting long-term changes in decision-making strategies to avoid future instances of regret altogether. While the short-term changes in valuation have been studied in human psychology, economics, neuroscience, and even recently in nonhuman-primate and rodent neurophysiology, the latter long-term process has received far less attention, with no reports of regret-avoidance in non-human decision-making paradigms.
We trained 31 C57BL/6J male mice in a novel variant of the Restaurant Row economic decision-making task, in which mice make decisions of whether to spend time from a limited budget to achieve food rewards of varying costs (delays, random 1-30s, indicated by tone pitch). Each trial contained a separate offer zone and wait zone. Delay was indicated on entry into the offer zone, but did not start counting down until entry into the wait zone. Mice could skip in the offer zone or quit in the wait zone. Importantly, we tested mice longitudinally for 70 consecutive days (60min daily sessions), on which the task provided their only source of food. Thus, decision strategies were interdependent across both trials and days.
We found that change-of-mind decisions in the wait zone corrected prior economically disadvantageous decisions to enter in the offer zone. Following change-of-mind decisions in subsequent trials, we found evidence for regret-like behaviors consistent with past reports (mice were more likely to accept the next offer, did so quickly, and rapidly consumed earned pellets before moving on). This reflects the immediate effects of regret on valuations that attempted to make up for lost efforts.
As mice were exposed to an increasingly reward-scarce environment, we found that they adapted and refined the economic efficiency of a distinct foraging strategy in the wait zone over the time course of weeks to maximize reinforcement rate. Interestingly, with prolonged training, mice transitioned from this early wait-zone strategy rooted in foraging, to a distinct strategy rooted in deliberation and planning in the offer zone without any further gains in reinforcement rate. We found that this latter strategy prevented future regret-inducing change-of-mind episodes from occurring. These data suggest that mice learned to avoid future regret, independent of and separate from reinforcement rate maximization.

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Poster

162. Animal Cognition and Behavior: Decision Making: Corticolimbic Circuits

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Program #/Poster #: 162.20/CCC13

Topic: H.01. Animal Cognition and Behavior

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MnDrive Neuromodulation Research Fellowship
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NIH Grant R01 DA019666

Title: Altering gain of the infralimbic to accumbens shell circuit alters economically dissociable decision-making algorithms

Authors: *B. SWEIS[1], C. E. HUTCHISON[1], A. E. MCLAUGHLIN[1], E. B. LARSON[2], A. D. REDISH[2], M. J. THOMAS[2]
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Abstract: The approach of many neuromodulation studies is to disrupt information flow during on-going behaviors, however this limits interpretation of endogenous encoding of information and the functional consequences of synaptic remodeling - a process often observed in neuropsychiatric disorders including addiction. Instead, targeting specific circuits with plasticity-altering manipulations can reveal more about the functional consequences of synaptic remodeling and could prove extremely useful to provide long-lasting treatments that can modify disease trajectory and prevent recovering addicts from making poor decisions that lead to relapse. However, manipulating plasticity to understand the functional consequences of circuit-specific synaptic remodeling on distinct aspects of decision-making information processing is only as useful as the task utilized is sensitive to circuit-computation-specific behaviors.
We trained 30 C57BL/6J male mice in a novel variant of the Restaurant Row economic decision-making task, in which mice make decisions of whether to spend time from a limited budget to achieve food rewards of varying costs (delays, random 1-30s, indicated by tone pitch). Each trial contained a separate offer zone and wait zone. Delay was indicated on entry into the offer zone, but did not start counting down until entry into the wait zone. Mice could skip in the offer zone or quit in the wait zone. The offer zone and wait zone uniquely captured distinct deliberative processes separate from change-of-mind foraging processes, respectively.
We then optogenetically altered the strength of synaptic transmission between glutamatergic IL-NAcSh projections by delivering 10Hz stimulation for 10min outside of behavioral testing. We found that induction of long-term depression in these synapses produced lasting changes in foraging processes without disrupting deliberative processes. Mice displayed inflated re-evaluations to stay when deciding whether or not to abandon continued reward-seeking investments but displayed no changes during initial commitment decisions. We also developed a novel ensemble-level measure of circuit-specific strength of synaptic transmission. We then used this novel assay and found individual differences in IL-NAcSh plasticity that could explain foraging but not deliberative valuations. Our results demonstrate that alterations in cell-type-specific synaptic strength between IL-NAcSh is capable of augmenting self-control economic valuations within a particular decision-making modality and suggests that the valuation mechanisms for these multiple decision-making modalities arise from different circuits.

Disclosures: B. Sweis: None. **C.E. Hutchison:** None. **A.E. McLaughlin:** None. **E.B. Larson:** None. **A.D. Redish:** None. **M.J. Thomas:** None.

Poster

163. Animal Cognition and Behavior: Executive Function: Learning and Memory I

Location: SDCC Halls B-H

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Program #/Poster #: 163.01/CCC14

Topic: H.01. Animal Cognition and Behavior

Support: DoD W81XWH-13-1-0377

Title: Thanks for being flexible: Cognitive flexibility training can attenuate the effects of a trauma model on fear learning and memory in rats

Authors: *L. CHABY[1], S. A. PERRINE[2], M. J. LISIESKI[3], K. KARAVIDHA[2], I. LIBERZON[4]
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Abstract: Stress exposure can cause lasting changes in cognition, but individual traits, such as strong cognitive abilities, can reduce the degree, duration, or severity of cognitive changes after stress. Here, we test whether cognitive training or high cognitive performance can attenuate changes in fear memory using a rodent trauma-model called single prolonged stress. Exposure to single prolonged stress, SPS, has been shown to heighten fear behavior (freezing) after a fear association has been extinguished, referred to as a deficit in extinction retention, which may reflect an impairment in context processing. We used cognitive flexibility training to assess individual variability in cognitive skills and to condition rats to discriminately use information in their environment. We found that cognitive flexibility training, alone or followed by exposure to SPS, can accelerate extinction learning and increase the rate of extinction retention, compared with rats not given cognitive training. These findings suggesting that cognitive flexibility training may attenuate context processing changes resulting from trauma. Individual performance during the reversal phase of cognitive training predicted subsequent context processing: individuals with high reversal performance exhibited a faster decrease in freezing during extinction retention testing than did individuals with low reversal performance, indicating that high reversal performance predicted an enhanced rate of safety information retention. We also quantified monoamines and their metabolites in brain regions important in fear expression, extinction, and cognitive flexibility using high performance liquid chromatography, because both stress exposure and cognitive flexibility training can affect decision making by modulating monoamine signaling. We found that in the prelimbic cortex, a region vital in both suppressing fear and maintaining cognitive flexibility, dopamine and norepinephrine levels are lastingly enhanced after cognitive flexibility training, with and without subsequent SPS stress exposure. Further, we found that SPS exposure can increase levels of dopamine and its metabolites in the striatum; dopamine signaling in the striatum is both dysregulated in PTSD patients and can

modulate cognitive flexibility. Detected increases in striatal dopamine and its metabolites following trauma were rescued in rats exposed to cognitive flexibility training prior to trauma. Overall, our results suggest that cognitive flexibility training can provide lasting benefits by attenuating the effects of trauma on behavior and monoamine changes in the brain.

Disclosures: S.A. Perrine: None. **M.J. Lisieski:** None. **K. Karavidha:** None. **I. Liberzon:** None.

Poster

163. Animal Cognition and Behavior: Executive Function: Learning and Memory I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 163.02/DDD1

Topic: H.01. Animal Cognition and Behavior

Support: NSFC 31471076

Title: Neuronal mechanisms supporting the context-dependent use of item-location association memory in the primate medial temporal lobe

Authors: *C. YANG[1,2], Y. NAYA[1,3,4,5]
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Abstract: The declarative memory system equips us to remember the information of past experience or knowledge so as to use this information flexibly for future action. Importantly, while numerous studies have investigated its encoding and retrieval processes, there is still room for exploration as to exactly how retrieved information is used. In the present study, we examined neuronal mechanisms supporting the utilization of declarative memory while separating this from the retrieval process. For this purpose, we devised a new memory task for macaques, a 'Prospective Brain for Monkey' (PBM) task, named after the "prospective brain paradigm" of human memory studies (Schacter et al., 2012). In the PBM task, 2 sets of 4 visual items were used as item cue (I-Cue) stimuli. Each I-Cue stimulus was associated with one particular location out of four relative to an orientation cue (O-Cue) stimulus. The O-Cue was a single visual object presented at different orientation angles (0-180 degrees). In each trial, an I-Cue (0.3 sec) and an O-Cue (0.3 sec) were sequentially presented in the center of a display with a delay (0.7 sec) in-between. Following an additional delay (0.7 sec), four dots were presented in the periphery of the display as choice. One of the dots was a target, while the others were distracters. The monkeys were required to saccade to the target dot in accordance with both I- and O-Cues. We recorded single-unit activities from the hippocampus (HPC, n = 153), parahippocampal cortex (PHC, n = 60) and perirhinal cortex (PRC, n = 166) of the medial

temporal lobe (MTL) as well as the area TE (TE, n = 82) while the monkeys performed the task. We first examined responses of individual neurons to the presentation of the I-Cue. Substantial number of neurons exhibited significant ($P <$ 0.01, ANOVA) stimulus-selective activities in all of the recording areas (I-Cue neurons, 20-29 % of the recorded neurons). The I-Cue neurons showed significantly correlated responses to the pairs of the I-Cue stimuli that were assigned to the same locations in both the MTL (median of correlation coefficients = 0.96, 0,82 and 0.76 in PHC, HPC and PRC) and TE (0.42). We next examined neuronal responses after the O-Cue presentation, and found that proportions of target-location-selective cells ($P < 0.01$, ANOVA) in HPC (19%) and PHC (13%) were larger than that in PRC (5%) and TE (5%) ($P < 0.005$, Chi-square test). These results may suggest that TE and PRC contribute to the retrieval of item-location association memory when the perceptual item signal enters MTL through the ventral pathway, while HPC and PHC contribute to the transformation from the to-be-retrieved location information to the target location for the action.

Disclosures: C. Yang: None. **Y. Naya:** None.

Poster

163. Animal Cognition and Behavior: Executive Function: Learning and Memory I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 163.03/DDD2

Topic: H.01. Animal Cognition and Behavior

Title: Efficacy of selective activators of SK channels to rescue attention deficit and memory in a mouse model of schizophrenia

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Abstract: Disordered memory and attention are common in schizophrenia, ADHD, and bipolar disease. Symptoms of disordered speech or writing, result from racing thoughts and flight of ideas. Rapid thought processing leads to difficulty filtering irrelevant stimuli, which impairs attention and memory encoding. Latent inhibition, the delayed acquisition of a conditioned response after pre-exposure to the conditioned stimulus, is impaired in schizophrenia. Mania, a positive symptom of schizophrenia, and impaired latent inhibition can be modeled in mice after pharmacological or genetic manipulation. Small conductance Ca^{2+}-activated K^{+} (SK) channels, expressed throughout the CNS, modulate neuronal excitability by mediating the medium component of the afterhyperpolarization, and by shaping excitatory postsynaptic responses. In an intact system, SK channels activated during learning and memory tasks modulate the timing of action potentials. SK channel modulation of action potential firing frequency may aid the processing of rapid and continuous input to attention circuits. Here, we examined the effect of SK channels activation on hippocampal-dependent object memory in a chronic ketamine (KET)

mouse model of schizophrenia. The objective of the study is, to determine if disordered memory and attention in KET-treated mice can be rescued by SK channel activation. Consistent with previous reports, we found that daily systemic administration of KET for 11 days led to the expression of mania-like behavior and impaired latent inhibition in male C57BL/6J mice. Studies are in progress to establish the effect of activating SK channels by systemic injection, with the SK2 activator, CyPPA and/or the SK1 activator, GW542573X, in the KET mouse model, on attention deficits and the encoding of object memory. Chronic KET- or vehicle-treated mice will receive systemic CyPPA or GW542573X, and then be placed in a familiar arena containing two identical novel objects. A test session will be presented 24 h later to assess strength of object memory. Latency to acquire 30 s of exploration of each sample object and test session object exploration will be analyzed to determine the efficacy of SK channel activation to affect attention, motivation and object memory in KET- and vehicle-treated mice. Future studies on the effects of systemic CyPPA and/or GW542573X on trace fear conditioning and a 5-choice serial reaction time task in KET-treated C57BL/6J mice are planned.

Disclosures: C.A. Rice-Kuchera: None. **R.W. Stackman:** None.

Poster

163. Animal Cognition and Behavior: Executive Function: Learning and Memory I

Location: SDCC Halls B-H

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Program #/Poster #: 163.04/DDD3

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant 2R01MH096274-05A1

Title: Novel experience impacts hippocampal-prefrontal synchrony and enhances learning

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Abstract: Prior work suggests that novel experience *primes* brain circuitry for an extended period, thereby enhancing memory retention. However, the circuit bases of priming and its effect on encoding remain elusive. We report that male and female C57BL/6J mice display enhanced learning in a T-maze task, in which they have to reverse established bias to get reward, after brief exposure to novel, not familiar, Open Field. To understand the circuit bases of priming, we performed simultaneous local field potential (LFP) recordings from the Hippocampus and LFP and single unit recordings from the medial Prefrontal Cortex (mPFC), areas respond to novel stimuli. Novelty induces prolonged increases in theta (4-12Hz) power in the ventral

Hippocampus (vHPC) (Wilcoxon rank sum test, $p<0.05$) and, surprisingly, decreases in vHPC-mPFC theta synchrony ($p<0.0005$). After learning the task rule, this dampened vHPC-mPFC theta synchrony normalizes, and mPFC cells deliver more task-relevant information. Finally, optogenetic labeling reveals that vHPC cells active during novelty exposure express dopamine D1 receptors, and inhibiting these receptors abolishes the novelty effect. Thus, novelty primes vHPC via D1 receptors, which weakens existing vHPC-mPFC connectivity to open a window for increased plasticity to encode new information.

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Poster

163. Animal Cognition and Behavior: Executive Function: Learning and Memory I

Location: SDCC Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Support: Wellcome Trust Strategic Award Grant WT101092MA
MRC Project Grant MR/K005480/1

Title: Contributions and interactions of prefrontal and temporal lobe cortical areas to recognition memory for novel and familiar visual stimuli in non-human primates

Authors: *Z. WU, M. O'NEILL, E. BOSCHIN, J. M. GALEAZZI, M. J. BUCKLEY
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Abstract: The medial temporal lobe (MTL) is known to support recognition memory; and many neuropsychological and neurophysiological studies have also emphasized the role of the prefrontal cortex (PFC) in maintaining information about stimuli for upcoming actions. However, the dynamics of neural interactions between PFC and MTL, and also inferotemporal cortex (IT), during encoding and retrieval stages of recognition memory for novel and familiar stimuli are not fully understood. Here we recorded neural activities in a visual recognition memory task using a simultaneous multi-area, multi-electrode recording paradigm in the awake behaving macaque monkey. We recorded simultaneously from 32-electrodes in dorsolateral prefrontal cortex (dlPFC) in PFC, 32-electrodes in in parahippocampal cortex (PhC) in MTL, and 64-electrodes in IT throughout the task. In each trial, a central sample image (object image) was initially shown to the monkey, then after a delay, either an identical or a novel (previously unseen) test image appeared on the screen together with a black circle; monkeys were rewarded for touching the test image if it matched the sample image, or rewarded for selecting the standard 'non-match button' (i.e. the black circle) if not match. In our study, we also changed the probabilities of cues being sample images, which are either familiar or novel to macaques. We analyzed the local field potentials (LFPs), single-unit (spikes), and multi-unit activities from the

three Utah arrays. A linear regression analysis for the behavioural data shows that accuracy in match trials (i.e. hit-rate) but not in non-match trials (i.e. correct rejection rate) positively correlated increased familiarity of sample images; response time decreased with familiarity in both hit and correct rejection trials. Moreover, during the encoding phase, brief bursts of beta oscillations (10-30 Hz) were observed in dlPFC, in which beta power was much stronger for the familiar images compared with the novel images. During the choice phase, beta oscillations were also found in dlPFC and signal power was stronger in association with correct choices compared with incorrect ones. In addition, some dlPFC neurons showed increased spiking activities when rewards were given for correct responses. Preliminary analyses indicate that prefrontal beta oscillations are related to encoding the stimuli and beta power signals how successful the stimulus has been remembered. Spike-spike, LFP-LFP and spike-LFP analysis across all the recorded regions will illustrate the dynamic interactions between dlPFC and PhC and IT in recognition memory and will facilitate decoding analyses of informational content.

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Poster

163. Animal Cognition and Behavior: Executive Function: Learning and Memory I

Location: SDCC Halls B-H

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Program #/Poster #: 163.06/DDD5

Topic: H.01. Animal Cognition and Behavior

Title: The domestic rabbit (oryctolagus cuniculus): A novel animal model for studying tactile object recognition memory

Authors: *K. L. HOFFMAN[1], P. RODRÍGUEZ XOCHICALE, 90740[2], E. BASURTO[2]
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Abstract: The novel object recognition (NOR) test has been used extensively in rodent models in order to study object recognition memory (ORM). This test is based on the laboratory rodent's natural tendency to investigate a novel object over a familiar one, and typically relies on visual memory. We recently developed a NOR test for use in the laboratory rabbit. The rabbit NOR test uses, as an index of object exploration, a scent-marking behavior ("chinning"; where the rabbit rubs its chin along the surface of an object) and appears to rely primarily on tactile memory. This test comprises: 1) a habituation phase (HP), during which the rabbit investigates two objects having similar characteristics (form, texture), in an open field arena; 2) a delay phase (DP), where the rabbit is returned to its home cage and the objects are replaced by one object identical to the first two and another object that differs with respect to form and texture; 3) a test phase (TP), when the rabbit is returned to the arena. A comparison of the number of chin marks

directed to the novel vs familiar object during the TP is considered an index of object recognition memory. In the present study, we varied several key parameters of this test (e.g., duration of HP; duration of DP; physical and visual characteristics of the objects), in order to more fully characterize this type of ORM. We found that these rabbits (adult males of the New Zealand strain) were unable to recognize the novel object when the HP was less than 10 min, or when the DP was greater than 20 min. When the objects differed with respect to visual characteristics alone (color), chinning directed toward the novel object was very much reduced, compared to when the object was novel with respect to both visual and tactile characteristics. The present results are consistent with the proposal that the rabbit NOR test taps into ORM processes that differ importantly from those involved in the rodent test, and suggest that tactile ORM requires a relatively prolonged period of object investigation and is relatively short-lived.

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Poster

163. Animal Cognition and Behavior: Executive Function: Learning and Memory I

Location: SDCC Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Support: FAPESP Grant 2016/01154-0
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Title: Evaluation of executive function and nociceptive response in rats exposed to neonatal status epilepticus

Authors: *S. P. B, P. B. DOS SANTOS, G. DA SILVEIRA, D. P. TAROZZO, R. M. CYSNEIROS
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Abstract: A single episode of neonatal *status epilepticus* (SE) in rats produces an autistic phenotype, characterized by social play impairment, low preference by novelty, social discrimination impairment, increased anxiety, with no changes in cognition function. We evaluated the cognition function using tasks with high cognitive demands (Barnes maze (BM) and octagonal maze (OM)) and the reaction time to a thermal stimulus in adults male Wistar rats subjected to neonatal SE by pilocarpine injection (380mg/kg, ip) at PN9. The reaction time to a thermal stimulus (RTTS) by the hot plate test (HP) was assessed in two situations: after a regular habituation and immediately after an exposition to a social novelty. BM was divided in 6 zones: 0-60, 60-120, 120-180, 180-240 and 240-360. The escape cage was located at the *0-60* in the training phase (TRP) and at *180-240* in the reverse phase (RP). In OM test, time spent to

complete the task did not differ between groups in none of the phases (TRP (U=33, *p*=0.81), test phase (TP) (t=1.160, *p*= 0.26), retest phase (U=23, *p*= 0.22). However, experimental animals (EA) exhibited less working memory errors in the TP (U=16.50, *p*=0.045), with no difference in reference memory errors in TRP (U=24.50, *p*= 0.2353), nor in TP (t= 1.62, *p*= 0.12) or in RP (U=25, *p*= 0.30). In BM, the time spent to find the escape hole in TRP decreased across sessions (F (11,165) = 5.25, p< 0.0001), but not differ between groups (F (1,165) = 0.11, *p*= 0.73). In TP, when the escape cage was removed, the time to reach the escape hole did not differ between groups (t= 1.08, *p*= 0.31), but EA seemed to use a different strategy to find the escape hole; they spent equal time among the zones, other than target, and control animals stayed next to the target hole. For HT test, RTTS did not differ between groups in both situations: without stress (t=0.55,p= 0.59) or immediately after stress challenge (t= 0.4904, p=0.638), but the second one increased the RTTS in both groups (F (1,15)= 48.03, p< 0.0001). A single neonatal SE produces a better performance in some aspects of cognitive function, as working memory and behavioral flexibility, may be related to the anxious character of the animal stimulated by anxiogenic aspect of the task, with no changes in response to other treats, like a thermal noxious stimulus.

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Poster

163. Animal Cognition and Behavior: Executive Function: Learning and Memory I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 163.08/DDD7

Topic: H.01. Animal Cognition and Behavior

Title: Evaluation of the neuroprotective effect of solid lipid nanoparticles of sesamol against radiation-induced cognitive impairment and mood disorders

Authors: *N. KUMAR[1], P. DUTTA[1], K. GOURISHETTI[1], L. KUMAR[2], S. CHERUKU[1], K. SHARAN[3], V. K. PARIHAR[4], C. RAO[1]
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Abstract: The radiation-induced cognitive deficit is increasingly gaining importance with increasing rates of cancer survivors. Sesamol is known for neuroprotective effect against ICV-STZ, radioprotective effect against whole-body gamma-irradiation, antioxidant and anti-inflammatory effects. However, its poor bioavailability restricts the usage. Considering the above points, the present study was designed to evaluate sesamol and its solid lipid nanoparticles (SLN) for neuroprotective effect against whole-body X-ray irradiation-induced cognitive impairment and mood disorders in mice. The optimized ratio of glyceryl monostearate (250mg), poloxamer (2%) and sesamol (100 mg) were selected for the SLN, which showed a particle size-249 nm,

PDI-0.441 and zeta potential-(-)16.2 mv. *In vivo* study was conducted in Swiss albino mice in four groups, namely, vehicle control, radiation control sesamol and formulation. Except for vehicle control animals, all animals were restrained in a well-ventilated perspex box and subjected to radiation 30 min after the respective tretments by Linear Accelerator in Kasturba Medical College, Manipal at 1.25 Gy/min dose rate (5Gy). Sesamol was administered *p.o.* at 100 mg/kg equivalent daily. Spatial memory was assessed by Morris water maze test and mood disorder was assessed by forced swim test (FST), tail suspension test (TST) and open field test (OFT) on the 21st day. Animals were sacrificed and assessed for acetylcholine esterase (AChE) activity and antioxidant (catalase & GSH) levels in brain homogenate. In Morris water maze test, radiation exposed animals showed a significant decrease in the total number of island entries and a significant increase in escape latency, which was significantly reversed by the formulation treatment. In FST & TST, radiation control animals showed a significant increase in immobility time and a similar trend of alteration was seen in OFT parameters. These parameters were significantly reversed by sesamol and its formulation treatments. The changes in memory and mood parameters were found to be correlated with AchE and antioxidant parameters. Radiation control group showed a significant increase in the AchE levels, reflecting the poor cognition, which was reversed by sesamol and its formulation treatments. A significant ($p<0.05$) depletion in catalase level was observed by the radiation which was significantly reversed by the free drug and its formulation. A similar trend was observed for GSH, though it was not significant. Thus, it can be concluded that sesamol and its formulation showed a potent reversal effect on the radiation-induced cognition impairment and mood disorders.

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Poster

163. Animal Cognition and Behavior: Executive Function: Learning and Memory I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 163.09/DDD8

Topic: H.01. Animal Cognition and Behavior

Title: Behavioral flexibility regulated by metabotropic glutamate receptor 5(mGluR5)

Authors: *H. NOH, J. LIM, C. KIM
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Abstract: Behavioral flexibility, defined as the ability to change preferences according to changing circumstances, is implicated as a key factor involved in the adaptive behavior to different environmental changes. Lack of behavioral flexibility appears in many different psychiatric and neurological disorders, yet the exact neural substrates underlying this mechanism are not fully understood. Metabotropic glutamate receptor 5 (mGluR5) is one of the group I

metabotropic glutamate receptor subtype that have been shown to play an important role in the modulation of synaptic plasticity. Previous studies have reported that genetic ablation or pharmacological inhibition of mGluR5 disrupts fear memory extinction and spatial reversal learning indicating that mGluR5 signaling is implicated in the regulation of behavioral flexibility. Most of these behavior studies exposed the testing mice to a highly-stressful condition like electric foot-shocks and water swimming. Considering that mGluR5 signaling has been reported to be involved in the stress response, investigating the animal behavior involving mGluR5 signaling under low-stressful conditions are crucial to clearly distinguish whether this cognitive rigidity is due to stress or the mGluR5 gene effect. So, our study examines the direct role of mGluR5 in behavioral flexibility at a low-stressful condition by using the appetitive operant conditioning of the touchscreen operant platform. We have used adult mGluR5 knock-out(KO) and wild-type(WT) male mice to examine behavioral flexibility by mainly focusing on the extinction and the visual discrimination-reversal paradigm under the altered stimulus-reward contingencies. Compared to mGluR5 WT controls, mGluR5 KO mice required more trials to reach 77% omission criterion in the extinction task and took more trials to reach 80% correct response criterion in the reversal learning task. Additionally, we were able to observe a similar trend when mGluR5 signaling was pharmacologically down-regulated by injecting the mGluR5 antagonist, 3-((2- Methyl-4thiazolyl)ehynyl)- pyridine(MTEP) in WT B6J mice. Together, our results indicate that mGluR5 signaling has a critical role in behavioral flexibility.

Disclosures: H. Noh: None. **J. Lim:** None. **C. Kim:** None.

Poster

163. Animal Cognition and Behavior: Executive Function: Learning and Memory I

Location: SDCC Halls B-H

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Program #/Poster #: 163.10/DP12/DDD9

Topic: H.01. Animal Cognition and Behavior

Support: NHMRC Project Grant 1126885

Title: Touchscreen testing elucidates specific cognitive abnormalities of learning and perseveration in mice lacking metabotropic glutmate receptor 5 and their rescue by environmental enrichment

Authors: *A. ZELEZNIKOW-JOHNSTON, T. RENOIR, E. BURROWS, A. HANNAN
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Abstract: Aim: To characterise the role of the mGlu5 receptor and environmental enrichment (EE) in cognition by examining the extent to which EE can rescue cognitive deficits in an mGlu5 knockout (KO) mouse model of schizophrenia-like cognitive impairment.
Background: Null homozygous mutation of the mGlu5 receptor in mice results in cognitive

impairments evocative of neuropsychiatric diseases. These mGlu5 KO mice have been shown to have long-term spatial memory impairments, however characterisation of their full cognitive phenotype has not been performed.
Methods: Following weaning, male mGlu5 KO mice and their WT littermate controls were housed in either standard or EE cages for the duration of touchscreen cognitive testing. Mice were tested on visual discrimination, reversal learning, pattern separation, working memory, and motivation through the progressive ratio test and extinction. NMDA receptor function was also assessed using pharmacological modulation probes during the working memory assessment.
Results: The mGlu5 KO mice had cognitive impairments across all tasks examined. EE was able to rescue and enhance performance in a select subset of cognitive tasks involving executive function. Acute pharmacological probes indicated that deficit induction and EE-mediated rescue was related to NMDA receptor function.
Conclusion: Absence of mGlu5 receptor expression results in generalised cognitive impairments which can be ameliorated by EE in specific cognitive domains. Acute pharmacological probes indicated that mGlu5 absence induces deficits, and EE housing produces benefits, in part through interaction with NMDA receptors. This work helps to constrain the cognitive role of mGlu5 and elucidate the effects of EE can in this model.

Disclosures: A. Zeleznikow-Johnston: None. **T. Renoir:** None. **E. Burrows:** None. **A. Hannan:** None.

Poster

163. Animal Cognition and Behavior: Executive Function: Learning and Memory I

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Topic: H.01. Animal Cognition and Behavior

Support: NIH NIMH R01 MH111499
NIH NINDS R35 NS097265

Title: Neuromodulator mediated learning in a closed loop reinforcement system

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Abstract: The brain machine interface paradigm involves reinforcement training of to achieve specific patternsets of neuronal activity an animal within a closed loop system. One goal of this approach is to train and use the activity of defined particular neuronal ensembles to eventually interface with prosthetic devices. Consequently, aA majority of ongoing research has focused on

volitional control of population of neurons involved in sensory or motor processingneuronal populations. Yet However, these populations are influenced by neuromodulatory systemsneuromodulators, which typically originatinge from i.e., systems characterized by small nuclei whose projections span a large and diverse array of circuits in the brain. This pattern of projections allows neuromodulators neuromodulatory systems to affect, e.g., by shifting membrane potential (Polack et al., Nat Neurosci 2013), spatially separated populations of neurons in a simultaneously and in a coordinated manner. Here we focus on the noradrenergic system, whose far-reaching projections emanate from the locus coeruleus, a small nucleus in the pons. This system has been implicated in the control of attention, mood, arousal, and stress. Moreover, phasic activity of neurons in the locus coeruleus has been associated with task-related decision processes (Aston-Jones et al., J Neurosci 1994). We thus hypothesize that the release of norepenephrinenorepinephrine (NE) from locus coeruleus can be volitionally controlled.
Here we use reinforcement learning to train head-fixed mice to lick for a liquid reward that is based on the cortical level concentration of NE. The change in cortical [NE] is measured in real time with α1a-CNiFERs implanted in frontal cortex (Muller, Joseph et al. Nat Meth 2014), and used to trigger a reward. Our preliminary data indicates that cortical [NE] increases with closed-loop training in a manner that is time-locked to the onset of licking. As a control, the concurrent motor act of running remains unlinked to cortical [NE]. Further, the cortical [NE] drops on a test day with no reward. These data imply that mice can volitionally control neuromodulator release. This work highlights how neuromodulation may be a controlling factor within various learning paradigms.

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Poster

163. Animal Cognition and Behavior: Executive Function: Learning and Memory I

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Program #/Poster #: 163.12/DDD11

Topic: H.01. Animal Cognition and Behavior

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Title: Neural codes underlying outcome encoding in anterior cingulate cortex

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Abstract: Information flow through the frontal-striatal system underlies flexible behavioral adjustment in the face of changing stimulus-reward contingencies [1]. Within this wider network, converging evidence implicates the anterior cingulate cortex (ACC) as a critical structure

encoding performance feedback and prediction errors necessary to shift behavioral strategy [2-4]. But it has remained elusive how the local neural circuit in the ACC encodes feedback-relevant information to flexibly influence connected areas in the wider network. We therefore set out to quantify how ACC spike counts, spike patterning over time, and spike associations with network-level, phasic local field potential (LFP) activity [5] convey feedback specific information during trial-end-error learning.To test the versatility of these distinct neural codes, we analyze concurrent spiking and LFP during a feature-based reversal learning task [4]. Monkeys had to correctly report the direction of motion of a target stimulus to receive reward. The target was defined by a color in a block-wise fashion. Monkeys had to learn through trial and error which color was rewarded. The color-reward contingency was reversed when performance criterion was reached, thus requiring multiple bouts of (re-) learning. Extracellular recordings were made in the anterior cingulate cortex simultaneously with lateral prefrontal cortex and striatum. We compare and contrast the decoding efficacy of different neural codes using support vector machines with 10-fold, stratified cross-validation, carefully controlling for differences in trial numbers. We found that ~30% of individual cells encoded outcome in their firing rate, in line with previous studies [4]. Conditioning the rate on the phase of the LFP increased decoding efficacy in up to ~45% of cells. This effect was observed using phase information in the theta band, as well as in the gamma band, but not in the beta band. Moreover, we found that the spike patterning in time was critically informative of the outcome in a substantial subset of cells, increasing the total encoding sites up to ~65%.Taken together, these results provide a path forward in understanding the underlying coding scheme of the anterior cingulate cortex during feature-based learning, and will be essential to understand how anterior cingulate cortex circuits affects the larger fronto-striatal network to guide learning and optimize goal-directed behavior.[1] Hikosaka et al (2017) J. Neural. Transm. [2] Shenhav et al (2016) Nature Neuro. Reviews [3] Kolling et al (2016) Nature Neuro. Reviews [4] Oemisch et al (2018) bioArxiv [5] Kayser et al. (2009) Neuron

Disclosures: B. Voloh: None. **M. Oemisch:** None. **T. Womelsdorf:** None.

Poster

163. Animal Cognition and Behavior: Executive Function: Learning and Memory I

Location: SDCC Halls B-H

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Program #/Poster #: 163.13/DDD12

Topic: H.01. Animal Cognition and Behavior

Support: ISF

Title: Activation of the eEF2 pathway in the dentate gyrus excitatory neurons enhances cognitive function and neurogenesis in young and old mice

Authors: *E. TAHA[1], S. PATIL[4], I. BARRERA[2], C. G. PROUD[5], C. R. BRAMHAM[4], K. ROSENBLUM[3]
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Abstract: Background: Regulation of the translation mRNAs into protein plays a pivotal role in learning and memory formation. Protein synthesis is a dynamic process, which is regulated at three main phases: initiation, elongation, and termination. While the initiation phase of translation is considered to be the rate-limiting step, regulation of the elongation phase via eukaryotic elongation factor 2 kinase (eEF2K) has also been suggested to be important for memory and synaptic plasticity consolidation (Taha E et al., 2013, 2016). During the elongation phase, eukaryotic elongation factor 2 (eEF2) promotes ribosomal translocation that leads to ribosomal movement along the mRNA. Phosphorylation of eEF2 on Thr56 by its specific kinase, eEF2K, inactivates eEF2 and leads to protein synthesis inhibition. Here, we aim at examining the function of the eEF2 pathway in the dentate gyrus (DG) of the hippocampus in mice. **Results:** Proteomic analysis of hippocampus from mice with genetic deletion of eEF2K (knock-out, KO), which leads to complete loss of eEF2 phosphorylation, revealed enriched fraction of proteins that are crucial for neurogenesis. Indeed, both neurogenesis and dentate gyrus-dependent context discrimination learning were enhanced in the eEF2K-KO mice compare to wild-type. Using injection of viruses harboring Cre recombinase under the neuron-specific synapsin or excitatory neuron-specific CaMKII promoters into the DG of eEF2K floxed mice, we observed enhanced neurogenesis. In addition, electrophysiological analysis of perforant path evoked transmission in the dentate gyrus in vivo identified enhanced synaptic excitability at baseline and altered LTP maintenance in CaMKII cre injected mice relative to GFP injected control. Importantly, enhanced neurogenesis and context discrimination can also be observed in aged CaMKII cre injected mice. **Conclusions:** Together, our findings reveal that the eEF2K pathway in granular DG excitatory neurons plays a specific and critical role in neurogenesis and DG-dependent behavior. In addition, rejuvenation of the DG by modulating eEF2 phosphorylation in adulthood and aging enhanced memory precision. Our study suggest that eEF2K inhibition has potential therapeutic significance in the cognitive decline associated with aging.

Disclosures: E. Taha: None. **S. Patil:** None. **I. Barrera:** None. **C.G. Proud:** None. **C.R. Bramham:** None. **K. Rosenblum:** None.

Poster

163. Animal Cognition and Behavior: Executive Function: Learning and Memory I

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Topic: H.01. Animal Cognition and Behavior

Support: PICT 2012-1519
PICT-2016-2145

Title: Social interaction is not necessary for learning reciprocal altruism in rats trained on the Iterated Prisoner's Dilemma

Authors: G. E. DELMAS[1], M. T. MARINO[3], S. E. LEW[2], *B. S. ZANUTTO[4]
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Abstract: Reciprocal altruism is a behavior for which non-related individuals favor others in detriment of their own benefit. What a subject lose for favoring another is rewarded by the reciprocity of another individual. In this way in the long term, the strategy that gives the greatest reward is to cooperate. Triver's theory of reciprocal altruism is able to explain how natural selection favors reciprocal altruism (reciprocity) among non-related individuals, but there is a controversy about the mechanisms behind this kind of behavior. While reciprocal altruism has been proven in monkeys by the Iterated Prisoner's Dilemma (iPD), birds and rats failed to reach high levels of cooperation. In our previous work, we have shown that by using positive and negative reinforcements and an appropriate contrast between rewards in a iPD framework, rats were able to learn reciprocal altruism with high mutual cooperation. In the payoff matrix, pellets and timeout were the positive and the negative reinforcement, respectively. In that experiments there were two subjects, the experimental and the opponent. Here the opponent was replaced by a lighting stimulus. We found that rats learned reciprocal altruism behavior with high level of mutual cooperation as in the previous experiments with an animal opponent . Without any doubt, social interactions are important, but here we showed that in the learning of reciprocal altruism under the use of the prisoner's dilemma paradigm, those interactions are not necessary.

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Poster

163. Animal Cognition and Behavior: Executive Function: Learning and Memory I

Location: SDCC Halls B-H

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Topic: H.01. Animal Cognition and Behavior

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Title: Do medial prefrontal neurons encode predicted value of a cue or the action elicited by a cue during classical conditioning?

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Abstract: Neurons in prelimbic (PL) and infralimbic (IL) medial prefrontal cortex (mPFC) are modulated by positive and negative cues and outcomes during learning and execution of learned behaviors. However, interpretation of valence encoding by PFC neurons can be compromised because behaviors vary depending on outcomes. Appetitive conditioning usually requires an action (reward-taking) to receive an appetitive outcome. In contrast, in aversive conditioning such as fear learning, outcomes are delivered in the absence of an action. This disparity raises the question of whether valence-selective neurons fire for predicted outcome value or presence or absence of an associated action. To address this question, we trained rats on a 1) passive classical conditioning task in which tone cues predict stimuli delivery directly into the mouth via intraoral catheters, and 2) on an active classical conditioning task in which tone cues predicted that stimuli were available at a reward port. Rats received interleaved trials of either a) appetitive (sucrose) vs. aversive (quinine) or b) appetitive vs. neutral (no outcome) cue-outcome pairings. 4 female and 4 male Wistar rats were implanted with intraoral catheters and drivable tetrode arrays. Here we describe preliminary results from 1 rat in the appetitive-neutral group with 47 neurons recorded from PL and 52 from IL. Over 60% of mPFC neurons responded significantly to one or more cue and/or outcome. In IL, 17% of neurons were modulated (equally excited or inhibited) during sucrose delivery in the active task. Nearly 50% of these neurons also modulated their firing when the rat entered the reward port after a neutral cue. A separate population of neurons (~10%) changed firing for sucrose delivery in the passive condition but not after neutral cue ceased. These profiles suggest that task requirements, like the necessity to move towards a reward port, not necessarily the predicted outcome value, change IL firing. In PL during the passive task, 20% of neurons were inhibited only directly after sucrose cue and sucrose delivery. In contrast, if the rat entered the reward port in the active task, 50% of these neurons reduced firing continuously from cue onset until after sucrose delivery. The differing activity patterns within the same PL neurons suggest that a rat's actions during tone and stimulus delivery play a large role in PL modulation. These current results and previous preliminary results (Kaminska & Moorman SfN 2017), indicate that mPFC representations of cue/outcome valence may be better characterized as, or at least significantly influenced by, representing execution or inhibition of actions driven by learned cue-outcome associations.

Disclosures: B. Kaminska: None. **D.E. Moorman:** None.

Poster

163. Animal Cognition and Behavior: Executive Function: Learning and Memory I

Location: SDCC Halls B-H

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Program #/Poster #: 163.16/DDD15

Topic: H.01. Animal Cognition and Behavior

Support: NARSAD Young Investigator #26718

Title: Attention selection in projection-defined prefrontal projection neurons

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Abstract: The capacity for volitional control of attention is a critical tool for the effective performance of self-directed behaviors, and deficits in this ability underlie the core symptoms of attention deficit hyperactivity disorder (ADHD), including difficulty in controlling attention, hyperactivity, and impulsiveness. Despite the prevalence of this disorder and the side effects associated with standard treatments, such as phenethylamine psychostimulants, the circuit mechanisms that underlie the control of attention within the mammalian brain remain unclear. Elucidating these mechanisms, therefore, is critical to the development of more targeted treatments for this disorder.
Attentional set-shifting is a switching behavior commonly used to model the context-specific control of attention in both human and translational rodent models. In a set-shifting task, a subject must inferentially learn to ignore a previously relevant stimulus feature and instead attend to a newly relevant feature. Successful execution of this behavior, which is impaired in patients with ADHD, has been linked to the activity of the dorsolateral prefrontal cortex in humans and its rodent functional analogue, the prelimbic cortex.
Theoretical circuit models have been proposed to explain how network architecture and plasticity in prefrontal circuits can give rise to the switching of cue processing across contexts. However, these models have not yet been tested against data from population-level physiology, due in part to technical obstacles to recording the activity of large populations of neurons in ways that capture their laminar and long-range connectivity profiles. To more clearly define the circuit mechanisms by which attentional set-shifting is accomplished in the mammalian cortex, we used in vivo calcium imaging and pharmacogenetic manipulations to examine attention-related activity in prefrontal output neurons defined by their long-range projection targets. These projection populations included neurons targeting ventromedial striatum (PFC-VMS, n=5 mice), amygdala (PFC-AMG, n=5 mice), dorsomedial striatum (PFC-DMS, n=5 mice), and the mediodorsal thalamus (PFC-MDT, n=5 mice). Population-level activity was found to simultaneously encode multiple task-related features, including both relevant and irrelevant

stimuli, response direction, trial outcome, and currently applicable task rule (context). These findings elucidate output-specific pathways within PFC that support the flexible selection of targets of attention.

Disclosures: T. Spellman: None. **C.M. Liston:** None.

Poster

163. Animal Cognition and Behavior: Executive Function: Learning and Memory I

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Topic: H.01. Animal Cognition and Behavior

Support: NWO 016.Veni.178.032

Title: A method for automatic partial sleep deprivation in songbirds

Authors: *S. M. TER HAAR, A. K. DWULIT, Jr, J. J. BOLHUIS, Sr, G. J. L. BECKERS
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Abstract: Songbirds are widely used as a model for vocal learning including human speech. Strong individual differences can occur in how well birds learn to imitate their tutors' song. Sleep is one factor potentially contributing to this variation in learning. Several findings suggest a role for sleep in vocal learning both in humans and in songbirds. However, most findings are based on correlative data. One way to directly study the causality of vocal learning is by means of sleep deprivation. Therefore, we developed a sleep deprivation device, aiming to balance sleep deprivation and animal welfare in zebra finches (*Taeniopygia guttata*). The device consists of a perch rotating at an interval of 1-3 seconds randomly, controlled by an Arduino that responds to an infrared detector when the bird is sitting on the perch. The perch rotated for 11 hours during the night. Zebra finches very rarely fell or flew off the perch, indicating their strong preference for the perch at night, despite the rotation. When they did fall off, they flew back up after the dim light was temporarily switched on. We determined the effectiveness of the device by quantifying the time the bird was awake during the night, measured as the percentage of time the bird had at least one eye open and/or showed other waking activity. In addition, we measured consequences of the (partial) sleep deprivation on behavior the following day by measuring locomotor activity and song output. The birds were awake 81% of the time 2 hours after dark and 65% of the time 6 hours after dark, indicating at least partial sleep deprivation. Later in the night, between each perch rotation the birds were usually able to close their eyes. Nevertheless, they had to move their feet and shortly opened their eyes upon rotation. Thus, unaffected sleep bouts were usually not longer than 3 seconds. There were strong individual differences in locomotor activity and singing the next day. Some birds showed an increase in activity and number of songs while others showed a decrease or no differences with the control situation. This may be explained by a

combination of stress, increased neural excitability and birds probably being quite resilient to the physiological effects of sleep deprivation. This method opens a new avenue for studying causality of sleep in birds. We will use this method to study vocal, auditory and spatial learning and memory and neurogenesis, but it may also be useful for studying effects of sleep on stress, health, neural and metabolic patterns during sleep and effects of locomotion in general.

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Poster

163. Animal Cognition and Behavior: Executive Function: Learning and Memory I

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Topic: H.01. Animal Cognition and Behavior

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McKnight Brain Research Foundation
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Claude D. Pepper Older Americans Independence Center Scholar Award and Pilot Grant (P30 AG028740)

Title: Nutritional ketosis enhances cognitive resilience in young and aged rats

Authors: *A. HERNANDEZ, C. M. HERNANDEZ, III, K. CAMPOS, L. M. TRUCKENBROD, Q. P. FEDERICO, B. M. MOON, J. A. MCQUAIL, A. P. MAURER, J. L. BIZON, S. N. BURKE
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Abstract: As the number of individuals living beyond the age of 65 is rapidly increasing, so is the need to develop strategies to combat the age-related cognitive decline that may threaten independent living. The incomplete link between cognitive decline and the neurobiological changes that accompany normative aging is further complicated by the existence of bidirectional changes in neural activity levels observed within aged subjects. This factor impedes the development and implementation of potential therapeutic strategies to combat age-related cognitive decline. Therefore, potential strategies must focus on mitigating the effects of aging by targeting processes ubiquitous to the entire brain, such as impaired glucose utilization and the neurometabolic deficits that commonly accompany the aging process. To do this, this study utilized a ketogenic diet as a metabolic strategy to switch the primary fuel source from glucose to ketone bodies, a process known as ketosis. Calorically and nutritionally equivalent ketogenic (KD) and control (CD) diets were utilized for a minimum of 3 months in 4- and 24-month old

male and female Fisher 344 x Brown Norway rats prior to behavioral testing and hippocampal (HPC) and prefrontal cortical (PFC) protein quantification. An elevated figure-8 maze, with one closed arm and one open arm, was utilized to test anxiety-like behavior within the context of a spatial alternation task. Rats on the KD learned to alternate throughout this asymmetrical maze in fewer training sessions relative to rats on the CD. Within the same testing apparatus, KD rats required fewer training sessions to acquire an object-in-place association than CD rats. Furthermore, CD rats selected the correct object significantly fewer times in the open arm relative to the closed arm, but the KD group did not differ in performance across arms. Together, these data demonstrate the potential of a KD to mitigate anxiety- and age-related cognitive deficits. Age- and region-specific changes were found in vesicular transporters for GABA and glutamate within PFC and HPC tissue, indicating changes in neuronal signaling properties within these regions following ketosis. Furthermore, glucose and monocarboxylate transporters were also differentially affected, suggesting metabolic capacities were also altered. While there were no changes in motor tests, positive changes in peripheral health markers including visceral adipose tissue and improved resting glucose levels were observed. Together, these data suggest utilizing a ketogenic diet to alter metabolic processes within the brain may confer resilience against the cognitive and neurobiological effects of advanced age.

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Poster

163. Animal Cognition and Behavior: Executive Function: Learning and Memory I

Location: SDCC Halls B-H

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Program #/Poster #: 163.19/DDD18

Topic: H.01. Animal Cognition and Behavior

Support: Simons Collaboration on the Global Brain
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Title: Inhibitory and excitatory populations have similar accuracy yet different redundancy in predicting the choice during perceptual learning

Authors: *F. NAJAFI[1], G. F. ELSAYED[2], E. PNEVMATIKAKIS[3], J. P. CUNNINGHAM[2], A. K. CHURCHLAND[1]
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Abstract: Decisions are driven by the coordinated activity of diverse neural populations in multiple structures. Inhibitory neurons play a critical role in many models of decision-making, but the difficulty in measuring large inhibitory populations in behaving animals has left their in vivo role mysterious. To understand the contribution of excitatory and inhibitory populations to decision-making, we used 2-photon imaging to measure neural population responses in behaving mice whose inhibitory neurons were genetically tagged (Gad2-Cre;Ai14). Mice were injected with the calcium indicator GCaMP6f in their posterior parietal cortex, and chronic imaging was performed throughout learning. To study decision-making we presented mice with a series of multisensory "events" (clicks and flashes), the rate of which fluctuated stochastically over one second. Mice were trained to lick to a left (right) spout to report that event rates were above (below) an abstract category boundary. We first evaluated choice representation in single excitatory and inhibitory neurons and found small but significant choice signal in both cell types. Interestingly the magnitude of choice tuning was slightly higher in inhibitory neurons. We then assessed choice representation at the population level by using linear classifiers. We found that total population activity reliably predicted the choice. Importantly, decoders trained on inhibitory vs. excitatory populations were remarkably similar in predicting the animal's choice. Also in both populations the choice decoder was similarly stable during the course of a trial. Furthermore, we found that inhibitory neurons were more correlated than excitatory neurons, providing a possible explanation for how inhibitory neurons outperformed excitatory neurons at the single-cell level but not at the population level. Finally, we showed that as mice transition from novice to expert decision makers, the accuracy of choice prediction increases; choice prediction shifts earlier in the trial, and the magnitude of noise correlation shrinks. Additionally, these effects occurred at the same time course in both excitatory and inhibitory populations. Altogether our findings demonstrate that: 1) inhibitory neurons represent choice reliably, advocating for specific connectivity between excitatory and inhibitory neurons. 2) Inhibitory neurons are more correlated, confirming their strong local connectivity pattern. 3) During perceptual learning, choice representation enhances and noise correlation decreases, indicating that behavioral performance relies on the accuracy of the population code.

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Poster

163. Animal Cognition and Behavior: Executive Function: Learning and Memory I

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant MH109548

Title: Consider the cascade- A classical physics turbulence description of LFP energy interaction in the hippocampus

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Abstract: The brain is often equated to a computer, with the processing of different functions occurring in localized regions. In this description, sensory input is "encoded" in lower cortical regions and relayed up through parallel pathways. In higher associational cortices, where this activity converges, it has been suggested that simultaneous cognitive functions can occur in a multiplexed fashion. For example, it is tempting to assign distinct hippocampal oscillatory frequencies to different pathways that subserve unique cognitive processes (e.g., encoding versus recall). The virtue of analogies is to derive information about something we know little, the brain, from something we know a great deal, a computer. It has been noted, however, that "While brains do indeed perform something akin to information processing, they differ profoundly from any existing computer in the scale of their intrinsic structural and dynamic complexity" (Koch and Laurent, 1999). In light of this, it is necessary to consider another framework in which the heavy lifting of cognition is not relegated to multiplexed oscillations, but rather a consequence of an energy cascade. Energy enters the hippocampus at low-frequencies (e.g., theta, slow oscillations/sharp-waves) and is re-purposed within smaller, densely interconnected networks, which in turn result in gamma, epsilon, and ripples. Simply, the brain supports cognition by moving activity on the macroscale and passing this energy into smaller scales. This framework, while sharing a similarity to the unitary slope 1/f power distribution observed in self-organized criticality, is more akin to the multi-sloped organization of power seen in wind, temperature, and water waves. The common theme between these domains and the hippocampus in terms of cascading energy is classical turbulence theory. Interestingly, this theory can explain why the integrity of hippocampal place cells and entorhinal grid cells require the theta paced input from the medial septum and the relationship between theta and gamma coupling to velocity (specifically, energy into the network). Moreover, it reinforces the idea that the LFP can often outperform behavioral decoding relative to multiple simultaneously recorded single units (Agarwal, Stevenson, Berényi, Mizuseki, Buzsáki, G., & Sommer, 2014). As the LFP is the result of coherent activity across a population of neurons, this theory connects single units to the macroscale, offering the ability to relate physics to cognition.

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Poster

163. Animal Cognition and Behavior: Executive Function: Learning and Memory I

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Topic: H.01. Animal Cognition and Behavior

Support: Wellcome Trust-Carnegie
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Department of Science and Technology South African Research Chair Initiative

Title: The role of CD4 positive T cells subsets in cognitive function

Authors: *T. BROMBACHER[1], K. S. DE GOUVEIA[2], O. TAMGUE[2], M. SCIBIOREK[1], N. MAKENA[1], J. WOMERSLEY[1], F. BROMBACHER[1]
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Abstract: Background
We (Brombacher et al. 2017) and others (Derecki et al. 2010) have demonstrated that T cells are important for cognitive functions by secreting Interleukin-4 (IL-4) and IL-13 during Morris water maze (MWM) training. These ligands stimulate astrocytes via IL-4Receptor alpha/gamma c (IL-4Ra/gc) and IL-4Ra/IL-13Ra1, respectively to produce brain-derived neurotrophic factor (BDNF) in the meninges to foster cognitive functions.
Materials and Methods
For the MWM task mice were given four trials per day from varying entry points for four days acquisition training followed by a single probe trial on day 5 to determine effects on memory formation (Brombacher et al. 2017). To gain insight into the possible downstream neural mechanism mediated by meningeal and brain parenchyma cytokine milieu, the effect of different cytokines and their combinations on astrocytic expression of pro- and anti-inflammatory products were examined following MWM training. Immunological and neurological cell populations influenced by specific IL-4Rα deficiency on T cells were characterized. EthoVision XT 8 was used to record data, with statistical analyses by ANOVA or Student t test. Protocols (No. 050/015) were approved by the animal ethics research committee of the University of Cape Town, South Africa.
Results
In order to better understand which T cell subsets are important we used a loss of function strategy by generating T cell specific IL-4Ra deficient mouse models. This included Pan T cell IL-4Ra deficient mice (Dewals et al. 2009), $CD4^+CD8^-$ T cell IL-4Ra deficient mice (Radwanska et al. 2007), $CD4^+g/d^-$T cell IL-4Ra deficient mice (unpublished), as well as $FoxP3^-$IL-4Ra deficient mice (Abdel Aziz et al. 2018) compared to wild type mice. Of interest, all T cell subsets showed reduced learning and memory compared to controls.
Conclusions
MWM studies using various T cell IL-4Ra deficient mice showed impaired learning and memory, suggesting that all T cell subsets, including regulatory T cells contribute to cognitive function.

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Poster

163. Animal Cognition and Behavior: Executive Function: Learning and Memory I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 163.22/DDD21

Topic: H.01. Animal Cognition and Behavior

Support: ERC STG dEMORY GA 311435
Alexander Onassis Foundation
Google fellowship

Title: Challenging the point neuron dogma: FS basket cells as 2-stage nonlinear integrators

Authors: *P. POIRAZI[1], A. TZILIVAKI[1,2]
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Abstract: Fast Spiking (FS) basket cells constitute one of the main types of hippocampal and neocortical interneurons. A growing body of work recognizes their importance in controlling executive functions. However, most studies have focused on their molecular and anatomical features and supported the dogma that these cells integrate inputs like linear point neurons, completely ignoring potential dendritic influences. Exciting new findings however, reveal that the dendrites of certain interneuron types are much more powerful than originally assumed. As a result, whether a linear point neuron or a more sophisticated abstraction can successfully capture their synaptic integration profile, remains an open question. To address this question, we developed detailed, biologically constrained biophysical models of FS basket cells using anatomical reconstructions of both hippocampal and cortical neurons. Synaptic stimulation within the dendrites, predicts the co-existence of two distinct integration modes; some dendrites exhibit supralinear synaptic integration while others operate in a sublinear mode. Morphological features such as dendritic length and/or diameter influence the integration mode and these features differ between hippocampal and cortical neurons. Dendritic volume appears to be a consistent discriminating feature among sub- and supralinear dendrites of both areas. By generating a large number of different spatial patterns of synaptic activation we find that spatially dispersed inputs lead to higher firing rates than inputs clustered within a few dendrites in both Hippocampus and PFC models. Moreover, a 2-layer Artificial Neural Network (ANN) with both sub- and supralinear hidden nodes can predict the firing rate of the aforementioned models much better than a linear ANN. This study provides a systematic, cross-area analysis of dendritic integration in FS basket cells. Our findings challenge the current dogma, whereby interneurons are treated as linear summing devices. We predict that the dendrites of FS basket cells in both Hippocampal and Neocortical regions can operate in distinct non-linear modes. As a result, FS basket cells, similar to pyramidal neurons, are better represented by a 2-stage integrator abstraction rather than a point neuron.

Disclosures: P. Poirazi: None. **A. Tzilivaki:** None.

Poster

163. Animal Cognition and Behavior: Executive Function: Learning and Memory I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 163.23/DDD22

Topic: H.01. Animal Cognition and Behavior

Support: NIA Grant 2RF1AG013622-21
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Dr. Miriam and Sheldon G. Adelson Medical Research Foundation

Title: Hotspots of dendritic spine dynamics facilitate learning and memory

Authors: *S. HUANG[1], A. FRANK[1], M. ZHOU[1], A. GDALYAHU[3], G. KASTELLAKIS[4], T. SILVA[1], E. LU[1], X. WEN[2], P. POIRAZI[4], J. TRACHTENBERG[1], A. SILVA[1]
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Abstract: Structural plasticity mediated by addition and elimination of dendritic spines is thought to underlie the formation of long-term memory. Modeling studies suggest that clustered structural plasticity of dendritic spines is an efficient mechanism of information storage in cortical circuits. However, why new clustered spines occur in specific locations and how their formation relates to learning and memory (L&M) remain unclear. Using in vivo two-photon microscopy, we track spine dynamics in the retrosplenial cortex before, during, and after two forms of episodic-like learning and find that learning-related spine clustering correlates with L&M performance. Importantly, spine turnover before learning predicts future L&M performance, as well as the rates of spine clustering. Consistent with the idea that these measures are causally related, a heterozygous Ccr5 null mutation that enhances pre-learning spine turnover also enhances both L&M and spine clustering. Remarkably, clustered new spines are mostly added on dendritic segments with rapid spine turnover, revealing the presence of hotspots on the dendritic tree where elevated rates of spine turnover facilitate clustered spine addition. Biophysically inspired modeling suggests that spine turnover increases clustering, network sparsity, and memory capacity. One implication of these findings is that increased spine turnover may allow neurons to more efficiently sample the synaptic space during L&M in order to optimize information acquisition. Once acquired, spine clustering may stabilize this information, thus strengthening memory circuits.

Disclosures: S. Huang: None. **A. Frank:** None. **M. Zhou:** None. **A. Gdalyahu:** None. **G. Kastellakis:** None. **T. Silva:** None. **E. Lu:** None. **X. Wen:** None. **P. Poirazi:** None. **J. Trachtenberg:** None. **A. Silva:** None.

Poster

163. Animal Cognition and Behavior: Executive Function: Learning and Memory I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 163.24/DDD23

Topic: H.01. Animal Cognition and Behavior

Title: Contribution of apical and basal dendrites of L2/3 pyramidal neurons to orientation encoding in mouse V1

Authors: *J. PARK[1], A. PAPOUTSI[2], R. T. ASH[3], P. POIRAZI[2], S. M. SMIRNAKIS[3]
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Abstract: Pyramidal neurons integrate synaptic inputs from basal and apical dendrites to generate stimulus-specific responses. Feed-forward inputs to basal dendrites are thought to drive a neuron's stimulus preference, while feedback inputs to apical dendrites sharpen selectivity. However, how a neuron's dendritic domains relate to its functional selectivity has not been directly demonstrated experimentally. We performed 2-photon dendritic micro-dissection on layer-2/3 pyramidal neurons in mouse primary visual cortex. We found that removing the apical dendritic tuft did not on average alter orientation-tuning. Furthermore, orientation-tuning curves were remarkably robust to the removal of basal dendrites: ablation of 2-3 basal dendrites was needed to cause a small shift in orientation preference, without significantly altering tuning width. In conclusion, neuronal orientation-tuning appears remarkably robust to loss of dendritic input. These observations are discussed in the context of a simple model as well.

Disclosures: J. Park: None. **A. Papoutsi:** None. **R.T. Ash:** None. **P. Poirazi:** None. **S.M. Smirnakis:** None.

Poster

163. Animal Cognition and Behavior: Executive Function: Learning and Memory I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 163.25/DDD24

Topic: H.01. Animal Cognition and Behavior

Support: DFG research unit FOR2143
ERC advanced grant 787450
DFG excellence initiative GSC-4 (SGBM)

Title: Two-photon calcium imaging of memory engrams throughout the hippocampal formation in behaving mice

Authors: *T. HAINMUELLER[1,2], M. BARTOS[1]
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Abstract: The hippocampus is essential for the storage and recall of conscious memories. Each of its major subfields is home to neuronal ensembles (engrams) which form abstract representations of memory-specific contents, such as places, sounds, time or individuals (O'Keefe and Dostrovsky, 1971; Quiroga et al., 2005; Aronov et al., 2017). Artificial activation of these engrams can be used to manipulate existing memories in the mouse brain or even create new ones (Ramirez et al., 2013). Despite their importance as a correlate of episodic memories in the brain, the circuit mechanisms by which memory engrams in the hippocampus evolve during different forms of learning are still poorly understood (Bittner et al., 2017; Sheffield and Dombeck, 2017).
To study the emergence and activity of hippocampal memory engrams in awake, behaving mice, we use two-photon calcium imaging with (sub-) cellular resolution in the various hippocampal subfields of behaving, head-fixed mice. During the imaging sessions, the mice perform different behaviours in a virtual environment displayed on screens around them (Hainmüller and Bartos, 2018). We investigate, how principal neurons in different hippocampal subfields represent the individual elements of episodic memories (places, objects, time etc.) and how these representations develop with learning. We also study the local circuit mechanisms underlying the formation of memory-bearing neuronal ensembles (Sheffield and Dombeck, 2017). By these means, we are trying to decipher how neuronal circuits in the hippocampal formation store and retrieve the contents of complex episodic memories.

Disclosures: T. Hainmueller: None. **M. Bartos:** None.

Poster

163. Animal Cognition and Behavior: Executive Function: Learning and Memory I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 163.26/EEE1

Topic: H.01. Animal Cognition and Behavior

Support: NSF Track-2 FEC1539041
NIH 1P50AA022534-01

Title: Knockdown of a cortical circRNA associated with psychiatric disorders impairs Homer1 mRNA isoform synaptic trafficking and executive control

Authors: *A. ZIMMERMAN[1], J. P. WEICK[2], N. MELLIOS[4], J. L. BRIGMAN[3]
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Abstract: Schizophrenia (SCZ) and Bipolar Disorder (BD) are multifactorial psychiatric disorders affecting together more than 3.5% of the adult U.S. population that result in major socioeconomic burdens. Both disorders are marked by impaired functioning of the prefrontal cortex (PFC) leading to behavioral deficits including impairments in executive functioning, such as cognitive flexibility. SCZ and BD have also been shown to have strong genetic components linking the diseases to protein-coding genes, specifically those associated with synaptic transmission and plasticity. Based on this evidence, it is hypothesized that genes at the post synaptic density (PSD) play an important role in these disorders. One likely candidate PSD protein, Homer protein homolog 1 (HOMER1), is a psychiatric disease-associated scaffolding protein known for its interaction with group 1 metabotropic glutamate receptors and its role in regulating synaptic transmission. Recently, circular RNAs (circRNAs) and other non-coding RNAs have been posited to regulate genes and lead to network changes and altered functional output. Although circular RNAs are highly enriched in the mammalian brain, little is known about their functions and interactions with protein-coding genes. Here, we show that *circHomer1*, a circRNA derived from *HOMER1*, is reduced in the orbitofrontal cortex (OFC) and in stem cell-derived neuronal cultures from patients with SCZ and BD and is inversely correlated to the relative abundance of *HOMER1B* mRNA isoform. By performing *in vivo circHomer1* knockdown in mouse OFC, we also uncover that *circHomer1* regulates the synaptic localization of specific *Homer1* isoforms. Lastly, using a discrimination-reversal touchscreen task that is a translational measure of cognitive flexibility, we find that mice with reduced *circHomer1* expression in their OFC exhibit significant learning impairments in the reversal stage similar to those seen in patients with psychiatric disorders. Together, our results indicate a novel psychiatric disease-associated circRNA capable of regulating synaptic gene expression and behavioral flexibility.

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Poster

163. Animal Cognition and Behavior: Executive Function: Learning and Memory I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 163.27/EEE2

Topic: H.01. Animal Cognition and Behavior

Support: 1P50-AA022534-01

5T32AA014127e13

Title: Exploring neuronal alterations mediating executive dysfunction as a result of prenatal alcohol exposure

Authors: *J. A. KENTON, JR[1], K. L. MARQUARDT[2], C. F. VALENZUELA[1], J. L. BRIGMAN[1,3]
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Abstract: Growing evidence demonstrates the negative effects moderate alcohol consumption during pregnancy has on executive function. Moderate prenatal alcohol exposure (PAE) via a drinking in the dark paradigm (10% EtOH available 4 hours every day throughout gestation; BAC: ~90mg/dL; SAC: saccharine controls) impairs executive function by significantly increasing perseverative responding on a touchscreen-based visual reversal learning task. Single unit and local field potential (LFP) activity during behavior was significantly altered in the orbitofrontal cortex (OFC) during early reversal. These alterations were concurrent with decreases in the synchronous activity between the OFC and dorsal striatum (dS). These findings suggest that deficits in behavioral flexibility after PAE occur as a result of impaired neuronal functioning and coordination during reversal learning. Here, we investigated whether alterations in excitatory signaling contribute to behavioral deficits and if precise stimulation of the cortical neurons following choice can reduce PAE-induced maladaptive perseveration and support behavioral flexibility. Recordings of spontaneous inhibitory post-synaptic currents in OFC pyramidal neurons in naïve age-matched PAE mice revealed a significant increase in the length and duration of the amplitude relative to SAC mice. A separate cohort was trained through discrimination and then microinfused with channelrhodopsin-expressing adeno-associated virus (AAV-CAMKαII-ChR2(H134R)-mCherry) and fitted with recording optrodes that targeted the OFC and dS for recording and stimulation. After 4 weeks of expression, PAE and SAC mice re-attained discrimination criteria and were then tested on the reversal. On the first 4 sessions of reversal, PAE and SAC mice received light pulses (10 Hz, 5mW, 5ms pulse for 1s) or no stimulation 1 second following a correct choice while waveform and LFP activity was recorded. Optogenetic stimulation increased in vivo OFC firing and coordinated activity following correct choices. Targeted stimulation also reduced perseveration in PAE mice compared to non-stimulated controls. These data provide evidence that PAE may alter excitatory/inhibitory balance at baseline and stimulation of specific populations of cortical neurons may reduce the impaired flexibility seen after PAE. Current studies are further examining the role of excitatory signaling in the regulation of efficient learning and reversal.

Disclosures: J.A. Kenton: None. **K.L. Marquardt:** None. **C.F. Valenzuela:** None. **J.L. Brigman:** None.

Poster

163. Animal Cognition and Behavior: Executive Function: Learning and Memory I

Location: SDCC Halls B-H

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Program #/Poster #: 163.28/EEE3

Topic: H.01. Animal Cognition and Behavior

Support: NIH-NIMH 1UH2-MH109168-01
NIH-NIGMS 1P20-GM109089-01A1

Title: Evaluating translational neurophysiological measures to improve efficacy of preclinical therapeutic target discovery

Authors: *J. F. CAVANAGH[1], D. C. GREGG, 87109[4], S. L. OLGUIN[2], G. A. LIGHT[5], J. W. YOUNG[6], J. L. BRIGMAN[3]
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Abstract: While preclinical studies provide numerous therapeutic targets for neuropsychiatric disorders, the failure to convert these to clinical treatments highlight that behavioral similarity alone without biomarkers of brain function is insufficient. In order to measure specific Research Domain Criteria (RDoC) aspects across species, we integrated touch-screen tasks with dura-resting electroencephalography (EEG)-like recording in the mouse to determine neural activity directly comparable to human data. C57BL/6J mice were first trained to initiate and respond for reward to visual stimuli in a touch-sensitive screen. Following pre-training, mice were fitted with fixed dura-resting EEG leads caps targeting medial PFC, posterior parietal cortex, primary motor cortex and cerebellum. After recovery, mice were trained on a series of behavioral tasks to RDoC-specific domains including the Five-Choice Continual Performance Task (5C-CPT) of cognitive control, Progressive Ratio Break-Point Task (PRBP) of effortful motivation, and a Probabilistic Discrimination Task (PrDT) of reward learning, all while EEG signal was recorded. Mice were able to successfully perform touch-screen variants of the tasks during tethered EEG recording. During 5C-CPT performance mice successfully differentiated between target and non-target trials with EEG exhibiting similarly differential frontal event-related components corresponding to trial type. Mice tested on the PRBP exhibited break-points similar to those in lever-based tasks and frontal EEG signal was linked to variability in willingness to exert effort to obtain reward. When tested on different probabilities of reward by stimulus-set, cortical EEG signal exhibited differential responses to reward by reward difficulty level. These studies demonstrate the capability of utilizing identical methods for investigating rodent neuronal activity performing the same behavioral tasks that are available in rats and humans and provide

important information regarding the validity rodent models to diseases in addition to the likelihood of translating drug-induced changes in performance across species.

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Poster

163. Animal Cognition and Behavior: Executive Function: Learning and Memory I

Location: SDCC Halls B-H

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Program #/Poster #: 163.29/EEE4

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant 1R01AA025652-01A1
NIH Grant 1P50AA022534-01
NIH Grant 1UH2-MH109168-01

Title: Cognitive control on the touch-screen five-choice continuous performance task is impaired by moderate prenatal alcohol exposure in mice

Authors: *J. L. BRIGMAN[1], S. L. OLGUIN[2], D. J. GREGG[2], C. F. VALENZUELA[3]
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Abstract: Evidence suggests that moderate alcohol intake can have lasting cognitive impact on offspring. These impairments, categorized as Fetal Alcohol Spectrum Disorder (FASD), persist across the lifespan and include deficits in cognitive control. In humans, cognitive control is typically measured via continuous performance tasks (CPT) and FASD populations have been shown to have decreased performance on these paradigms. A rodent CPT has recently been developed and EEG recording demonstrates that both humans and mice utilize medial prefrontal (mPFC) and posterior parietal cortex (PPC) when attending to target stimuli and control responding to non-targets, respectively. While clinical data suggest that prenatal alcohol exposure (PAE) impairs the ability to withhold responding on CPT tasks, the mechanisms underlying these deficits are not understood. We tested PAE (limited access 10% sweetened EtOH available 4 hours/day, throughout gestation; BAC: ~90mg/dL) and saccharin control (0.066% sweetened water, “SAC”) mice on the 5C-CPT task. Mice were trained to respond to a brief (1.5 sec.) target stimulus (white square) in 1 of 5 available locations on a touch-screen. After stable performance was reached, mice were tested on the 5C-CPT in which non-target trials (all 5 locations illuminated) were added and reward was only delivered if a response was withheld. Mice were first tested on a 2:1 target to non-target ratio and then moved to 5:1 ratio. There was no effect of exposure or sex on the ability to acquire the target trial only task as measured by sessions to criterion or secondary response measures. When tested on the 5C-CPT

male and female PAE mice made significantly increased false alarms (responses to non-target stimuli) versus control animals regardless of task difficulty. Compared to SAC, PAE had a significantly decreased sensitivity index, indicating they responded more often to non-target stimuli than target stimuli. Next, PAE and SAC control animals were fitted with dura-resting EEG-like recording caps targeting mPFC, PPC, primary motor, and cerebellar cortex. Results suggest that PAE altered cortical signaling seen during target and non-target responding on the 5C-CPT. Together, these findings suggest that moderate exposure to alcohol during development can have long lasting effects on attention, vigilance, and inhibition and closely models that seen in FASD. This data provide an important framework for examine the mechanisms by which exposure to alcohol during development alters cortical signaling required for cognitive control.

Disclosures: J.L. Brigman: None. **S.L. Olguin:** None. **D.J. Gregg:** None. **C.F. Valenzuela:** None.

Poster

164. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 164.01/EEE5

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant R01MH109170
NIH Grant R21NS088798

Title: Paradoxical response of interneurons in both CA3 and CA1 during optogenetic inactivation of CA3

Authors: *L. WATKINS DE JONG[1,2], D. LYTTLE[1], K. DIBA[2]
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Abstract: The hippocampus plays a vital role in episodic memory. In area CA1 of the rat hippocampus, excitatory pyramidal cells encode the representations of spatial memories and provide output to cortical and subcortical regions. However, little is understood about how excitatory and inhibitory inputs to CA1 neurons interact to generate their firing patterns. In order to assess the contribution of CA3 inputs in the processing of information by area CA1, we combined high-density electrophysiological recordings of CA1 with optogenetic (AAV2/CaMKIIa-ARCHT-GFP) silencing of CA3 in urethane anesthetized and freely-moving rodents. While, our initial hypothesis predicted an overall decrease in activity in CA1 due to the expected decrease in excitatory drive from CA3, few cells showed decreased firing. Surprisingly, suppression of excitatory drive from CA3 resulted in a strong disinhibition of interneurons in CA1. Of the 98 recorded CA1 interneurons, 29% showed disinhibition, 3% decreased firing and

68% had no response to light stimulation in CA3. These findings are largely consistent with predictions of Tsodyks et. al. J Neurosci (1997). To examine the dynamics in CA3 that contribute to this disinhibition in CA1, we recorded from CA3 from optrodes while silencing CA3 neurons. Similar to CA1, recordings in CA3 showed disinhibition of interneurons at sites distal to the focal silencing region along with suppression of firing of cells in both excitatory and inhibitory cells proximal to the location of silencing (84 interneurons, 23% disinhibited, 41% inhibited, 36% unaffected),. Taken together, these results suggest focused perturbation of the recurrent CA3 network results in inhibitory-stabilization within the network that is transferred to downstream targets. Preliminary results from chronic recordings has shown similar disinhibition within the CA1 interneuron population, however further analysis is needed to determine how this increased inhibition changes the rate and temporal coding within the area.

Disclosures: L. Watkins De Jong: None. **D. Lyttle:** None. **K. Diba:** None.

Poster

164. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 164.02/EEE6

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant R01MH109170

Title: Prolonged reactivation of neuronal activity following novel experience

Authors: *B. K. GIRI[1], H. MIYAWAKI[3], K. DIBA[2]
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Abstract: The role of the hippocampus in encoding and consolidation of memory is well established. While encoding is attributed to animals' waking experience, reactivation of these wake-induced neural activities during sleep is considered important for memory consolidation. However, it is still unclear how long the brain continues to reactivate newly formed memories. Previous studies reported a time window of 15-30 minutes of replay in sleep immediately following a task(Kudrimoti et. al. J Neurosci 1999, Tatsuno et. al. J Neurosci 2006). Contrasting the timescale of few minutes, in cellular consolidation, it takes hours for neurons to strengthen their synapses. On the other hand, memories are vulnerable to sleep deprivation for up to several hours following learning (Prince et. al. N Learning Memory 2013). However, we noted that many of these earlier replay studies involved either highly trained animals or behavior in environments that were familiar to the animals. Using recordings from silicon probe electrodes implanted in the CA1 area of the rat hippocampus, we re-examined the extent and duration of reactivation in naïve animals following novel maze exploration. Employing a widely-used

“explained variance” method based on pairwise correlations of spiking activity (Kudrimoti et. al. J Neurosci 1999), that controls for existing correlations in PRE-task sleep. we found that reactivation endures for several hours into sleep as opposed to ~15 minutes. To visualize a detailed time course of replay at much finer time scale, we also employed a recently developed PCA-ICA based technique (Lopes-dos-Santos et. al. J Neurosci Methods 2013). Using this, we extracted independent cell assemblies formed during maze exploration and inspected their activation during sleep before (PRE sleep) and after (POST sleep) the task. In comparison to PRE, these cell assemblies showed increased reactivation persisting for multiple hours into POST. As expected, this enhanced replay of assemblies was also locked to hippocampal sharp-wave ripples. Together our results suggest a much longer timescale for processing of newly encoded memory in the hippocampus. This timescale also more closely matches timescales reported for cellular consolidation, suggesting that the mechanisms for these two processes may be reconcilable.

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Poster

164. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 164.03/EEE7

Topic: H.01. Animal Cognition and Behavior

Support: NIH R01NS39600
NIH U01MH114829

Title: Hippocampome.org: A dynamic open-access knowledge base of rodent hippocampal neuron types and their properties

Authors: *D. W. WHEELER[1,2], C. M. WHITE[1], A. O. KOMENDANTOV[1], C. L. REES[1,3], D. J. HAMILTON[1], S. VENKADESH[1], K. MORADI[1], S. M. ATTILI[1], C. TECUATL[1], G. A. ASCOLI[1,2]
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Abstract: Hippocampome.org, an open-access knowledge base of the rodent hippocampus, uses peer-reviewed literature to define over 140 neuron types by the patterns of their axonal and dendritic presence across the parcels of the rodent hippocampal formation: dentate gyrus, CA3, CA2, CA1, subiculum, and entorhinal cortex. The knowledge base also encapsulates information on molecular markers based on direct evidence, including some Allen Brain Atlas (ABA) gene expression data, and inferential evidence relating the expression of one marker to another; electrophysiology, which encompasses membrane biophysics and firing patterns; and known and

potential connectivity. All data are linked to extracted quotations and figures, which facilitates revisions and opens the way for users' personal interpretations. As we continue to endeavor toward a simulation of the complete hippocampal formation, we are expanding into several new dimensions of information. For example, we derive estimated counts of each of the neuron types from multiple constraints including the cellular distributions in Nissl images from the ABA. In addition, we now leverage the quantified presence of the axons and dendrites across a given parcel to estimate statistically the probability of potential connectivity between neuron types. We also collect data on the amplitude, kinetics and short-term plasticity of synapses between the types. Furthermore, we include fully parameterized single- and multi-compartmental Izhikevich models for each neuron type, along with code-snippets for direct computer simulation. Finally, we present an enhanced user experience through an improved web portal, which now boasts an advanced connectivity search engine and an application program interface (API). As we grow, we also expand the underlying foundations of Hippocampome.org by continually data mining the literature for new neuron types and neuronal properties, making sure to cross-link and integrate these new data with prior data.

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Poster

164. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 164.04/EEE8

Topic: H.01. Animal Cognition and Behavior

Support: NIH R01NS39600
NIH U01MH114829

Title: Hippocampome.org: The integrated electrophysiological data of hippocampal synapses

Authors: *K. MORADI[1], G. ASCOLI[1,2]
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Abstract: The cellular and synaptic architecture of the rodent hippocampus has been the subject of more than 1200 peer-reviewed published studies, since the first electrophysiological recording of mammalian synapses more than 70 years ago. The advent of new tools has given scientists a more fine-grained access to neuronal microcircuits, which has led to modifications in the definition of neuronal types over time. To our knowledge, no significant attempt has been made to integrate, digitize, and interpret this huge dataset. Harnessing state-of-the-art information

technology, we have devised a systematic method to translate any relevant synaptic recording from the experimental literature into machine-readable search queries that are based on the morphology, electrophysiology, and biomarker profile of the underlying neurons. Specifically, we map experiments to a dynamic, self-organized set of neuronal pairs continuously and seamlessly updated as more information is collected. Our work leverages Hippocampome.org, which provides a growing list of neuronal types by keeping track of their properties and a custom-made connectivity search engine based on Peters' rule. A major challenge is that the synaptic data are multimodal. Our collected data show that researchers have used 8 different recording modalities and more than 70 different measurement methods to quantify the amplitude, kinetics, and short- and long-term plasticity of synapses. In other words, a data structure of at least 8 times 70 columns per experiment is needed to organize these data. Including metadata, we have used a data structure of more than 700 columns to collect experimental data. We have developed a cloud-based tool to automatically find relevant data in scientific papers, while still enabling user input to interpret the experiments, devise search queries, and correctly input the automatically extracted information in the data structure. Thus, while automation is used, where suitable, to avoid user errors, our data are highly hand curated. We have also digitized all the experimental synaptic traces and assigned each trace a membrane potential, a calculated or measured synaptic reversal potential, a liquid junction potential, and a set of presynaptic stimulation times, which are needed for normalization and computational modeling. The data will be publicly released via the Hippocampome.org project portal, supporting researchers from diverse disciplines including neurology, psychiatry, artificial intelligence, machine learning, data science, computer science, and bioinformatics.

Disclosures: K. Moradi: None. **G. Ascoli:** None.

Poster

164. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 164.05/EEE9

Topic: H.01. Animal Cognition and Behavior

Title: Interconnections of horizontal cells in CA1 stratum oriens

Authors: *N. KOGO[1], É. KÓKAI[2], P. SZUCS[2], Y. ISOMURA[3], T. AIHARA[4]
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Abstract: O-LM cell is one of the interneurons in hippocampus that has a large horizontal cell body in stratum oriens and sends an axon in long distance to stratum lacunosum-moleculare. The characteristic morphology implies its role in controlling signals from entorhinal cortex that

pyramidal cells receive at the distal part of the apical dendrites. Its importance in neural coding in hippocampus has been suggested by the reports showing that, for example, they are active during place field depolarization, they are involved in the generation of theta oscillation, and they are recruited during sharp wave ripple activities linked to memory consolidation.
At the network connectivity level, O-LM cell receives excitatory inputs from pyramidal cells and, hence, pyramidal cells and O-LM cells form a feedback circuit. It is also known that O-LM cells form a network by electric couplings among them. Furthermore, it has been known that axons of O-LM cells have collaterals that leave the main axon within stratum oriens or its vicinity and project back to stratum oriens, implying interconnections among the neurons in stratum oriens. We investigated the possibility that these axon collaterals mediate interconnections between O-LM cells. For this purpose, we performed double patch clamp recordings from the cells in striatum oriens with large horizontal cell bodies. The recording was done from CA1 of mouse hippocampus (age p12-21) under DIC-IR visualization. The patch pipettes contained biocytin and were filled with either low or high Cl^- solution. Most of the cells showed characteristic physiological properties of O-LM cells such as depolarization sag in response to hyperpolarizing current step and sharp rising phase of after-hyperpolarization, indicating the presence of *Ih* current, and non-fast spiking firing pattern. Among the recorded pairs, 32 of them showed inhibitory monosynaptic connection. Furthermore, 3 of them had mutual connections. Application of picrotoxin in the bath blocked the PSPs. Majority of the IPSPs showed short-term potentiation. We will report the physiological properties of the monosynaptic IPSPs and the morphologies of the connected pairs.

Disclosures: N. Kogo: None. **É. Kókai:** None. **P. Szucs:** None. **Y. Isomura:** None. **T. Aihara:** None.

Poster

164. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 164.06/EEE10

Topic: H.01. Animal Cognition and Behavior

Support: IBS-R002-A1

Title: Dissociable effects of reward and navigation history on forward and reverse replays

Authors: *B. BHATTARAI[1,2], J. W. LEE[2], M. W. JUNG[1,2]
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Abstract: Sequential firing of hippocampal place cells during navigation is replayed in forward and reverse directions during sleep and awake immobility. Currently, functional roles of and

factors influencing forward and reverse replays are not clearly understood. We trained rats in a spatial sequence memory task and examined effects of reward and navigation history on forward and reverse replays of CA1 place cells during awake immobility. Reward enhanced both forward and reverse replays, but in different ways. Reward increased reactivation fidelity of forward replays while increasing the rate of reserve replays. In addition, reactivation was stronger for place cell sequences associated with future than past rewarding trajectories in forward, but not reverse, replays. Our results show differential effects of reward and navigation history on forward and reverse replays, and support the proposed role of forward replays in planning future navigation.

Disclosures: B. Bhattarai: None. **J.W. Lee:** None. **M.W. Jung:** None.

Poster

164. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits I

Location: SDCC Halls B-H

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Program #/Poster #: 164.07/EEE11

Topic: H.01. Animal Cognition and Behavior

Support: IBS-R001-D1
IBS-R002-G1

Title: Impact of mossy fiber stimulation on CA3 activity during spatial exploration is inhibitory and transient

Authors: *J. LEE[1,2], M. YUN[2], E. CHO[1], J. LEE[1], D. LEE[3], M. JUNG[1,2]
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Abstract: The hippocampus is critically involved in encoding masses of past experiences as distinct memories. It has long been hypothesized that the dentate gyrus minimizes overlaps among similar input patterns and imposes new patterns to learn onto CA3 network via strong mossy fiber synapses during memory encoding. Such ‘detonator’ hypothesis was directly tested by optogenetically stimulating mossy fibers in freely-behaving mice. We found that optogenetic stimulation of mossy fibers can drive CA3 neuronal firing in mice navigating along a circular track, but their effects are overall inhibitory and transient. Spatially restricted mossy fiber stimulation was more likely to suppress than enhance CA3 neuronal activity. Also, changes in spatial firing induced by optogenetic stimulation reverted immediately upon stimulation termination, leaving CA3 place fields unaltered. Our results argue against the view that mossy fibers convey teaching signals, and show the robustness of established CA3 spatial representations.

Disclosures: J. Lee: None. **M. Yun:** None. **E. Cho:** None. **J. Lee:** None. **D. Lee:** None. **M. Jung:** None.

Poster

164. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits I

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Topic: H.01. Animal Cognition and Behavior

Support: ISF
Adelis foundation
Allen and Jewell Prince Center for Neurodegenerative Disorders of the Brain

Title: Hippocampal place cells demonstrate pattern completion and separation deficits in Alzheimer's disease model mice in a novel tactile gradient task

Authors: *O. RECHNITZ, D. DERDIKMAN
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Abstract: Pattern completion and separation deficits have been used as measures for dementia in both humans and rodents. Here we relate pattern completion and separation to remapping of hippocampal place cells and demonstrate that deficits in pattern completion and separation may be determined from the population of recorded place cells in mice. We developed a novel tactile gradient paradigm tailored to create a strong remapping effect of place cells in wild-type (WT) and Alzheimer's disease (AD) model mice. In this paradigm, mice were trained to run on an elevated linear track with interchangeable floors of fine or coarse texture. Training was complete when the population of recorded cells achieved clear distinction between fine and coarse conditions, while also presenting a stable representation of each condition (attractors). Following the training phase, mice were presented with a gradient of textures- fine to coarse and vise-versa. The cell population showed clear difference in representation dynamics between the WT and AD model mice across the contextual-tactile gradient. One key difference, is greater tendency of AD mice to have a consistent time-dependent representation through the gradient, suggesting a pattern separation deficit. These results hold importance for both diagnosis and treatment of dementia.

Disclosures: O. Rechnitz: None. **D. Derdikman:** None.

Poster

164. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits I

Location: SDCC Halls B-H

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Adelis Foundation - joint Technion and Weizmann program

Title: VTA dopaminergic stimulation controls “spatial foveation” in the hippocampus

Authors: D. KHATIB[1], G. TOCKER[1], J. GROSS[1], G. MORRIS[2], *D. DERDIKMAN[1]
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Abstract: Hippocampus and associated structures are related to perception of space. An important projection to the hippocampus is the dopaminergic input from the VTA, which is considered to be part of the reward learning system. In the current study we used excitatory DREADDs in conjunction with TH-Cre mice, to specifically stimulate dopaminergic neurons in the VTA. We subsequently recorded single units from dorsal CA1 of awake-behaving mice. The mice ran along two linear tracks distinguished by different textures. Running on one of the tracks was preceded by CNO injections inducing VTA stimulation. We hypothesized that VTA stimulation would affect spatial representation in place cells, which receive projections from the VTA. Our data demonstrates that indeed CA1 place cells display smaller place fields in the arena associated with VTA stimulation, despite overall higher speed in this arena. Thus, we suggest that dopaminergic activity in the VTA modulates properties of hippocampal place cells by creating a “spatial foveation” on fragments of space with high valence.

Disclosures: D. Khatib: None. **G. Tocker:** None. **J. Gross:** None. **G. Morris:** None. **D. Derdikman:** None.

Poster

164. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits I

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Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 164.10/EEE14

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant 1R21MH109796

Title: Optogenetic stimulation of the prefrontal cortex induces spatial alternation deficits and alters hippocampal local field potentials in rats

Authors: *K. S. KIDDER[1], P. M. BAKER[2], Z. M. RIVERA[2], S. M. LEWIS[1], D. H. GIRE[2], S. J. MIZUMORI[3]
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Abstract: A current challenge in our understanding of the neurobiology of learning and memory is the nature of interactions between memory systems. The hippocampus (HPC) is important for context-dependent episodic memory and the medial prefrontal cortex (mPFC) plays a role in action selection and flexible decision making. Both structures are implicated in working memory. Without a direct mPFC to HPC connection, mPFC may exert influence over HPC via the nucleus reuniens, effectively updating HPC with information about planned actions and outcomes. In order to probe the interaction between mPFC and HPC, the current project used a 64-channel micro-drive with bilateral optic fibers, to investigate the effects of optogenetic stimulation of mPFC on HPC local field potentials (LFP) during a delayed alternation task in rats. Rats were trained on a plus-maze with two arms designated as reward arms (east and west) and two arms designated as start arms (north and south). The task consisted of 60 trials with the start arm chosen pseudo-randomly, contained a 10 second inter-trial interval (to engage working memory), and rewarded animals with 2 (45mg) sucrose pellets upon correctly alternating choices. Rats were not rewarded if they chose the previously selected reward arm. Laser stimulation was held at 10 or 20Hz (1-4mW). Four experimental protocols consisted of 1) three blocks of 20 trials (baseline, stimulation, recovery) in which stimulation was applied during the entirety of the middle block. 2) two blocks of 30 trials (baseline, stimulation) in which stimulation was applied for 10 sec during the inter trial interval (start location condition). 3) two blocks of 30 trials (baseline, stimulation) in which stimulation was applied during the second block for 10 seconds upon reward delivery (reward location condition). 4) two blocks of 30 trials (baseline, stimulation) in which stimulation was applied during the second block upon initiation of each trial for 5 seconds (reward approach condition). For Protocol 1, preliminary analysis reveals that animals committed on average more errors during stimulation trials than baseline and recovery. Animals committed slightly more errors in recovery than baseline trials. Initial analyses reveal increased average errors during stimulation trials in Protocols 2 (start) and 4 (reward). HPC LFP analysis reveals significant power increases during stimulation trials in all conditions. The power increase was observed at the frequency of stimulation. These data show that optogenetic stimulation of mPFC caused alterations in HPC LFP at the stimulation frequency, and that task phase specific stimulation may deferentially cause deficits in behavior.

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Poster

164. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits I

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Program #/Poster #: 164.11/EEE15

Topic: H.01. Animal Cognition and Behavior

Support: NIMH grant MH109796

Title: Hippocampus and lateral habenula interact to support delayed alternation performance in rats

Authors: *Z. M. G. RIVERA[1,2], P. M. BAKER[1], S. J. Y. MIZUMORI[3]
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Abstract: The medial prefrontal cortex (mPFC) is implicated in behavioral flexibility, utilizing afferent bilateral signals from the hippocampus (HPC) to contextualize current decisions based on past outcomes. Bilateral efferent signaling from the mPFC to the lateral habenula (LHb) enables integration of response instructions with internal state information to direct voluntary behavior. Thus, LHB may indirectly interact with HPC, creating a mPFC-LHb-HPC circuit which is essential for learned tasks that require dynamic, quick decision-making. In an initial study of this circuit, a LHb-HPC disconnection study was conducted to verify that LHb and HPC work in tandem. A delayed alternation task was used. Trained rats were rewarded with 2 45-mg sucrose pellets for using an allocentric navigational strategy, requiring rats to visit the arm opposite from the preceding trial when presented with the option of visiting one of two possible reward arms. Rats were not rewarded if they returned to the arm that they had previously visited. There was a 10s delay between trials to engage working memory. The study was conducted on an automated plus-maze with two arms designated as reward arms (west and east) and two arms were designated as start arms (north and south). Rats began on the north start arm. Subsequent start arms were generated psudeorandomly after each trial. Prior to behavioral testing, male and female Long Evans rats were implanted with two sets of bilateral cannula aimed at LHb and HPC. Once rats recovered from surgery, they were pre-trained to run the task until they reached asymptotic levels (80% accuracy for three consecutive sessions). In order to disconnect function between LHb and HPC, GABA agonists (baclofen and muscimol; 50ng/0.2µL), or a saline control were administered 8 minutes prior to testing. The four inactivation administrations were: **IPSI**: ipsilateral inhibition of LHb and HPC, **CONTRA**: contralateral inhibition of LHb and HPC, **UNI LHb**: unilateral inhibition of LHb, and **UNI HPC**: unilateral inhibition of HPC. Saline infusions occurred according to the same injection pattern as baclofen/muscimol. All 8 treatment groups were randomly assigned (without replacement) to each rat. Break days were interspersed between treatment days where rats ran the task without drug or saline infusion.

There was a significant decrease in accuracy for rats in the IPSI and CONTRA conditions but not the UNI LHb or UNI HPC conditions. Impairment was not influenced by the order of infusions, and no gender differences were observed. These data show that LHb and HPC work in tandem to make appropriate decisions in dynamic environments, supporting the mPFC-LHb-HPC circuit hypothesis.

Disclosures: Z.M.G. Rivera: None. **P.M. Baker:** None. **S.J.Y. Mizumori:** None.

Poster

164. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits I

Location: SDCC Halls B-H

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Program #/Poster #: 164.12/EEE16

Topic: H.01. Animal Cognition and Behavior

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Title: The rat lateral habenula contributes to flexible responding during maze-based delayed discounting

Authors: *Y. RAO, J. CAVALLI, M. MANAVALAN, Y. JO, J. DAVIS, K. E. WRIGHT, J. J. KIM, S. J. Y. MIZUMORI
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Abstract: The lateral habenula (LHb) is thought to provide an aversive or 'anti-reward' signal in the mammalian brain under Pavlovian conditions. However, recent evidence suggests that the LHb also controls more complex decision-making during appetitive behaviors since LHb disruption impairs hippocampal (HPC)-dependent memory. This study tested whether LHb's role in appetitive behaviors is at least in part due to its integration of internal state information and limbic cortical information to enable flexible responding that reflects the most beneficial choice under changing task conditions. Since delay-discounting performance relies on an analysis of subjective values of reward (i.e. a cost-benefit interpretation) to determine choice preferences as task conditions change, the current study tested rats' performance on a maze-based delay discounting task. Male and female Long-Evans rats were trained to make choices between two options on an elevated T-Maze: a short delay (3 sec) followed by delivery of a small reward (1 sugar pellet), or longer delays (10, 20, or 40 sec) followed by a large reward (4 sugar pellets). Rats showed the typical delay discounting function that illustrates their strong preference for the large reward option after the 10 sec delay, but a significantly reduced preference for the large reward option after a 20 or 40 sec delay. Muscimol, (MUS, a GABAA receptor agonist) microinfusion into the LHb eliminated delay discounting behavior such that LHb inactivated rats showed no preference for the large reward at any delay interval (p<.01). There were no gender

differences in the LHb effect, and whether the rat received left or right arm as the large reward arm were counterbalanced across animals. Similar elimination of delay discount behavior was found after hippocampal inactivation. The employed reward magnitude discrimination task further indicated that, MUS inactivation did not impair animal's ability to distinguish between a small reward and a large reward with equal delay (3 sec). Confirming that LHb is functioning in a higher order flexible responding process, our results reveal that the LHb plays a necessary role in discounting behaviors since rats exhibited chance performance when choosing between a sooner-smaller reward and a later-larger reward regardless of the delay between choice and reward acquisition. Further these data show that the LHb is critically important in a HPC-dependent appetitive task. Although there are no known anatomical connections between LHb and HPC, these results are consistent with our hypothesis that these brain areas are functionally connected to enable animals to flexibly respond in adaptive ways.

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Poster

164. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits I

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Topic: H.01. Animal Cognition and Behavior

Support: KIST grant 2e27850
University of Science and Technology

Title: Impact of spatial and behavioral input on place coding mechanism in CA1

Authors: *F. SHARIF[1,2], J.-Y. LEE[1,2], B. TAYEBI[3], A. SARIEV[1], S. ROYER[1]
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Abstract: Parallel sub-circuits coexist within the hippocampal CA1; however, spatial or behavioral stimulus parsing them functionally and the underlying mechanisms are unclear.Here, we record CA1 and CA3 cells in mice that are running sequentially through zones of a treadmill belt enriched and impoverished in visual-tactile cues, for water rewards delivered either in a fixed or repetitively moved position. We find that local cue and self-motion information differentially encoded via sub-circuits in deep and superficial depth of the CA1. Moving the reward location increases CA3 place field precision and shifts CA1 place field generation toward superficial regions of the radial axis, which association with CA3 inputs is supported by matching spike theta phase relationships, increased low gamma oscillations, confined temporal range of spike during phase precession. In this condition, CA1 and CA3 PCs show similar spatial

selectivity properties undergoing related plasticitymechanism.Furthermore, increased participation of CA3 in CA1 place coding follow by more synchronized activity of spikes population in the second half the place field. These findings suggest that superficial CA1 is functionally associated with CA3 and gain precedence over deep CA1 when memory demand increases and landmark information is lacking. (Furthermore, place field profiles become more asymmetric in both CA1 and CA3, but in prospective versus retrospective direction for the enriched versus impoverished zone, respectively.)

Disclosures: F. Sharif: None. **J. Lee:** None. **B. Tayebi:** None. **A. Sariev:** None. **S. Royer:** None.

Poster

164. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits I

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Program #/Poster #: 164.14/EEE18

Topic: H.01. Animal Cognition and Behavior

Support: Korea Institute of Science and Technology Institutional Pogram 2E27850

Title: Local remapping responses of dentate granule and mossy cells to change in object layout

Authors: *D. JUNG[1,2], S. KIM[1], A. SARIEV[1,3], F. SHARIF[1], D. KIM[2], S. ROYER[1,3]
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Abstract: Detecting changes in the spatial configuration of objects, potentially signaling passage of predators, is critical for survival. Rodents can detect changes in object locations and make decisions based on the spatial configuration of objects, and this ability is compromised when lesions of the dentate gyrus (DG) are conducted. However, the mechanisms are unclear. Here, we use silicon probes with integrated light guides to record and photo-stimulate DG neurons in mice expressing channelrhodopsin in either granule cells (GC) or mossy cells (MC). We record GCs and MCs, identified by spike features and optogenetics, as mice run on a treadmill belt enriched with visual-tactile cues. We observe that fixing another cue on the belt induces a remodeling of spatial representations (through place field emergence, loss and rate remapping), which is maximal in the vicinity of the cue, comparatively weaker and gradual in GCs, and asymmetrically organized as firing rates in positions before/after the cue are decreased/increased in GCs and reduced differentially in MCs. We show that a network model of dentate gyrus incorporating modulation of feedback inhibition by MCs reproduces the strength, spatial spread

and asymmetric profile of GC remapping, suggesting that GCs encode changes in object layouts through local remapping, which strength and spatial extent is controlled by MCs.

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Poster

164. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits I

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Program #/Poster #: 164.15/EEE19

Topic: H.01. Animal Cognition and Behavior

Support: KIST Grant 2E27850

Title: Dentate gyrus place map genesis via competitive learning and information binding

Authors: *S. KIM[1], D. JUNG[1,2], S. ROYER[1]
[1]Ctr. for Functional Connectomics, Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of; [2]Korea Advance Inst. of Sci. and Technol., Daejeon, Korea, Republic of

Abstract: In the dentate gyrus (DG), a form of encoding suggested to take place is the binding of object and spatial information, as diverse DG lesions affect animals' ability to differentiate the location and spatial configuration of objects. It is also hypothesized that granule cells (GCs) operate as a competitive network in which strong inhibitory feedback allows only few GCs to be active at any time, via a 'winner-take-all' process, as such network property could generate the sparse activity and high spatial selectivity of GCs reported and required for DG implementation of pattern separation. Interestingly, computer modeling studies have suggested that discrete place representations could emerge naturally in GC competitive network, when the synaptic weights of inputs are recursively updated by simple hebbian learning. Although the time scale of this process is unclear, a prediction is that discrete GC place fields should progressively emerge during repeated exposure to an environment. Yet, a physiological validation is missing. Here, we record over 27 days putative GCs and mossy cells (MCs), identified by spike features and optogenetic, as mice run repetitively through a sequence of objects fixed on a treadmill belt. GCs initially encode specific object locations or periodic patterns, suggesting excitation from single object or grid cell inputs, and progressively develop discrete context-specific or multiple non-specific place fields. MCs display large firing fields and strong firing rate increases in object locations. We show that a competitive network model incorporating hebbian learning reproduces GC transformations, predicts gradual integration of object and grid cell information, and requires modulation of inhibition by MCs to control object representations. Hence, GC competitive network progressively encodes conjunctions of object and spatial information, and MCs regulate information representation.

Disclosures: S. Kim: None. **D. Jung:** None. **S. Royer:** None.

Poster

164. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits I

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Topic: H.01. Animal Cognition and Behavior

Support: Grants-in-Aid for Science Research on Innovative Areas "Brain Information Dynamics" (18H05114)

Title: Hilar mossy cells decentralize hippocampal information back into in dentate circuits

Authors: *A. OUCHI, Y. IKEGAYA
The Univ. of Tokyo, Tokyo, Japan

Abstract: Sparse activity of dentate granule cells is a hallmark feature of hippocampal function, such as distributed processing and pattern separation. This concept has been proposed based mainly on observations of sparse firing activity of dentate granule cells. Therefore, the subthreshold activity under sparse coding is poorly understood. We previously demonstrated that the subthreshold membrane potentials of hilar mossy cells reflect the activity of hippocampal sharp waves (SWs)/ripples initiated in the CA3 subregion in acute brain slice preparations. Moreover, a recent study demonstrated that simulation of mossy cells' axons increases the firing activity of dentate granule cells. We thus hypothesis that mossy cells relay CA3 SWs backward to the dentate gyrus. More specifically, information encoded by CA3 pyramidal cells is convergently registered to a subset of mossy cells and then is sparsely distributed to granule cells; note that mossy cells are less numerically dominant than pyramidal cells or granule cell. Using the whole-cell current-clamp technique, we simultaneously recorded the membrane potentials of multiple mossy cells in combination with recordings of local field potentials from the CA3 stratum pyramidale in acute slices. Information theoretical analysis revealed that the activity patterns of SWs predict the combinatorial dynamics of the membrane potentials of three hilar mossy cells. For further confirmation, we conducted *in vivo* whole-cell recordings from mossy cells together with recordings of local field potential of the CA1 subregion in urethane anesthetized mice. The recorded cells were morphologically confirmed based on *post-hoc* biocytin-labeled morphology and immunoreactivity of GluR2/3, a mossy cell marker in the dentate hilus. Our findings shed light on a novel mechanism underlying sparse coding focusing on mossy cells.

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Poster

164. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits I

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Ellison Medical Foundation New Scholar in Aging
Whitehall Foundation
Inscopix Decode award
NARSAD Independent Investigator Award

Title: Dentate granule cell recruitment of feedforward inhibition governs engram maintenance and remote memory generalization

Authors: *N. GUO[1,2,3], M. SODEN[4,5], C. HERBER[1,3], M. KIM[1,2,3], A. BESNARD[1,2,3], P. LIN[1,2,3], X. MA[6,7], C. CEPKO[6,7], L. ZWEIFEL[4,5], A. SAHAY[1,2,3,8]
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Abstract: Memories become less precise and generalize over time as memory traces re-organize in hippocampal-cortical networks. Increased time-dependent loss of memory precision characterizes overgeneralization of fear in post-traumatic stress disorder (PTSD) and age-related cognitive impairments. In the hippocampal dentate gyrus (DG), memories are thought to be encoded by so-called “engram-bearing” dentate granule cells (eDGCs). Here we show using rodents that contextual fear conditioning increases connectivity between eDGCs and inhibitory interneurons in the downstream hippocampal CA3 region. We identify actin-binding LIM protein 3 (abLIM3) as a mossy fiber terminal localized cytoskeletal factor, whose levels decrease upon learning. Downregulation of abLIM3 in DGCs was sufficient to increase connectivity with CA3 stratum lucidum interneurons (SLINs), promote parvalbumin (PV) SLIN activation, enhance feed-forward inhibition onto CA3, and maintain a fear memory engram in the dentate gyrus (DG) over time. Furthermore, abLIM3 downregulation in DGCs conferred conditioned context-specific reactivation of memory traces in hippocampal-cortical and amygdalar networks and decreased fear memory generalization at remote time points. Consistent with age-related hyperactivity of CA3, learning failed to increase DGC-SLIN connectivity in 17 month-old mice,

whereas abLIM3 downregulation was sufficient to restore DGC-SLIN connectivity, increase PV-SLIN activation and improve remote memory precision. These studies exemplify a connectivity-based strategy targeting a molecular brake of feedforward inhibition in DG-CA3 that may be harnessed to decrease time-dependent memory generalization in PTSD and improve memory precision in aging.

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Poster

164. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 164.18/EEE22

Topic: H.01. Animal Cognition and Behavior

Support: ERC Grant 694539

Title: Presynaptic inputs to hippocampal ventral mossy cells

Authors: *M. A. SILVA SIFUENTES, Y. BEN SIMON, R. SHIGEMOTO, F. FREDES
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Abstract: Hippocampal mossy cells (MCs) are the only glutamatergic neurons within the hilus of the dentate gyrus (DG). At least two different MCs subpopulations can be distinguished along the dorso-ventral hippocampal axis; MCs located in the dorsal pole of the DG (dMC) are calretinin negative and have a net inhibitory effect on dorsal granule cells (GCs), by feedforward inhibition (Hsu et al., 2016). They play an essential role in spatial pattern separation (Danielson et al., 2017; GoodSmith et al., 2017; Senzai and Buzsáki, 2017) and also control epileptic seizures (Bui et al., 2018). In contrast, MCs located in the ventral pole of the DG (vMC) are calretinin positive with a net excitatory effect on dorsal GCs and are crucial for novelty detection and contextual memory formation (Fredes et al., unpublished).
These contrasting functionalities suggest that dorsal and ventral MCs could have different synaptic connectivity. Recently, it has been shown that dMCs receive strong excitatory input from dorsal GCs and sparse input from CA3 and the medial septum (Sun et al. 2017). To investigate whether vMCs receive different inputs, we labeled the various afferent inputs onto these neurons using G-deleted, EnvA-pseudotyped rabies viral vectors. To specifically target ventral MCs for retrograde labeling, we first injected an adeno associated virus (AAV) expressing the avian receptor protein TVA together with the rabies CVS-N2c glycoprotein (N2cG), in a Cre-dependent manner, to the ventral DG of calretinin-Cre mice. Two weeks later, we injected G-deleted, CVS-N2c rabies viral vectors- a rabies strain with improved transsynaptic transfer (Reardon et al., 2016), pseudotyped with the avian sarcoma leucosis virus glycoprotein

EnvA and expressing tdTomato (CVS-N2C$_{EnvA-\Delta G}$-tdTomato).
Our results show that, in addition to input from the medial septum and the raphe nucleus, vMCs receive robust bilateral input from CA3 pyramidal neurons spanning the whole dorso-ventral hippocampal axis, with only sparse input from ventral GCs. Along with our previous findings showing that vMCs strongly excite dorsal GCs, these results suggest a novel organization of the information flow within the hippocampus, where assemblies of CA3 neurons can directly influence excitatory transmission in the dorsal DG-CA3 pathway.

Disclosures: M.A. Silva Sifuentes: None. **Y. Ben Simon:** None. **R. Shigemoto:** None. **F. Fredes:** None.

Poster

165. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 165.01/EEE23

Topic: H.01. Animal Cognition and Behavior

Support: DECRA RG150689 RE694 PSYC

Title: The effect of diets high in fat and sugar on spatial- and context-dependent memory in rats

Authors: *K. N. ABBOTT, Y. CHEN, R. F. WESTBROOK, D. P. BEGG
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Abstract: The hippocampus is critical for coding place information necessary for spatial navigation, and for coding configural representations of the physical cues comprising a context. Excessive fat and sugar consumption impairs hippocampal-dependent spatial memory in a place recognition memory task, and this deficit may be mediated by diet-induced neuroinflammation or disruption to CNS insulin signalling. We used a rodent model to confirm that exposure to a high fat and/or high sugar diet impaired place recognition memory and assess whether it also affected context fear conditioning. We additionally examined the effects of diet on neuroinflammation and activation of insulin signalling in the hippocampus. Rats were provided ad libitum access to either chow (control diet; CD), semi-purified high fat diet (HFD), chow and 10% (w/v) sucrose solution (High sugar diet, HSD), or HFD and 10% sucrose solution (High fat and sugar diet; HFSD). Spatial memory was assessed after four weeks of the diet using an object-and-place recognition memory test. At six weeks of diet, rats were assessed for their ability to form a configural representation of context in a context pre-exposure fear conditioning (CPFC) procedure. Rats were perfused after CPFC following priming of CNS insulin signalling pathways with an intraperitoneal injection of insulin (10 U/kg). This allowed for the effect of diet on activation of downstream targets of insulin signalling and neuroinflammation within the hippocampus to be assessed using immunohistochemistry. We found that rats consuming a HFD,

HSD, or HFSD had impaired place but not object recognition memory. Moreover, this deficit in place recognition memory did not extend to context fear conditioning as comparable levels of freezing behaviour were seen across groups. This result suggests that high fat and high sugar diet consumption does not influence configural processing of context cues.

Disclosures: K.N. Abbott: None. **Y. Chen:** None. **R.F. Westbrook:** None. **D.P. Begg:** None.

Poster

165. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 165.02/EEE24

Topic: H.01. Animal Cognition and Behavior

Support: SFB1089
Studienstiftung des deutschen Volkes

Title: Optogenetic stimulation of hippocampal PV+ interneurons ameliorates memory of APP/PS1 transgenic mice

Authors: *E. AMBRAD GIOVANNETTI[1], S. POLL[1], D. JUSTUS[2], H. KANEKO[2], F. FUHRMANN[2], J. STEFFEN[1], S. REMY[2], M. FURHMANN[1]
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Abstract: Disrupted neural oscillatory activity is among the earliest symptoms observed in patients affected by Alzheimer's disease (AD). In particular, a slower frequency of theta oscillations (4-12 Hz) has been detected in both patients and mouse models of the disease. As synchronous neural activity is promoted by the interneuron-mediated recruitment of cellular ensembles, aberrant oscillations reflect a defective inhibition-excitation balance between and/or within the brain areas involved in the generation of brain rhythmic activity. Interestingly, restoring the function of parvalbumin-positive (PV+) interneurons in the hippocampus of hAPPJ20 transgenic mice was beneficial for their cognitive functions.
Based on the aforementioned findings, we formulated the hypothesis that restoring theta defects in the hippocampus of APPswe/PS1dE9 (APP/PS1) mice would ameliorate the behavioral deficits displayed by this mouse model. We therefore employed a precisely timed closed feedback-loop optogenetic stimulation of hippocampal PV+ interneurons based on the intrinsic theta oscillations detected in the hippocampal local field potential (LFP), while the mice performed a novel object recognition task. Moreover, two stimulation protocols enabled us to discern between the effect of inhibition and the effect of precise theta entrainment.
Upon rhythmic optogenetic stimulation of PV+ interneurons, PV-Cre::APP/PS1 mice injected with the excitatory opsin C1V1 performed better than sham injected controls. Instead, the same

stimulation had detrimental effects for C1V1 injected PV-Cre::WT mice compared to controls. Instead, arrhythmic optogenetic stimulation was not sufficient to improve the behavior of APP/PS1 transgenic mice. Hence, we propose that the beneficial effect of the stimulation was rather dependent on the interneuron-promoted improvement of temporal coding, rather than on the increase in inhibition that results from PV+ interneuron stimulation.

Disclosures: E. Ambrad Giovannetti: None. **S. Poll:** None. **D. Justus:** None. **H. Kaneko:** None. **F. Fuhrmann:** None. **J. Steffen:** None. **S. Remy:** None. **M. Furhmann:** None.

Poster

165. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

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Topic: H.01. Animal Cognition and Behavior

Support: The ETH Domain for the Blue Brain Project (BBP)
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he BlueBrain IV BlueGene/Q system is financed by ETH Board Funding to the Blue Brain Project as a National Research Infrastructure and hosted at the Swiss National Supercomputing Center (CSCS)

Title: Reconstruction and simulation of a full-scale model of rat hippocampus ca1

Authors: *A. ROMANI[1], N. ANTILLE[2], J.-D. COURCOL[2], A. DEVRESSE[2], A. ECKER[2], J. FALCK[3], C. P. H. FAVREAU[2], M. GEVAERT[2], A. I. GULYAS[4], R. MIGLIORE[5], M. PEZZOLI[6], O. HAGENS[6], J. V. HERNANDO[2], L. KANARI[7], J. G. KING[2], S. LANGE[3], C. A. LUPASCU[5], S. RAMASWAMY[2], A. POVOLOTSKY[2], M. W. REIMANN[2], C. A. RÖSSERT[2], S. SÁRAY[4], Y. SHI[2], W. A. H. VAN GEIT[2], T. F. FREUND[4], S. KALI[4], H. MARKRAM[2], M. MIGLIORE[5], A. M. THOMSON[3], E. B. MULLER[2]
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Abstract: We present a full-scale cellular level model of the CA1 area of the hippocampus of a rat. The model is built using a bottom-up, data-driven workflow, along the same lines followed

to implement a cortical column (Markram et al., 2015). Starting from a set of reconstructed morphologies, a set of electrophysiological traces, and data-driven channel kinetics, we implemented many biophysically accurate neuron models for pyramidal cells and interneurons able to reproduce the experimental traces. The circuit was then built using connectivity rules and synaptic properties validated against a number of experimental findings. The current release is composed of 42 morphologically and biophysically accurate neurons (24 excitatory and 18 inhibitory) divided into 13 morphological types and 17 morpho-electrical types, 156 potential pathways, and 7 intrinsic synapse types. Simulations of the network show interesting emergent properties, such as theta oscillations in a LFP-like signal. The oscillations emerge from the intrinsic connectivity of the CA1 circuit driven by the spontaneous miniature events without any external input, as observed experimentally (Goutagny et al 2009). Furthermore, the network activity propagates along the septo-temporal axis, consistent with what has been observed experimentally (Lubenov and Siapas, 2009). Phenomena like oscillations and traveling waves in the theta rhythm range can play important roles in shaping the hippocampal function, but their mechanisms are not completely understood. The full-scale CA1 model represents an important tool to shed light on those phenomena, understand in which physiological conditions they can occur, their origins, their maintenance, and eventually their role in the brain. References: Goutagny R, Jackson J, Williams S. Nat Neurosci. 2009 Dec;12(12):1491-3. Lubenov EV, Siapas AG. Nature. 2009 May 28;459(7246):534-9. Markram H, Muller E, Ramaswamy S, Reimann MW⋯ DeFelipe J, Hill SL, Segev I, Schürmann F. Cell. 2015 Oct 8;163(2):456-92.

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Poster

165. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 165.04/EEE26

Topic: H.01. Animal Cognition and Behavior

Support: FIRB 2013
PRIN 2015

Title: An increase in NCX1 expression and/or activity induces anxiety and ameliorates hippocampal-dependent spatial learning and memory

Authors: S. NATALE[1], *P. MOLINARO[2], S. ANZILOTTI[4], T. PETROZIELLO[1], R. CICCONE[1], A. SERANI[1], L. CALABRESE[1], F. FRECENTESE[3], A. SECONDO[1], A. PANNACCIONE[1], V. SANTAGADA[3], G. CALIENDO[3], L. D'ESPOSITO[2], G. DI RENZO[1], L. ANNUNZIATO[1]
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Abstract: Three different Na^+/Ca^{2+} exchanger (NCX) isoforms, NCX1, NCX2, NCX3 are expressed in CNS, where they participate in the maintenance of Na^+ and Ca^{2+} homeostasis. Among these three isoforms, NCX1 is highly expressed in hippocampus, cortex and amygdala, three brain regions involved in long-term spatial memory and social behavior. Recent results showed that the overexpression, or the downregulation, of NCX1 is accompanied with an increase or a decrease of Akt1 phosphorylation, respectively, in cortical and hippocampal neurons of genetic modified mice1. Since Akt1 is part of the PTEN/Akt1/CREB signaling pathway that regulates synaptic plasticity and long-term memory, we hypothesized that NCX1 expression, or activity, might affect some hippocampal-dependent learning and memory tasks. To this aim, we used two genetic modified mice: (1) one mouse strain overexpressing (*ncx1.4*^{over}), and (2) another one downregulating selectively (*ncx1*^{neuko}) NCX1 in neurons of hippocampus, cortex and amygdala. In addition, we also designed and synthesized a new drug, CN-PYB2, that selectively increases NCX1 activity. These genetic modified mice and wild-type mice treated with CN-PYB2 were studied in several hippocampal-amygdala-dependent spatial learning and memory tasks. Results showed that an increase of NCX1 expression/activity augmented, whereas NCX1 downregulation decreased, the active forms of CREB and Calmoduline Kinase II in the hippocampus. In addition, CREB was also upregulated, or downregulated, in both cortex and amygdala subregions of *ncx1.4*^{over} or *ncx1*^{neuko} mice, respectively. More interesting, mice overexpressing NCX1 in neurons of hippocampus, cortex and amygdala and mice treated with CN-PYB showed an increase of anxiety levels, long-term spatial learning and memory performance, as measured by open field, novel object recognition test, zero maze, dark/light box, Barnes maze, trace fear conditioning. Accordingly, mice knock-out for NCX1 in neurons of the same brain regions showed a decrease in long-term spatial learning and memory performance.Altogether, these results demonstrate that NCX1 participates in the hippocampal-amygdala memory consolidation and anxiety behavior.
1. Molinaro P, Sirabella R, Pignataro G, Petrozziello T, Secondo A, Boscia F, Vinciguerra A, Cuomo O, Philipson KD, De Felice M, Di Lauro R, Di Renzo G, Annunziato L. J Cereb Blood Flow Metab. 2016 ;36:1790-1803.

Disclosures: S. Natale: None. **P. Molinaro:** None. **S. Anzilotti:** None. **T. Petroziello:** None. **R. Ciccone:** None. **A. Serani:** None. **L. Calabrese:** None. **F. Frecentese:** None. **A. Secondo:** None. **A. Pannaccione:** None. **V. Santagada:** None. **G. Caliendo:** None. **L. D'Esposito:** None. **G. Di Renzo:** None. **L. Annunziato:** None.

Poster

165. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 165.05/FFF1

Topic: H.01. Animal Cognition and Behavior

Support: FAPESP 2016 18039-9

Title: A proteomics approach of effects of gingko biloba treatment in the dorsal hippocampal formation

Authors: R. B. GAIARDO, T. F. ABREU, A. K. TASHIMA, M. M. TELLES, *S. M. CERUTTI
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Abstract: Recent studies from our laboratory have shown that CREB-1 upregulation in the dorsal rat hippocampal formation (dHF) is essential to acquisition of lick suppression. Additionally, we have recently demonstrated that acute and long-term treatments with standardized extract of the *Ginkgo biloba* (EGb) modulated the acquisition of fear memory in a dose dependent manner, which was associated with CREB-1 upregulation in the dHF. To better understand the molecular changes subjacent to these processes, we employed a proteomic analysis on dHF samples of adult male Wistar rats (n=6/group) treated with a single dose of the vehicle (control) or EGb (0.25, 0.50 or 1.00 g.kg-1) 30 minutes before conditioning. The procedure comprised of 5-days of pre-conditioning sessions (baseline), a conditioning session on day 6 (tone-shock pairing - 4x), a reshaping session on day 7 (rebaseline) and a test session on day 8. During test session, all rats were subjected to ten tone (CS) presentation and suppression ratio (SR) of licking response was calculated for each trial as percentage of the time required to complete ten licks before and during tone. The animals were decapitated 24 hours after completion of the test session and dHF samples were removed and resolved in a 2D-SDS-PAGE followed by identification in an ESI-q-TOF mass spectrometer. Data regarding the SR means on first trial (SR1) demonstrated that all groups experienced the acquisition of conditioned suppression [EGb 0.25 g.kg-1 (SR_1=0.77), 0.50 g.kg-1 (SR_1=0.78) and 1.0 g.kg-1 (SR_1=0.82) and vehicle (SR_1=0.89)]. The 2DE gels of dHF showed 351±6 spots in all groups. Thirty-two spots showed significant optical density changes between control and EGb groups. The comparison between the EGb-treated groups indicated that the 0.50 $g.kg^{-1}$ dose had a higher number of altered proteins in relation to other doses. The pathways analysis showed that EGb altered proteins involved on composition of structures found in the membrane of somatodendritic and axonal compartments, with cellular morphogenesis, size and form of dendritic spines, myelin sheath formation, proteasome system and ribosomal small subunit assembly. Our results suggest that differential protein expression in dHF sustain the modulatory effects of EGb on the

acquisition of conditioned lick suppression through. In addition, the present data support the EGb neuroprotective effects previously described by our group.

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Poster

165. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 165.06/FFF2

Topic: H.01. Animal Cognition and Behavior

Support: SFB779

Title: Functional coherence between proximal CA3, distal CA1 and proximal CA1 during non-spatial memory processes

Authors: *S.-P. KU[1,2], E. ATUCHA[2], P. VAVRA[3], K. KAEFER[4], M. SAUVAGE[2,3]
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Abstract: Electrophysiological and *Arc* imaging studies have recently reported a proximodistal functional segregation of CA1 and CA3 (Henriksen et al, Neuron, 2011; Nakamura et al, J. Neurosc., 2013; Flaschbeck et al, 2018). Based on these findings and the fact that proximal CA3 preferentially projects to distal CA1 and distal CA3 to proximal CA1, we have suggested the existence of hippocampal subnetworks that preferentially process either spatial or nonspatial information: the distal CA3- proximal CA1 and the proximal CA3-distal CA1 subnetworks, respectively. Here, we further investigate the cross-area functional interactions within these subnetworks by first investigating non-spatial information processing in the proximal CA3-distal CA1 subnetwork. We simultaneously record spiking activities and local field potentials in distal CA1 and proximal CA3 (the non-spatial network) and proximal CA1 as a control in rats performing a delayed non-matched to odor task (Nakamura et al, J. Neurosc., 2013) using *in-vivo* electrophysiology technique. We especially evaluate whether neural populations in proximal CA3 and distal CA1 work coordinately during the encoding and the retrieval of memories by comparing spike-field or field-field coherences between proximal CA1, distal CA1 and proximal CA3. These results will contribute to further characterization of proximodistal differences in CA1 and might provide the first evidence of a coordinated proximal CA3- distal CA1 subnetwork, thus giving further support to the existence of ‘spatial’ and ‘non-spatial’ subnetworks across CA1 and CA3 segregated along the proximodistal axis of the hippocampus.

Disclosures: **S. Ku:** A. Employment/Salary (full or part-time):; Leibniz Institute for Neurobiology. **E. Atucha:** A. Employment/Salary (full or part-time):; Leibniz Institute for Neurobiology. **P. Vavra:** A. Employment/Salary (full or part-time):; Otto von Guericke University, Medical Faculty. **K. Kaefer:** A. Employment/Salary (full or part-time):; Institute of Science and Technology Austria. **M. Sauvage:** A. Employment/Salary (full or part-time):; Leibniz-Institute for Neurobiology.

Poster

165. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 165.07/FFF3

Topic: H.01. Animal Cognition and Behavior

Support: SFB 874 Project B12

Title: Median raphe projections specifically alter hippocampal information processing

Authors: *J. BAUMANN, O. A. MASSECK
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Abstract: The hippocampus is the main structure where cognitive representations of our environment are formed and stored. Importantly, the ability and effectiveness to store and recall these representations is regulated by the affective state of the system, such as arousal, attention, mood or saliency. The affective state of the system regulates how this storage and forming works. The serotonergic system has been closely associated with the regulation of the affective state and is known to influence memory acquisition within the hippocampus. We hypothesize that serotonergic neurotransmission is able to change global excitability patterns of the hippocampus. In a first series of experiment we figured out the direct impact of serotonin on memory acquisition, retrieval and consolidation in object recognition. The injection of a retrograd tracer (Fluorogold) in the CA1 region showed that the hippocampus receives its main serotonergic input from the *median raphe nucleus* confirming results described in the literature. As the main input from the hippocampus arises from the MRN a double floxed Channelrhodopsin 2 (ChR2) was injected in the MRN of ePet-Cre mice. Subsequently, the opsin is exclusively expressed in serotonergic neurons and in axon terminals targeting the hippocampus. A control group received an injection with a fluorphore only. Bilateral implantations of optical fibres above the hippocampus enabled the precise release of serotonin during behavioural tests. We performed an object recognition task and modulated serotonin release either during training, consolidation or retrieval. Throughout the first two days mice were habituated to the arena and the optical fibre for 10 min. On the third day, two identical objects were presented. Mice were allowed to explore them free for 10 minutes. One hour later, mice were again placed into the arena but now a novel object replaced one of the identical objects.

Mice are again allowed to explore them for 3 minutes. Serotonin release during training impaired object recognition. Whereas serotonin release during consolidation and retrieval seems to have no effect on object recognition. Our results imply that serotonin plays a key role in information processing within the hippocampus.

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Poster

165. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 165.08/FFF4

Topic: H.01. Animal Cognition and Behavior

Title: The hippocampus of birds in a view of evolutionary connectomics

Authors: *C. HEROLD[1], D. GRÄßEL[2], P. SCHLÖMER[2], I. MAFOPPA-FOMAT[2], J. MEHLHORN[3], K. AMUNTS[2,4], M. AXER[2]
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Abstract: Understanding the routes of brain evolution in the context of cognition seems to be a challenging task for comparative neuroscientists given the fact that there are still many gaps to fill with a limited amount of species living in the 21st century. Nevertheless, in the past 50 decades' researchers have presented data reporting that birds, cephalopods and even other taxa without a "six-layered" neocortex, show complex and intelligent behavior and far more other skills comparable to or even exceeding those of some mammals. Together all of these cognitive functions and skills involve a functional cortico-basal-ganglionic circuit, which seemed to be highly conserved between species as shown for example for the song system of birds and speech production in humans. These findings however, have increased comparative studies and ongoing discussions dealing with the evolution of the "six-layered" neocortex. In contrast, debates about hippocampal evolution are relatively tame, although hippocampal (archicortical) structures evolved sometime in between the paleo- and the neocortex, and a lot of cognitive functions involve hippocampal circuits. The hippocampal formation in birds can be divided into several subdivisions, however, there is an ongoing discussion, which subdivisions of the hippocampal formation in birds correspond to their mammalian counterparts, i.e. the dentate gyrus, the cornu ammonis fields, the subiculum and the entorhinal cortex. New theories of the evolution of the dentate gyrus as a late evolving structure and exclusive add to the hippocampus of mammals have further questioned whether a dentate gyrus as strict as in mammals even exists in birds or not. Yet, further insights of functionally different domains in the hippocampal formation of

mammals provide another clue in the evolution of hippocampal circuits. Therefore, we analyzed two different components of hippocampal functional circuity, adult neurogenesis and long-range connections along the rostro-caudal axis of the hippocampal formation in pigeons (*Columba livia*). For this purpose, we used immunohistochemical analysis of different neurogenic markers, and the recently developed method of polarized light imaging. Polarized light imaging has shown to be a powerful tool to analyze the overall fiber architecture, and the course of fibers and fiber tracts in mammals at the microscale level. Our approach reveals differences in DCX+ and BrdU+/NeuN+ cells as well as differences in connectivity of the several subdivisions of the hippocampal formation of pigeons along the rostra-caudal axis, thereby providing further insights into hippocampal evolution.

Disclosures: C. Herold: None. **D. Gräßel:** None. **P. Schlömer:** None. **I. Mafoppa-Fomat:** None. **J. Mehlhorn:** None. **K. Amunts:** None. **M. Axer:** None.

Poster

165. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 165.09/FFF5

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Title: Distinct subpopulations of somatostatin interneurons in dorsolateral septum relay fear signals to govern activation of subcortical circuits

Authors: *A. BESNARD[1], Y. GAO[2], M. TAEWOO KIM[1], T. LANGBERG[1], W. FENG[1], X. XU[3], D. SAUR[4], I. G. DAVISON[2], A. SAHAY[1]
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Abstract: Adaptive fear responses to ambiguous environmental threats rely upon hippocampal contextual encoding and subsequent relay of these computations to limbic circuits mediating behavioral fear responses. We combined longitudinal *in vivo* calcium imaging, retrograde mono-synaptic tracing, electrophysiological whole-cell recordings and *in vivo* optogenetic tools to uncover a non-canonical dentate gyrus (DG)-CA3-Dorsolateral septal (DLS) circuit that targets

somatostatin-expressing interneurons (SST-INs) to calibrate fear responses. We demonstrated that SST-INs are functionally heterogeneous, receive direct CA3 inputs and recruit diverse subcortical targets to decrease expression of fear. Together, these observations uncover previously unrecognized functional heterogeneity in subcortical SST-INs and suggest a role for distinct DLS SST-INs subpopulations in sensing and relaying computations from higher cortical areas to govern context specific activation of fear circuits.

Disclosures: A. Besnard: None. **Y. Gao:** None. **M. TaeWoo Kim:** None. **T. Langberg:** None. **W. Feng:** None. **X. Xu:** None. **D. Saur:** None. **I.G. Davison:** None. **A. Sahay:** None.

Poster

165. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 165.10/FFF6

Topic: H.01. Animal Cognition and Behavior

Title: Reasons why Alzheimer's disease is “diabetes of the brain”

Authors: *A. S. SHINGO[1], T. KANABAYASHI[2], S. KITO[3], T. MURASE[4]
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Abstract: The object of this study is showing evidence that the intraventricularly streptozotocin injected rat (brain diabetes rat) is a definite Alzheimer disease model. Behavioural, immunohistochemical studies were performed together with dendritic spine analysis of the hippocampal granular cells. As methods, 1) Spatial cognitive function tests were performed on intraventricularly streptozotocin injected “brain diabetes” rats by the Morris water maze (n=7). Controls were injected with PBS (n=10). 2) Quantitative immunohistochemical examinations of amyloid β protein, insulin-degrading enzyme, somatostatin, AKT, p-CREB were performed using the rat hippocampal tissue (brain diabetes: n=7, control: n=10). 3) Dendrites of granular cells of the hippocampal dentate gyrus were quantitatively evaluated. 4) A single injection of detemir, a long-acting insulin analogue, into the third ventricle of the rat was done (brain diabetes: N=3, n=1, control: N=3, n=15). The results were summarised as follows. The spatial cognitive function of rats with “brain diabetes” has been impaired. Immunohistochemistry of the hippocampus revealed an increase in amyloid β protein and decreases in all other tested values. Spine densities of the granular cells were significantly decreased. By intraventricular single injection of the insulin analogue, all the tested values approached to those of control rats. From the results above, we concluded that the “brain diabetes” rat is a definite model of Alzheimer disease.

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Poster

165. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits II

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Ville de Paris Programme Émergences (RAP)
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Title: Examination of how hypothalamic supramammillary activity alters area CA2 output to modulate area CA1

Authors: *V. ROBERT[1], L. THERREAU[1], R. BOEHRINGER[2], A. HUANG[2], T. J. MCHUGH[2], V. CHEVALEYRE[1], R. A. PISKOROWSKI[1]
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Abstract: Hippocampal theta and gamma oscillations are critical for learning and memory. While activity in area CA3 and CA1 during these brain states has been extensively studied, area CA2 has rarely been examined. Recently, in vivo studies have revealed a role for area CA2 in sharp-wave / ripple generation and spatial coding. Thus, area CA2 actively contributes to hippocampal network function, and the underlying local circuitry and synaptic properties of area CA2 need to be examined. Of note, Area CA2 receives theta-locked long-range input from the hypothalamic supramammillary nucleus (SuM) and sends strong excitatory output to area CA1. However, the effect of SuM input on area CA2 activity and the consequences on CA1 remain unexplored. To address this, we used optogenetics to stimulate SuM axons projecting to area CA2 and performed patch-clamp recordings of CA2 and CA1 pyramidal neurons (PNs) on acute hippocampal slices following application of the cholinergic agonist carbachol (CCh). We found that SuM excitation drives direct excitation and feedforward inhibition onto CA2 PNs. Upon application of 10 µM CCh, CA2 PN fired action potentials (AP) in bursts, the properties of which could be controlled by SuM input. When examining the consequences of SuM activity on CA2 output transmission in area CA1, we observed an inhibitory post-synaptic current (IPSC) in CA1 PNs occurring approximately 15 ms after SuM light-stimulation. The delay of this IPSC matches a reduction of CA1 PN AP firing seen in vivo and indicates that it results from the recruitment of several synapses. At a longer time scale, we observed a reduction of action potential firing in CA1 stratum pyramidale (SP) that lasted several seconds following light-stimulation of SuM inputs. Whole-cell recordings of CA1 PNs revealed that this reduction of field activity was paralleled by a hyperpolarization of CA1 PN membrane potential (VM) that prevented action potential firing in a seconds-long time window after SuM axon

photostimulation. Altogether, our data demonstrate that the SuM inhibitory drive received by CA2 PNs controls the timing of their burst firing, resulting in a lasting silencing of CA1 PNs during CCh-induced network activity. These results indicate that the hippocampal output from area CA1 is controlled by SuM input through the modulation of area CA2 activity.

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Poster

165. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 165.12/FFF8

Topic: H.01. Animal Cognition and Behavior

Support: NIBIB 5R01EB018297

Title: Neuromodulation of spike timing-dependent plasticity controls memory stability - a local excitation attractor model

Authors: *M. R. ZOCHOWSKI[1], J. P. ROACH[2], N. OGNJANOVSKI[4], S. J. ATON[3], L. SANDER[1]
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Abstract: Sleep-wake cycles are associated with dynamic changes in neuromodulatory release; amongst these are acetylcholine (ACh) and norepinephrine (NE). NE is released in its highest level during wakefulness and is low in both REM and NREM sleep. At the same time, the highest level of ACh is seen during periods wakefulness; during NREM sleep ACh is at its lowest; in REM ACh is at a moderate level. These neurotransmitters significantly affect the quality of synaptic plasticity by gating either the LTP (NE) or LTD (ACh) components of spike timing-dependent plasticity (STDP). Due to this modulation there are likely to be large changes in the shape of the STDP envelope across the sleep-wake cycle. During wakefulness STDP is biased toward LTP, in NREM it is still biases to LTP but both magnitudes are reduced, and in REM it is balanced or biased to LTD. Here we use a biophysical model to investigate how neuromodulation of STDP, as well as the effects of ACh on neural excitability, impact the formation, strengthening, and stability of memories stored in a bump attractor network. In these networks spiking activity is highly localized in space, forming a bump of firing which can be pinned to a location by external input or through enhanced recurrent excitation. We take the location of the bump to be representative of information and therefor STDP should bias the bump to stay a location to successfully store a memory. When the network is under conditions associated with wakefulness the bump location is stabilized, while during REM conditions the

bump location is destabilized. We show that shape of the STDP envelope is responsible for this by controlling in a reciprocally connected cell pair, whether the connecting synapses strengthen toward a cell with a higher frequency of firing (the case of wakefulness) or the lower frequency cell (the case of REM). During the NREM state (Low ACh, Low NE) bump location is inherently unstable due to the effects of low acetylcholine inducing increased spike-frequency adaptation (SFA) and firing dynamics become a traveling wave. In this case STDP strengthens synapses in the direction of the traveling wave, allowing previously encoded memories to connected. When we take these results as a whole, we show that the neuromodulatory environment of wakefulness is optimized for encoding new memories and strengthening existing one. On the other hand, during sleep REM and NREM work together to consolidate multiple memories into larger ensembles by cyclically destabilizing and connecting stored locations into reliable sequences of activation. This work provides a mechanistic insight into how modulation of neuronal processes contributes to sleep-dependent memory consolidation.

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Poster

165. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits II

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Program #/Poster #: 165.13/FFF9

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant 5R01AG048172-03
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Title: Hippocampal Homer1b/c is necessary for contextual fear conditioning and for group 1 metabotropic glutamate receptor mediated long-term depression

Authors: *K. GIMSE, A. OLIN, S. OSTING, C. BURGER
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Abstract: Previous research in our lab and others have indicated a role for coiled-coil forms of Homer1, including Homer1b and c (H1b/c), in hippocampal learning, memory and synaptic plasticity. H1b/c are important mediators of group 1 metabotropic glutamate receptor (mGluR1/5) signaling. This signaling mediates long-term depression (mGluR1/5-LTD) and potentiation, shown to underlie learning and memory in the hippocampus. To determine if H1b/c is necessary for successful learning and memory and mGluR1/5-LTD, a small hairpin RNA targeting H1b/c (rAAV-shH1b/c) was used to knockdown H1b/c expression in the hippocampi of adult (4-6 month-old) male Sprague-Dawley rats. Sham injected and animals injected with a non-targeting shRNA expressing rAAV vector (rAAV-shCTL) were used as controls. We carried

these initial studies in young male rats based on our previous studies overexpressing Homer1c in *Homer1* knockout mice that showed no differences in behavioral or cellular phenotype when comparing males to females. In this study, behavioral analyses including the Morris Water Maze, contextual (CFC), auditory cued fear conditioning (ACFC), and novel object recognition tasks were used to assess learning and memory ability. Electrophysiological field recordings were used to measure mGluR1/5-LTD. Prior to the investigation, a statistical power analysis was performed for sample size estimation. The projected sample size needed was calculated with G*Power 3.1 software as n=7 for each experimental group. Based on these numbers, behavioral analyses were performed on a total of 17 rAAV- shH1b/c injected, 9 sham injected and 8 rAAV-shCTL injected animals. Additional sham injected animals (total of n=21) were added to facilitate investigation of mGluR-LTD mechanism. We found that a 60% reduction in hippocampal H1b/c results in deficits in CFC (n= t-test, p=.025), but not in other hippocampal dependent learning tasks or ACFC. Additionally, knockdown of H1b/c completely ablates expression of mGluR1/5-LTD (shH1b/c: n= 7 slices (4 animals); control: n= 5(4); p= .004). In conclusion, the results of this study indicate that Homer1b/c is necessary for contextual fear conditioning and mGluR1/5-LTD.

Disclosures: K. Gimse: None. **A. Olin:** None. **S. Osting:** None. **C. Burger:** None.

Poster

165. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits II

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Support: Natural Sciences and Engineering Research Council
Heart and Stroke Foundation
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Title: Investigating the effects of selective pacemaker channel (I_h) blocker ZD7288 in epileptogenesis and adenosine A1 receptor mediated hippocampal neuronal damage

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Abstract: Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels or pacemaker channels conduct the I_h current in the brain. Several important studies found that pharmacological inhibition of I_h or genetic deletion of HCN1 channels increases excitability in neocortical and hippocampal pyramidal neurons. Previously, the Cayabyab lab showed that

prolonged adenosine A1 receptor (A1R) stimulation induced persistent hippocampal synaptic depression and neurodegeneration. We hypothesize that the inhibition of HCN1 channels with ZD7288 in presence of A1R agonist N^6-Cyclopentyladenosine (CPA) serves a neuroprotective role by prolonging the latency to pilocarpine-induced status epilepticus (SE) and enhancing synaptic function. Male Sprague-Dawley (SD) rats (200-250g) 18-20 days postnatal, were given intraperitoneal (IP) injections of 5 mg/kg of the A1R selective agonist CPA or DMSO/Saline, as well as intranasal (IN) administration of 1 mg/kg of the selective HCN1 blocker ZD7288. The perfused brain tissue was cut at 40 μm and was probed with A1R and HCN1 antibodies or Fluoro-Jade B (FJB). FJB staining showed that IP CPA + IN DMSO increased neurodegeneration in the hippocampus, which was blunted by IN ZD7288 co-treatment with IP CPA. These changes in neuronal health were associated with reduced A1R and increased HCN1 expression (IP CPA + IN DMSO) or increased A1R and decreased HCN1 expression (IP CPA + IN ZD7288). Results obtained from FJB and Immunohistochemistry suggest that inhibition of HCN1 or I_h current with ZD7288 serves a neuroprotective function against the neuronal damage caused by chronic A1R stimulation. The rats that received IP CPA + IN DMSO spent less time in the novel arm during the Y maze test, which examines hippocampal-dependent spatial memory. Whereas, the rats that received IP DMSO + IN ZD7288 spend the majority of their time in the novel arm. Lastly, we found that IP DMSO + IN ZD7288 administration decreases the latency to pilocarpine-induced SE as opposed to prolonging it. Since adenosine elevation occurs after stroke and post-stroke seizures are observed in some stroke survivors, these results suggest a role for A1R-mediated changes in HCN channels associated with epileptogenesis.

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Poster

165. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits II

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Title: Characterization of “non-significant” sharp wave ripple associated events

Authors: *J. CHU, E. ACKERMANN, C. KEMERE
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Abstract: The place cell activity of hippocampal pyramidal cells during exploration has been described as the cognitive map substrate of spatial memory. Replay- time-compressed reactivation of place cell sequences-is sometimes observed during hippocampal sharp wave ripples and is critical for consolidation and recall-guided behaviors. However, only a small fraction of the sharp wave ripple associated events are consistent with observed animal behavior, the so-called "significant" replay events, leading to the question of what the rest of those events might correspond to. These "non-significant" events have been variously hypothesized to represent remote experiences, interrupted (failed) replay events, extra-spatial content, neural noise, or some combination of these. To investigate the likely composition, we carefully characterized all sharp wave ripple associated events from animals while they were exploring two environments for liquid reward (Karlsson, Carr and Frank, CRCNS hc-5 2015). More specifically, we used a recently developed hidden Markov model (HMM) based approach that is able to learn and identify repeated patterns of neural activity independent of animal behavior. Using this approach, we were able to identify local and remote replay events (which have been previously reported), but in addition, we were able to find some model-congruent extra-spatial sequences that are impossible to detect with a traditional Bayesian decoding approach. Furthermore, through simulation, we try to answer the important questions of how many sequence prototypes are needed for us to learn a consistent representation, as well as how many simultaneous representations we can recover with our approach. Together, these insights can help us to re-interpret some of our experimental data, to better untangle the composition and understand the potential role of sharp wave ripples in the formation of hippocampus-dependent memories.

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Poster

165. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 165.16/FFF12

Topic: H.01. Animal Cognition and Behavior

Support: NIH #R01NS101108

Title: Recordings in awake behaving rodents show disruptions in hippocampal laminar field structure and CA1 spike-field interactions post-TBI

Authors: *K. GAGNON[1], C. D. ADAM[2], C. COTTONE[3], P. KOCH[4], J. ALAMAR[3], R. J. RUSSO[5], J. A. WOLF[2,6]
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Abstract: Hippocampal-dependent memory dysfunction is a major sequela of TBI, yet the network level mechanisms of memory impairment are poorly understood. Previous work using the fluid percussion injury (FPI) model have reported reduced theta power and a loss of total power across frequency bands in the hippocampus, as well as learning and memory deficits in spatial memory tasks. Long Evans rats injured by FPI at a moderate severity (1.6-1.8 ATM) were able to remember a pre-trained pattern of rewarded arms in the radial arm maze. However, sham injured rats were able to learn a shift in reward locations, whereas injured rats were unable to adapt to the new spatial relationship. Learning and memory impairment following TBI may be due in part to communication disruptions within the hippocampus via altered oscillations and disorganized unit firing. To examine these potential neuronal mechanisms, rats were chronically implanted in the hippocampus with 64 channel double-shank silicon probes on a high resolution microdrive following injury, which allowed for high quality recordings of single units and simultaneous laminar field potentials across CA1. Wireless recordings were performed while the animal was exploring both a familiar (open field) and a novel (radial arm maze) environment to examine the effects of injury on place cells and their formation. Preliminary results show place cells in the familiar environment were maintained in injured animals. Surprisingly, new place cells were formed in the novel environment over multiple recording days post-injury. In addition, specific laminar dependent changes in power were observed, with a substantial loss of theta power in stratum oriens, a loss of theta power in the stratum radiatum, and a decrease in ripple band power (120-250 Hz) in the pyramidal layer. However, CA1 firing rates remain unchanged compared to sham, suggesting disorganized timing of neuronal activity may lead to the observed ripple band power loss. Spike-field interactions also demonstrated a shift in the entrainment phase angle to theta in injured animals. Loss of theta and ripple power, as well as the disordered timing of CA1 unit firing, may disrupt hippocampal encoding resulting in learning and memory deficits post injury.

Disclosures: K. Gagnon: None. **C.D. Adam:** None. **C. Cottone:** None. **P. Koch:** None. **J. Alamar:** None. **R.J. Russo:** None. **J.A. Wolf:** None.

Poster

165. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits II

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Topic: H.01. Animal Cognition and Behavior

Support: NIMH 5T32MH015174

Title: Physiological role of hyperpolarization-activated, cyclic nucleotide-gated channels in parvalbumin-positive basket cell terminals

Authors: *E. W. BUSS, F. LEROY, B. SANTORO, S. A. SIEGELBAUM
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Abstract: Hyperpolarization-activated, cyclic nucleotide-gated (HCN) channels generate the cationic Ih current in neurons and regulate the excitability of neuronal networks. The function of HCN channels depends, in part, on their subcellular localization. HCN1 is abundantly expressed in the dendrites of pyramidal neurons in hippocampal area CA1 where they act as an inhibitory constraint on dendritic integration and synaptic plasticity. HCN1 is also strongly expressed in presynaptic terminals of parvalbumin-positive (PV+) inhibitory neuron basket cells (PVBC), which provide strong inhibitory control over hippocampal activity. Yet, less is known about the specific role of HCN1 channels in these cells and the extent to which they regulate evoked GABA release from basket cell terminals. Here, we investigate how the electrophysiological properties of PV+ basket cells are regulated by HCN1, including how HCN1 activity at presynaptic terminals regulates the release of the inhibitory transmitter GABA onto pyramidal neurons (PN) in the CA1 region of the hippocampus. To selectively express channelrhodopsin 2 (ChR2-YFP) in PV+ interneurons, we crossed PV-Cre mice with a Cre-dependent ChR2-YFP line enabling us to produce large light-evoked IPSCs in CA1 pyramidal neurons under voltage clamp. Bath application of the HCN blockers ZD7288 and ivabradine produced a striking inhibition of the IPSC over a wide input-output range. We also found that ZD7288 inhibits electrically-evoked IPSCs in CA1 PNs in the presence of glutamate blockers to examine direct inhibition. Overall, our results suggest that Ih and HCN1 powerfully enhance evoked GABA release from PVBCs forming synapses on hippocampal CA1 pyramidal neurons.

Disclosures: F. Leroy: None. **B. Santoro:** None. **S.A. Siegelbaum:** None.

Poster

165. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits II

Location: SDCC Halls B-H

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Program #/Poster #: 165.18/FFF14

Topic: H.01. Animal Cognition and Behavior

Title: Investigating GABAergic neurons of the lateral septum using calcium imaging in freely behaving mice

Authors: *S. VAN DER VELDT, G. ETTER, B. RIVARD, F. MANSEAU, S. WILLIAMS
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Abstract: The lateral septum (LS) is the main subcortical target of the hippocampal formation, receiving strong projections from the pyramidal neurons of CA3, CA1 and subiculum neurons. A large body of evidence has underlined the role of the hippocampus in constructing a detailed internal spatial representation of the environment. However, there is little evidence on how this

hippocampal spatial map is processed in downstream regions such as the LS for goal directed navigation. The LS consists of a large heterogenous population GABAergic cells. Spatially tuned cells have been previously reported in the LS (Zhou et al., 1999; Leutgeb and Mizumori, 2002; Takamura et al., 2006), yet how their firing characteristics relate to hippocampal inputs remains unknown. Using cutting edge viral tracing techniques and calcium imaging with miniaturized fluorescence microscopy, we characterized the spatial firing properties of cells in the LS based upon their anatomical connections and hypothesized functional relationship with the hippocampal formation and medial septum. For this, we recorded activity of pathway defined GABAergic LS subpopulations using both open field and goal directed navigation tasks. We have identified spatially tuned cells and followed their activity pattern over the course of multiple days.

Disclosures: S. Van Der Veldt: None. **G. Etter:** None. **B. Rivard:** None. **F. Manseau:** None. **S. Williams:** None.

Poster

165. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits II

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Program #/Poster #: 165.19/FFF15

Topic: H.01. Animal Cognition and Behavior

Support: NSF BRAIN 1533598

Title: The effect of teleportation in virtual reality on the hippocampal place code

Authors: *E. R. REDINGTON, S. SOLTANIAN-ZADEH, A. SILBERSTEIN, M. ZHANG, H. ZHAO, S. FARSIU, Y. GONG
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Abstract: As an animal performs spatial tasks a subset of pyramidal neurons in CA1 of the hippocampus act as places cells. Activity of place cells depends upon the integration of a combination of internal and external cues. The prevailing hypothesis for how external and internal cues drive place cell activity states that internal cues help perform path integration, while external cues like the visual scene help correct for errors in path integration that accumulate as a function of time (McNaughton et al., 2006). Testing the separate roles of internal and external cues requires that movement is uncoupled from its external consequences, a difficult task to accomplish in a physical setting. Fortunately, virtual reality (VR) provides a unique opportunity to perform experiments with spatial tasks that are impossible in the physical world. Previous experiments making use of VR tested how half speed traversals of a virtual reality setting effect the hippocampal place code (Chen et al., 2012). During half speed runs roughly half of place cells make use of visual cues to correct for the errors induced in path integration by half speed

runs. How vision corrects for these errors is still a question that is unanswered in the field. Given the likely connections between regions of the brain related to optic flow and the hippocampus (Wylie et al., 1999; Gasbarri et al., 1994) we hypothesize that the hippocampus makes use of optic flow to correct for motion related errors in path integration. To test this hypothesis, we designed a behavioral paradigm that uncouples movement from the expected sequence of changes in the visual scene. We accomplished this uncoupling by teleporting male c57BL/6 mice without warning on randomly determined laps of a VR track. As mice traversed the VR track, we optically recorded calcium activity from CA1 of the hippocampus using GCaMP6f. We found that unexpected teleportation alters short-term the stability of the hippocampal place code. From this we conclude that the hippocampus integrates visual information to correct for errors in path integration.

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Poster

165. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits II

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Program #/Poster #: 165.20/FFF16

Topic: H.01. Animal Cognition and Behavior

Support: 5TL4GM118977
2R15NS060117-02

Title: Effects of brassicaceae and asteraceae plants on high-fat diet induced deficits in spatial learning and memory impairment in the Morris water maze on pre-diabetic C57BL/6 mice

Authors: *S. SHATELA, T. SIMON, D. HICKS, B. TENG, L. R. BANNER
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Abstract: Statistical data from the CDC exhibits a dramatic rise in obesity rates in U.S. adults over the past few decades. One of the reasons for this gradual increase in obesity rates is due to a persistent consumption of high fat diets. Diets high in fat lead to obesity, greatly increase the risk for developing type-2 diabetes, and influence changes to the central nervous system. Obesity also promotes a pro-inflammatory response in an individual, our main focus being the neuroinflammation aspect of it. When neuroinflammation occurs, pro-inflammatory cytokines are being upregulated in the central nervous system, leading to problems like disruptions in neuronal signaling and impairments in cognitive memory. Neuroinflammation also paves the way for neurodegenerative and neurological diseases, such as dementia. We propose that the supplementation of kale, arugula (f. Brassicaceae), and dandelion (f. Asteraceae) plants in an obese, C57BL/6 mouse model diet will ameliorate the inflammatory response and have an

overall impact on learning and memory. Mice were fed either a control low fat diet, high sugar, or high fat diet (60%) for 18 weeks followed by greens supplementation for an additional 22 weeks. To assess possible outcomes, we perform the Morris Water Maze on these mice to determine their spatial and related forms of learning and memory. The mice must learn to use distal cues to navigate a direct path to the hidden platform when started from different, random locations around the perimeter of the tank. We use the tracking software ImageJ to examine the movements of the mice and analyze different variables, such as time. These experiments will provide evidence for the role of diet in cognition. Results demonstrate that compared to animals fed a control diet, the mice fed the high fat diet had the slowest average time in reaching the platform, with the high sugar diet group following. Looking at the high fat diet group, mice that were fed the arugula and dandelion found the platform sooner when compared to the control group, indicating that the incorporation of these greens improved overall performance.

Disclosures: **S. Shatela:** Other; NIH BUILD PODER Undergraduate Research Program. **T. Simon:** None. **D. Hicks:** None. **B. Teng:** None. **L.R. Banner:** None.

Poster

165. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 165.21/FFF17

Topic: H.01. Animal Cognition and Behavior

Support: HFSP
Cain Foundation
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Title: Characterization of a novel head-fixed paradigm for navigation in virtual acoustic space

Authors: ***S. GAO**[1], A. BANTA[1], Z. H. MRIDHA[2], W. ZHANG[2], C. KEMERE[1], M. J. MCGINLEY[2]
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Abstract: Auditory cues play an important role in navigation for animals especially when the visual system cannot detect objects or prey in the dark. Decades of work in many labs have led to a precise understanding of how acoustic spatial stimuli are encoded in the auditory brainstem and represented in cortex in stationary animals. However, little is known about how acoustic spatial stimuli are processed and used by the brain to aid in navigation. In particular, we know very little about how the auditory system and the hippocampus interact to use spatially-modulated auditory stimuli for motivated navigation. Spatial representations in the rodent hippocampus are thought to underlie memory-guided navigation. Recent advances have come from the use of head-fixed mice navigating in virtual space, primarily defined by visual stimuli. Investigators have created

structured sensory stimuli that create a virtual reality in which rodents can navigate while head-fixed. In these experiments, animals navigate by moving on a ball or wheel, with the structure of the environment being presented with visual stimuli. In this study, we present a new experimental paradigm where sensory stimuli associated with space are auditory rather than visual. After mice were implanted with stable head posts, we examined the walking and licking behavior of mice as they walk on a virtual track while fixed on a cylindrical treadmill in a well-controlled acoustic environment. When mice were rewarded in a zone defined by auditory stimuli, we find that after several sessions mice reduces their velocity and express anticipatory licking in an acoustically defined zone preceding the location where reward is delivered. Consequently, we can conclude that memory-guided navigation is possible with exclusively auditory stimuli. This behavioral approach can serve as a platform for the study of hippocampal-cortical interaction and spatial hearing during virtual navigation.

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Poster

165. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits II

Location: SDCC Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Support: NIH R01 MH082930
UCI Faculty Research & Travel Award
James L. McGaugh Chair in the Neurobiology of Learning & Memory

Title: Opening the black box: Probing the role of neural gene expression and hippocampal circuit activity in neuroinflammation-induced memory retrieval impairment

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Abstract: It is thought that neuroinflammation is a common factor and primary driver of cognitive impairments associated with neurological disorders and brain injuries with diverse etiologies. The “black box” between neuroinflammation and cognitive impairments is understanding how proinflammatory mediators modulate the functions of neurons and neural circuits. This understanding is imperative in order to develop better clinical therapies for such disorders. We have demonstrated previously that acute peripheral immune stimulation elevates expression of proinflammatory cytokines in brain, alters hippocampal pattern separation, and impairs context discrimination memory retrieval. Here, we test the hypothesis that neuroimmune

activation within the hippocampus alters the expression of neural-synaptic genes, leading to dysregulation of circuit function and memory retrieval impairment. To manipulate the neuroimmune response of subjects, we used lipopolysaccharide (LPS) as an immune challenge in conjunction with the drug minocycline, which inhibits activation of microglia to a proinflammatory state. First, we trained adult male rats in a 2-environment context discrimination conditioning task. After reaching a predetermined performance criterion, each subject received peripheral injections of minocycline (50 mg/kg, i.p.) or saline, followed by LPS (150 µg/kg, i.p.) or saline 6 h before retrieval testing. After testing, brains were harvested for circuit activity analysis (using the immediate-early gene method "catFISH") and for RNA analysis (using a custom NanoString assay for selected neuroimmune and neural-synaptic genes). As predicted, systemic minocycline injection attenuated the negative impacts of immune challenge on measures of both CA1 hippocampal circuit activity and context discrimination memory. For subsequent analyses, we generated a neuroimmune score from the transcriptomic data for each subject. This neuroimmune score correlated strongly with expression of neural-synaptic genes associated with glutamatergic neurotransmission and plasticity, and these neural-synaptic genes correlated with memory function. In addition, local minocycline infusion within dorsal hippocampus (2 µg) prevented neuroinflammation-induced impairment in context discrimination. Together, these findings indicate that the neuroinflammatory state of the hippocampus drives changes in neural-synaptic gene expression regulating context memory retrieval. Ongoing analyses are focused on further defining and modeling the relationship of neural-synaptic gene expression to CA1 circuit activity, and CA1 circuit activity to behavior.

Disclosures: J.F. Guzowski: None. **T.E. White:** None. **J. Czerniawski:** None.

Poster

165. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits II

Location: SDCC Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Support: NIH T32 Sleep and Genetics

Title: Single prolonged stress leads to deficient retention of fear extinction and corresponding changes in hippocampal function

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Abstract: Posttraumatic stress disorder (PTSD), which presents with hypervigilance, avoidance of trauma-related contexts and memories, and intrusive re-experiencing symptoms such as

flashbacks, occurs in a subset of individuals after exposure to serious trauma. PTSD is understood as an increase in fear reactivity likely involving abnormal fear generalization and deficient retention of fear extinction. These cognitive processes involve overlapping regions of neurocircuitry composed of the amygdala, the medial prefrontal cortex (mPFC), and the hippocampus. The hippocampus is responsible for contextual processing, which is necessary for fear extinction by means of projections to the mPFC. Previously, our laboratory has developed the single prolonged stress (SPS) rodent model of PTSD, composed of three stressors in series (restraint, a forced swim, and exposure to ether) followed by a 7-day quiescent period. SPS and control rats were tested for fear conditioning (FC), fear extinction (FE), and fear extinction retention, respectively. Contextual cues, including specific scents, light conditions, and ambient sounds, were provided in order to differentiate FC (Context A) from FE and the extinction retention test (both Context B). Control and SPS rats responded similarly to fear conditioning and fear extinction in these experiments, but SPS rats exhibited a deficit in fear extinction retention. To confirm the hypothesis that differential hippocampal-mPFC function underlies this deficit, we injected SPS and control rats with radioactively-labeled 2-deoxyglucose (C14-2DG) prior to the test of fear extinction retention. In neurons, C14-2DG is taken up in proportion to the cells' metabolic demands, and quantitative measurement of regional activity in the brain can be assessed by autoradiographic analysis of brain sections. As expected, the SPS group had comparative fear conditioning and extinction but a significant deficit in fear extinction retention. Normalized C14-2DG uptake was unchanged across all areas tested except the hippocampus where a significant increase was found in CA3 activity as measured by autoradiography. Our results provide evidence that abnormal hippocampal activity is associated with the deficiency in retention of fear extinction in our animal model of PTSD. Since contextual information is provided by the hippocampus, we propose that patients with PTSD are unable to adequately incorporate safety cues into pre-existing schemata, leading to the disorder's characteristic symptoms. Understanding the neurobiological mechanisms of these symptoms will facilitate the development of improved treatments and therapies for PTSD.

Disclosures: J.J. Winters: 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.; Sunovion. **E. Rodriguez:** None. **I. Liberzon:** 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.; Sunovion.

Poster

165. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits II

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 165.24/FFF20

Topic: H.01. Animal Cognition and Behavior

Support: University of Wisconsin-Milwaukee Research Growth Initiative

Title: Ventral hippocampal neuronal excitability and immediate early gene expression following trace fear learning

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Abstract: Associative learning triggers neuronal plasticity in brain regions that are critical for storing and retrieving memory. Trace fear conditioning is an associative learning paradigm that induces plasticity in several brain regions, including the amygdala, prefrontal and retrosplenial cortices, and the dorsal hippocampus. One region whose role in trace fear learning and memory remains elusive is the ventral hippocampus (VH). Due to its proximity to and connections with other brain regions involved in the fear circuit, VH is well-suited to support trace fear learning. Indeed, several inactivation and lesion studies suggest that VH has a major role in this learning task. Trace fear learning is disrupted following excitotoxic lesions of VH (Yoon & Otto, 2007), or when VH is inactivated using the $GABA_A$ agonist muscimol (Czerniawski et al., 2009). VH may also be involved in maintaining long-term trace fear memory, as trace fear expression is impaired when VH is inactivated up to 42 days after training (Cox et al., 2013). These studies suggest there could be a definitive role for VH in supporting associative trace fear learning. However, there is currently very little understanding of how trace fear learning can alter VH plasticity. By examining immediate early gene (IEG) expression using Western blots, as well as intrinsic excitability using whole-cell patch-clamp electrophysiology, our lab is exploring how trace fear learning affects VH plasticity. Adult male F344 rats (3 mo.) were randomly assigned to one of four behavioral groups: 1) home-cage (NAIVE), 2) pseudo-conditioning (PSEUDO), 3) trace fear conditioning (TRACE), or 4) context fear conditioning (CTXT). Animals underwent a single training session on day 1, followed by a brief behavioral test on day 2. *In vitro* patch-clamp recordings of brain slices from TRACE animals suggest trace fear learning increases spiking activity of VH CA1 neurons. Western blot analysis also suggests Arc expression is elevated in VH of CTXT animals. The extent to which neuronal IEG expression co-varies with changes in the excitability of those neurons to represent circuit-specific (e.g., context vs trace memory) is currently unknown. These findings suggest that neuronal plasticity within VH is altered following acquisition and/or retrieval of trace and context fear conditioning.

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Poster

166. Animal Cognition and Behavior: Learning and Memory: Dentate Gyrus and Neurogenesis

Location: SDCC Halls B-H

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Program #/Poster #: 166.01/FFF21

Topic: H.01. Animal Cognition and Behavior

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HDRF RGA-13-003

Title: The role of ventral dentate gyrus mossy cells in anxiety-related behavior

Authors: *J. BERRY[1], G. F. TURI[2,3], N. MANFRED[1], M. OGBU[1], S. YUSUFOV[1], R. HEN[2,3]
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Abstract: The ability to recognize dangerous situations and environments is crucial for survival, but overvaluing risk can lead to pathological avoidance of normal activities, potentially leading to anxiety disorders. Many studies over the past several decades have begun to identify the brain regions underlying threat detection and anxiety behavior. In particular, the ventral hippocampus has emerged as a critical structure for emotional behaviors, including innate anxiety and fear learning. Recent work from our lab and others has shown that neurons in ventral CA1 encode information about anxiety, and these neurons preferentially target downstream structures such as hypothalamus and medial prefrontal cortex. The mechanism by which this anxiety representation arises in the ventral hippocampus is still unknown, but it is likely that interneurons play a critical role in shaping this activity. Here, we use chemogenetics and freely-moving calcium imaging to test whether excitatory interneurons in the ventral dentate gyrus called mossy cells are preferentially active during exploration of anxiogenic contexts and are necessary for anxiety-related behavior. We find that mossy cells are highly active when exploring novel and anxiogenic environments, and that chemogenetic inhibition disrupts fear learning. These results suggest mossy cells may play a critical role in the dentate gyrus circuit, and could be important for anxiety-related and mnemonic behaviors.

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Poster

166. Animal Cognition and Behavior: Learning and Memory: Dentate Gyrus and Neurogenesis

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 166.02/FFF22

Topic: H.01. Animal Cognition and Behavior

Support: NIH MH-109305
NSERC- PDF
NIH MH-110165
New York State Office of Mental Health

Title: Excitation and inhibition of granule cells by mossy cells of the adult mouse dentate gyrus

Authors: *H. E. SCHARFMAN[1,2], H. L. BERNSTEIN[2], Y.-L. LU[1], J. J. BOTTERILL[1]
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Abstract: It has been debated whether mossy cells (MCs) of the dentate gyrus (DG) excite or inhibit granule cells (GCs). We addressed the question using two different transgenic mouse lines with specificity for MCs: dopamine D2 receptor-Cre and calcitonin receptor-like-receptor-Cre mice. Adult males and female transgenic mice received a stereotaxic injection of Cre-dependent adenoassociated virus encoding channelrhodopsin-2 tagged with yellow fluorescent protein either in dorsal, mid or ventral left DG. Hippocampal slices (350-400 µm-thick) were prepared >5 days later and recordings were made in either an interface chamber (field potentials or sharp electrode recordings of GCs) or submerged chamber (whole-cell recordings of GCs). Brief (<5 msec) light pulses to the terminal zone of the MCs (the inner molecular layer; IML) led to field EPSPs (fEPSPs) at that location in almost all slices tested (n=3-5 slices/mouse, 20 mice), suggesting that there is common and widespread excitation of GCs by MCs. Differences related to the site of the injection were not detected, suggesting a similarity of dorsal and ventral MCs. Effects were also similar ipsilateral and contralateral to the viral injection site, suggesting similarity in the projection of MCs to GCs. Interestingly, fEPSPs were small and population spikes were hard to detect compared to the perforant path input. Individual GCs showed variable responses to light pulses but predominantly EPSPs, which did not reach threshold when the GC was at its normal resting potential. However, depolarization of the GC often led to action potential generation. EPSPs in GCs began within 2 msec of the start of the light pulse and therefore were likely to be monosynaptic (direct MC-to-GC excitation). IPSPs began within 4 msec of the start of the light pulse and therefore were likely to be disynaptic (indirect MC-to-interneuron-to-GC inhibition). Inhibitory effects were generally strong, which was demonstrated by triggering a pre-pulse of light to the IML before a perforant path electrical stimulus. The pre-

pulse inhibited the population spike elicited by perforant path stimulation but did not inhibit the underlying subthreshold potential, consistent with a role of perisomatic-targeting interneurons. The data collectively suggest that, under normal conditions, MCs excite GCs weakly and they strongly inhibit GC firing.

Disclosures: H.E. Scharfman: None. **H.L. Bernstein:** None. **Y. Lu:** None. **J.J. Botterill:** None.

Poster

166. Animal Cognition and Behavior: Learning and Memory: Dentate Gyrus and Neurogenesis

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 166.03/FFF23

Topic: H.01. Animal Cognition and Behavior

Support: NIH R21 DC016746-01A1

Title: Dentate granule cell representation is modulated by reinforcement learning

Authors: *J. SHEN[1], S. GE[1], Q. XIONG[2]
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Abstract: The dentate gyrus (DG) is believed to serve as a codon layer in the pattern separation neural network, where the output neuronal activity is less similar than the input. However, whether the DG is separating contextual information, or general sensory inputs that is required for episodic memory is largely unclear. To address this question, we designed an auditory discrimination-reinforcement learning task in a three-armed maze. In this context, the spatial input stays the same, however, the association between the location and auditory cue evolved during the learning process. Using *in vivo* Ca^{2+} imaging of the dentate granule cell (DGC) in rats, we found that the firing pattern of the DGCs was modulated by the learning. In general, the spatial coding of the DGC became more precise after the animal learned the auditory discrimination task. The place fields of DGCs were fairly diffuse in the beginning of reinforcement training. Following learning, their place fields shifted (i.e. remapping) and/or converged to become relatively precise. Surprisingly, a subpopulation of DGCs exhibited stable spatial representation within the maze across time. Our results indicate that the DG may have at least two distinct functions: one population of DGCs is in charge of associating spatial information with other sensory inputs to form episodic memory, while another population represents the cognition map that is related to physical location (i.e. similar to CA1 place cells).

Disclosures: J. Shen: None. **S. Ge:** None. **Q. Xiong:** None.

Poster

166. Animal Cognition and Behavior: Learning and Memory: Dentate Gyrus and Neurogenesis

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NIH/NCI grant U54CA132378/ U54CA137788 (KSR)
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Title: The effect of exercise on cognitive function in gonadectomized male rats

Authors: *C. VASQUEZ[1], A. ELZANIE[2], M. AZIZ[2], T. IRVING[2], A. COLE[2], U. AKPARA[2], T. ADEBOWALE[2], K. Y. SALAS-RAMIREZ[3]
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Abstract: Prostate cancer, one of the most common cancers in the United States, is a condition in which there is uncontrollable growth of cells from the prostate glands. Because testosterone sustains proliferation of prostate glandular cells, androgen ablation therapy is one way to control this mechanism. Though various interventions are available for patients with early stage prostate cancer, patients with advanced prostate cancer are recommended to use systemic androgen ablation therapy. This intervention, though effective in decreasing prostate gland cell proliferation, has been proven to impair cognitive function in men. Studies support that exercise is an effective intervention for cognitive decline after androgen ablation therapy as seen by improved working, visual and spatial memory, as well as increased in BDNF in the hippocampus. The objective of this study was to determine whether hippocampal neurogenesis is the mechanism by which these behaviors are fully restored after an exercise intervention. Adult male rats were divided in four groups (1) intact exercise, (2) intact no exercise, (3) gonadectomized (gdx) exercise and (4) gdx no exercise. The exercise group was exposed to twenty minutes of forced exercise on a rat treadmill for five days/week for a total of six weeks. On the last day of the exercise intervention, males were injected with bromodeoxyuridine (BrdU; 300 mg/kg; ip), a marker for cell birth, for three consecutive days. Half of the animals were sacrificed immediately after the exercise training and the other half were sacrificed 30 days after the last BrdU injection. Brains were harvested and stained for BrdU-immunoreactivity (BrdU-ir). Neurolucida software was used to trace the subdivisions of the hippocampus: CA1, CA2, CA3 and dentate gyrus (DG). A one-way ANOVA revealed that intact males exposed to exercise had increased BrdU-ir cells in the hippocampus ($p < 0.05$), particularly in the DGs. We hypothesize

that gonadectomized males who exercised will exhibit a significantly higher number of BrdU-ir cells compared to those who did not exercise. This finding would support that increased hippocampal neurogenesis is a mechanism by which cognitive function can be restored after androgen ablation therapy. Further studies will assess whether these changes are specific to cell proliferation or cell survival, in addition to a specific kind of cells in the brain.

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Poster

166. Animal Cognition and Behavior: Learning and Memory: Dentate Gyrus and Neurogenesis

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 166.05/GGG1

Topic: H.01. Animal Cognition and Behavior

Title: Memory modification by beta-adrenergic receptor activation via intracellular Zn^{2+} signaling in the amygdala

Authors: *H. TAMANO, M. KUBOTA, R. SHIMAYA, R. ITOH, M. SUZUKI, A. TAKEDA
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Abstract: Amygdala modulates hippocampus-dependent memory such as object recognition memory. Our previous study demonstrated that intracellular Zn^{2+} signaling in the hippocampal dentate gyrus is required for object recognition memory via long term-potentiation (LTP) induction at perforant path-dentate granule cell synapses. On the basis of the findings, we examined the possibility that intracellular Zn^{2+} signaling in the amygdala is involved in formation of object recognition memory. Rats were injected with ZnAF-2DA (0.1 mM, 2 μl), an intracellular Zn^{2+} chelator, into the basolateral amygdala 20 min before the training of object recognition task. One hour after the training, object recognition memory was impaired in ZnAF-2DA-injected rats, suggesting intracellular Zn^{2+} signaling in the amygdala is required for object recognition memory. In agreement with the results, in vivo dentate gyrus LTP was attenuated by injection of ZnAF-2DA into the basolateral amygdala 20 min before LTP induction, suggesting that intracellular Zn^{2+} signaling in the amygdala is required for object recognition memory via dentate gyrus LTP induction. On the basis of the findings that noradrenergic activation of the basolateral amygdala enhances object recognition memory, furthermore, we tested whether intracellular Zn^{2+} signaling in the amygdala is involved in modulation of hippocampus-dependent memory formation via adrenergic receptor activation in the amygdala. Injection of phenylephrine (0.5 mM, 2 μl), a α_1-adrenergic receptor agonist, into the basolateral amygdala did not influence in vivo dentate gyrus LTP induction. In contrast, injection of isoproterenol (0.5 mM, 2 μl), a β-adrenergic receptor agonist enhanced dentate gyrus LTP induction. The

enhancement was blocked by co-injection of ZnAF-2DA, suggesting that intracellular Zn^{2+} signaling is required for activation of β-adrenergic receptors in the amygdala, resulting in enhancement of dentate gyrus LTP. On the other hand, dentate gyrus LTP was attenuated by injection of human $A\beta_{1-42}$(25 μM, 2 μl)into the dentate gyrus 1 h before LTP induction. Interestingly, $A\beta_{1-42}$-induced attenuation of dentate gyrus LTP was rescued by activation of β-adrenergic receptors in the amygdala in the same manner. The present study suggests that hippocampus-dependent memory is modulated by activation of β-adrenergic receptors via intracellular Zn^{2+} signaling in the amygdala. It is likely that β-adrenergic receptor activation in the basolateral amygdala is a strategy for rescuing $A\beta_{1-42}$-induced cognitive decline that is associated with hippocampal synaptic plasticity.

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Poster

166. Animal Cognition and Behavior: Learning and Memory: Dentate Gyrus and Neurogenesis

Location: SDCC Halls B-H

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Argentine Research Council PIP0597
DFG Center for Nanoscale Microscopy and Molecular Physiology of the Brain (CNMPB)

Title: A hippocampal age-related eleven gene signature conserved across the species

Authors: *J. PARDO[1], M. C. ABBA[2], E. LACUNZA[2], L. FRANCELLE[3], G. R. MOREL[4], T. F. OUTEIRO[5], R. G. GOYA[4]
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Abstract: Even though some descriptive studies on transcriptomics have been conducted on the rodent senile hippocampus, most of them were on males. Here, we studied age changes in learning and memory, hippocampal morphology and transcriptome in female rats.
In the Barnes Maze Task, senile rats displayed significant deficits in the learning parameters latency (two-way RM ANOVA age factor $P=0.044$) and errors (two-way RM ANOVA age factor $P<0.001$). In the probe trials, both groups showed a clear preference for exploring the

escape hole, but the explorations of the young rats in this hole was significantly higher (Student's t-test P=0.015 and Mann–Whitney test P<0.001 for the first and second probe trials, respectively). In the hippocampus, we observed a dramatic reduction in the neurogenesis rate, as assessed by doublecortin positive neuroblast count in the Dentate Gyrus (Student's t-test P<0.001). On the contrary, we found a significantly increased number of reactive microglial cells in the senile hippocampal Stratum Radiatum region (ANOVA group factor P=0.04, LSD test 0.03).
Our hippocampal RNA-Seq data showed that several genes involved in the immune response are overexpressed in the hippocampus of senile rats, for example TYROBP, CD11b, C3, CD18, CD4, and CD74. By means of enrichment analysis we found that the pathways overrepresented in the senile rats matched those of an exacerbated inflammatory environment, which reinforced our morphologic findings.
When comparing our results with public data of human and mouse hippocampal gene expression, we found an 11-gene signature of overexpressed genes related to inflammatory processes conserved across species, which we hypothesize may be involved in a common biological process. Furthermore, when analyzing other brain transcriptome public data, we found that this signature is overexpressed in Alzheimer´s Disease, Huntington´s Disease and some low-grade brain tumours. We conclude that age-related hippocampal deficits in female rats share commonalities between human and rodents. Our study further supports neuroinflammation as a promising target to treat cognitive dysfunction in old individuals
and some brain tumors. In this regard, our identified signature will be further characterized in future studies

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Poster

166. Animal Cognition and Behavior: Learning and Memory: Dentate Gyrus and Neurogenesis

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Program #/Poster #: 166.07/GGG3

Topic: H.01. Animal Cognition and Behavior

Title: New-born-neuron-mediated social experiences regulate retrieval of contextual fear memory

Authors: *B. LEI, B. KANG, H. CHEN, Y. ZHONG
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Abstract: In contrary to current view of a consolidated memory with static amplitude, our analysis revealed that retrieval of hippocampus-dependent contextual fear memory is perturbed

bi-directionally by social experiences that incur immediately before recall. Such social experiences-mediated bidirectional regulation of memory retrieval is abolished with suppression of hippocampal neurogenesis through local X-RAY irradiation and pharmacologicaltreatments. Conversely, increased hippocampal neurogenesis led to significantly strengthened effects in retrieval. Involvement of neurogenesis was linked to new-born neurons (NBNs) in the hippocampus for excluding NBNs from engram cells, through chemogenetical inhibition of their activity during contextual fear conditioning, could block social experiences-dependent regulation of memory retrieval. Our data suggest that even consolidated memory is plastic for its retrieval is subject to bidirectional regulation by experiences immediately before recall and such regulation is mediated through activity of NBNs engram cells.

Disclosures: B. Lei: None. **B. Kang:** None. **H. Chen:** None. **Y. Zhong:** None.

Poster

166. Animal Cognition and Behavior: Learning and Memory: Dentate Gyrus and Neurogenesis

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 166.08/GGG4

Topic: H.01. Animal Cognition and Behavior

Title: Sex-specific effect of maternal exercise on promoting adult hippocampal neurogenesis, improving learning and memory and depression-like behaviour in adult offspring

Authors: *H. LEE, C. LI, S. YAU
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Abstract: Clinical studies showed that maternal exercise in pregnancy is beneficial to brain development and connectivity of new-born babies and cognitive functions in later-life. Using mouse model, we sought to explore if voluntary wheel running during pregnancy improves depression-like behavior, learning and memory, and adult hippocampal neurogenesis in both female and male offspring. Pregnant mice were allowed to run voluntarily by introducing running wheels into the housing cages throughout the gestational period. Eight- to nine-week-old male and female offspring were then subjected to object recognition task and Porsolt swim test, then were sacrificed for immunohistochemistry to examine changes of adult hippocampal neurogenesis. Our results demonstrated that maternal exercise reduced depression-like behavior in despair state in both male and female pups, whereas only male offspring demonstrated improvement in object recognition in temporal order task. Cell quantification revealed that maternal exercise promotes neuronal differentiation in the ventral hippocampus in male offspring and cell proliferation in the dorsal hippocampus in female offspring. Nevertheless, maternal exercise did not affect the levels of brain-derived neurotrophic factor (BDNF) in the hippocampus in both male and female offspring. Collectively, these illustrated that maternal

exercise benefits both female and male offspring in association with enhanced hippocampal adult neurogenesis, but independent of changes in hippocampal BDNF levels.

Disclosures: H. Lee: None. **C. Li:** None. **S. Yau:** None.

Poster

166. Animal Cognition and Behavior: Learning and Memory: Dentate Gyrus and Neurogenesis

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 166.09/GGG5

Topic: H.01. Animal Cognition and Behavior

Support: ERC 692692
FWF Z312-B27

Title: Parvalbumin-expressing interneurons permit pattern separation in the dentate gyrus by lateral inhibition and structured connectivity

Authors: C. ESPINOZA[1], S. J. GUZMAN[2], *J. L. CSICSVARI[1], P. JONAS[1]
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Abstract: A hallmark of the dentate gyrus is its ability to convert overlapping input patterns into non-overlapping output patterns; a phenomenon referred to as pattern separation. A winners-take-all process, in which Parvalbumin-expressing (PV^+) interneurons separate patterns by lateral inhibition, is an attractive network mechanism for such a computation. However, the functional connectivity rules between PV^+ interneurons and granule cells (GCs) remain unknown. To address this question, we performed simultaneous in vitro patch-clamp recordings from up to seven GCs and up to four PV^+ interneurons in the dentate gyrus. In total, we tested 8279 possible connections. We found 107 excitatory GC-PV^+ interneuron connections (out of 1285 tested connections), 318 inhibitory PV^+ interneuron-GC connections (out of 1285), 45 inhibitory PV^+-PV^+ interneuron connections (out of 154), and 33 gap junction PV^+-PV^+ interneuron connections (out of 77). Excitatory connections between GCs were never detected (0 out of 5539 tested connections). We found that different connectivity rules apply for excitatory and inhibitory principal neuron-interneuron connections. At the macroscopic level, both types of synapses showed high focal connectivity. For excitatory GC-PV^+ interneuron synapses, the connection probability was 12% at the maximum and declined to the half-maximal value (length constant) at a distance of 141 µm, whereas for inhibitory PV^+ interneuron-GC synapses the maximal connection probability was 28%, and the length constant was 223 µm. The length constant for excitation was significantly shorter than for inhibition ($P < 0.0001$). Overall, lateral inhibition (in which a GC disynaptically inhibits a neighboring GC) was 9.2 times more abundant than

recurrent inhibition (in which a GC disynaptically inhibits itself). At the microscopic level, we found that out of 25 possible disynaptic motifs, convergence, divergence, and mutual inhibition motifs were significantly overrepresented in comparison to random networks. To study the mechanisms of pattern separation, we developed network models, which incorporated either random connectivity or experimentally determined connectivity rules. Whereas a network model with random connectivity was a poor pattern separator, a full-size model with a high abundance of lateral inhibition and distance-dependent connectivity efficiently decorrelated overlapping patterns. In addition, networks containing microscopic connectivity motifs performed better than random models in pattern separation. Thus, PV^+ interneuron-containing microcircuits of the dentate gyrus appear to be optimized for pattern separation.

Disclosures: C. Espinoza: None. **S.J. Guzman:** None. **J.L. Csicsvari:** None. **P. Jonas:** None.

Poster

166. Animal Cognition and Behavior: Learning and Memory: Dentate Gyrus and Neurogenesis

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 166.10/GGG6

Topic: H.01. Animal Cognition and Behavior

Support: ERC 692692

Title: Sparse coding in identified dentate gyrus granule cells in head-fixed running mice

Authors: *X. ZHANG, A. SCHLOEGL, P. JONAS
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Abstract: Sparse activity is a hallmark feature of the dentate gyrus granule cells (GCs) and promotes pattern separation. Data acquired by tetrode recording and calcium imaging showed that only a minority of granule cells was activated at a very low frequency when animals explored the open field environment (Senzai and Buzsaki, 2017, Neuron 93: 691; Danielson et al, 2017, Neuron 93: 552; GoodSmith et al, 2017, Neuron 93: 677). However, the mechanism underlying sparse activity of the dentate granule cells remains unknown. To uncover this mechanism, we performed whole-cell patch-clamp measurements from morphologically identified GCs, together with local field potential (LFP) recordings in head-fixed mice running on a linear belt (Bittner et al, 2015, Nat Neurosci 18: 1133; Pernía-Andrade and Jonas, 2014, Neuron 81:140). GCs were identified and reconstructed based on post-hoc biocytin labeling. We found that the activity of GCs varied over a wide range: 58% (23 out of 40) of cells were silent during the 10-30 min recording period, while 42% (17 out of 40) were sparsely active, generating either single spike (4 out of 40), bursts (8 out of 40), or superbursts of action potentials (APs) (5 out of 40). Interestingly, active GCs branched further away from the soma

with a slightly longer total dendritic length at order 4-10, whereas silent GCs branched mostly close to soma with significantly longer total dendritic length at order 1-3 (n = 9/12, p = 0.21, 0.03, Wilcoxon rank-sum test). Besides this, active GCs had notably lower input resistance (n = 18/18, p = 0.03). Those results suggest that the activity of GCs may correlate with both structural and functional maturity. Moreover, active GCs were intrinsically more excitable than silent GCs, indicated by significantly higher AP amplitude, larger maximal dV/dt of AP rising phase, and shorter AP half-width (n = 18/18, p = 0.02, 0.01, 0.03, respectively). All GCs showed abundant excitatory postsynaptic potential (EPSP) activity that enhanced during running periods and was phase-locked to the peak of theta-gamma oscillations. In a subpopulation of GCs, subthreshold EPSPs displayed spatial preference. In conclusion, our results suggest that the activity in identified GCs is sparse but heterogeneous. Sparse activity might be associated with more complex dendritic trees and higher intrinsic excitability. The subthreshold EPSPs are embedded in a precise temporal and spatial structure during running periods**.**

Disclosures: X. Zhang: None. **A. Schloegl:** None. **P. Jonas:** None.

Poster

166. Animal Cognition and Behavior: Learning and Memory: Dentate Gyrus and Neurogenesis

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 166.11/GGG7

Topic: H.01. Animal Cognition and Behavior

Title: Relationships between home range size and markers of cellular turnover in the dentate gyrus of wild male meadow voles (microtus pennsylvanicus)

Authors: *M. D. SPRITZER, M. R. SINKS, D. E. MORRISON, R. A. RAMDEV, S. LENTZOU
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Abstract: There is growing evidence that a combination of neurogenesis and cell death within the dentate gyrus region of the hippocampus is necessary for spatial learning and memory among rodents. To date, the study of neural plasticity within the hippocampus has relied heavily on experiments involving captive rodents, but to fully understand how new neurons function, field experiments are needed in which animals are allowed to express their full behavioral repertoire under natural conditions. We examined multiple markers of cellular turnover (cell proliferation, neurogenesis, and cell death) within the dentate gyrus of free-ranging male meadow voles. Home range size has been previously shown to correlate positively with spatial ability in male meadow voles, so we predicted that range size would also correlate with markers of cellular turnover. Wild adult male voles (n = 19) were captured using live traps in a field (Middlebury, Vermont, U.S.A.) and fitted with radio collars. Over five consecutive evenings, 40 spatial fixes were

collected in the field for each collared vole using radio-telemetry and a handheld GPS device. Two common measures of home range size (95% convex polygon and 95% kernel density) were calculated for each vole (ArcGIS ArcMap 10.3 software). After the home range data were collected, voles were recaptured and brought into the lab for euthanasia, brain tissue collection, and tissue sectioning (40 µm). Four histological markers were used: cresyl violet staining (pyknotic cells), Ki67, pHisH3, and doublecortin (DCX). Marked cells were counted on every tenth section throughout the extent of the dentate gyrus, using either fluorescent or light microscopy. Male home range size showed a significant positive relationship with cell proliferation (pHisH3-labeling) and cell death (pyknotic cells) within their dentate gyri. Neurogenesis (DCX-labeled cells) was not significantly predictive of home range size. Analyses of another cell proliferation marker (Ki67) are still in progress. We collected blood serum at the time of perfusion and measured testosterone concentrations using ELISAs. We found no significant relationships between testosterone concentrations and any of our markers of home range size or cellular turnover within the dentate gyrus. Thus, testosterone does not seem to be the physiological cause of the observed relationships between range size and cellular turnover. Our results suggest that individuals that range more widely face increased spatial memory demands, which may be met through increased cellular turnover (proliferation and cell death) within the hippocampus.

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Poster

166. Animal Cognition and Behavior: Learning and Memory: Dentate Gyrus and Neurogenesis

Location: SDCC Halls B-H

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Program #/Poster #: 166.12/GGG8

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant NS093866
NIH Grant MH109305

Title: Interrogating hilar mossy cell circuits using a genetically-encoded hybrid optical voltage sensor

Authors: *Y. MA[1], P. O. BAYGUINOV[2], M. B. JACKSON[3]
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Abstract: Mossy cells located in the hilar region of the dentate gyrus receive diverse inputs, and project widely in the hippocampus to innervate both excitatory and inhibitory neurons. The functions of mossy cells and their roles in hippocampal information processing remain unclear. Here we targeted a genetically-encoded hybrid Voltage Sensor (hVOS) to mossy cells in order to visualize their electrical activity. hVOS employs Förster resonance energy transfer with a small organic molecule, dipicrylamine, to transduce membrane potential changes into changes in fluorescence intensity. We crossed our previously-described hVOS Cre-reporter mouse (Bayguinov et al., 2017) with a conditional calretinin (Calb2) Cre driver, and a calcitonin receptor-like receptor (Calcrl) Cre driver (Jinde et al., 2012), to express hVOS probe in mossy cells. In both Calb2CreER^{T2}/hVOS and CalcrlCre/hVOS animals hVOS probe labeled large neurons with thorny excrescences, consistent with mossy cell morphology. By stimulating different pathways and recording optical signals from the hilus in hippocampal slices, we were able to investigate the circuits that activate mossy cells. First we verified that electric stimulation in the perforant path evoked responses in mossy cells. This response often originated in the CA3c/hilar border and propagated back towards the granule cell layer, suggesting a back projection from CA3 pyramidal cells to mossy cells. The mGluR 2/3 agonist DCG-IV, which reduces glutamate release from granule cell nerve terminals, inhibited mossy cell responses elicited by perforant path stimulation, but not responses elicited by granule cell stimulation. Directly stimulating CA3 pyramidal cells also evoked responses in mossy cells, with preferential activation in the infrapyramidal blade. To rule out antidromic activation of mossy fibers, we stimulated the fimbria to activate pyramidal cells, and this also excited hilar mossy cells. To check that CA3 pyramidal cell layer stimulation did not activate mossy fibers in the nearby stratum lucidum, we used hVOS 2.0 mice, which express hVOS probes preferentially in axons (Ma et al., 2017). The responses were localized to approximately 100 μm from the site of stimulus, demonstrating that it was unlikely to elicit antidromic activation of mossy fibers. These studies assessed the back projection of CA3 pyramidal cells to mossy cells using a novel technique. Genetically-targeted hVOS imaging provides an opportunity to study mossy cells, and to ask questions about their circuit interactions that are difficult to address with electrophysiological methods. (Supported by NIH grants NS093866 and MH109305)

Disclosures: Y. Ma: None. **P.O. Bayguinov:** None. **M.B. Jackson:** None.

Poster

166. Animal Cognition and Behavior: Learning and Memory: Dentate Gyrus and Neurogenesis

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 166.13/GGG9

Topic: H.01. Animal Cognition and Behavior

Support: INSERM

PHC IMHOTEP N° 38044YF
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Title: Two distinct populations of neurons from the lateral supramammillary nucleus, both displaying a dual glutamatergic and GABAergic neurotransmittion phenotype, innervate the dorsal dentate gyrus

Authors: H. ELSEEDY[1,2], J. SCAPULA[1], A. GHESTEM[1], H. BRAS[3], S. A. SALAM[2], N. E. ABDELMEGUID[2], *M. ESCLAPEZ[1]
[1]Aix Marseille Univ, INSERM, INS, Inst. Neurosci Sys, Marseille Cedex 05, France; [2]Alexandria University, Fac. of Science, Zoology Dept., Alexandria, Egypt; [3]Aix Marseille Univ, CNRS, INT, Inst. Neurosci Timone, Marseille Cedex 05, France

Abstract: The lateral region of the supramammillary nucleus (SuML) is the main sub-cortical region sending substantial projections to the dentate gyrus (DG). Several studies have provided evidence that this hypothalamic nucleus could play a crucial in the control of several hippocampal-dependent activities and associated functions, including theta rhythms, REM sleep as well as emotional learning and memory (for review see, Soussi et al., 2010; Renouard et al., 2015). However, the anatomical and physiological substrate by which such control occurs is not clearly understood. Previous studies have demonstrated in rat (Soussi et al., 2010) and mouse (Castillo et al., 2015) that the neurons from the SuML innervating the dorsal DG (dDG) display a unique dual phenotype. Their axon terminals co-express markers for both glutamatergic (vesicular glutamate transporter 2; VGLUT2) and GABAergic (glutamate decarboxylase 65, GAD65 and the vesicular GABA transporter, VGAT) neurotransmission and establish asymmetric (excitatory) and symmetric (inhibitory) synapses on DG granule cells (GC). We (Castillo et al., 2015) and others (Petersen et al. 2017) further demonstrated using in vitro optogenetic experimental approach performed in transgenic mouse that these SuML axon terminals indeed co-release monosynaptically Glutamate (Glu) and GABA in GC and parvalbumin-containing interneurones of the DG. However, it is still unclear whether or not these neurons form a heterogeneous population. In this study, we examined the potential heterogeneity of the SuML neuronal population innervating the dDG by coupling tracing methods with rabies virus, RNAscope fluorescent in situ hybridization technology for detection of VGLUT2 and VGAT mRNAs and immunohistofluorescence for detection of calretinin, VGLUT2 and VGAT in mouse. We demonstrate that the population of SuML neurons expressing both VGLUT2 and VGAT is heterogeneous. Among these GABA/GLU SuML neurons projecting to the dDG, 67% contain calretinin whereas 33% do not. This subpopulation of GABA/GLU SuML neurons containing calretinin is more numerous in the anterior region than in the posterior region of the SUML, representing respectively 97% and 41% of the GABA/GLU SUML neurons. We will discuss whether these two subpopulations of GABA/GLU SuML neurons target different neurons of the dDG.

Disclosures: H. Elseedy: None. **J. Scapula:** None. **A. Ghestem:** None. **H. Bras:** None. **S.A. Salam:** None. **N.E. Abdelmeguid:** None. **M. Esclapez:** None.

Poster

166. Animal Cognition and Behavior: Learning and Memory: Dentate Gyrus and Neurogenesis

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 166.14/GGG10

Topic: H.01. Animal Cognition and Behavior

Support: NSERC Discovery Grant

Title: Adult-born neurons inhibit developmentally-born neurons

Authors: *A. ASH[1], J. CLEMANS-GIBBON[2], T. P. O'LEARY[3], D. R. SEIB[4], E. CHAHLEY[2], J. S. SNYDER[1]
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Abstract: Recent reports indicate that lateral inhibition plays a powerful role in selecting which dentate gyrus (DG) neurons are recruited during memory formation. This raises the question of whether developmentally-born and adult-born DG neurons have distinct roles for inhibition, particularly in vivo when neuronal ensembles are selected during memory encoding. To address this we combined chemogenetics and immunohistochemistry for BrdU+Fos to silence and measure activity in developmentally and adult-born neurons as rats learned a spatial water maze task. Specifically, retrovirus was injected into the DG of male rats at 6 weeks of age to express the inhibitory DREADD receptor, HM4Di, in neurons born in adulthood. The same rats were also injected with BrdU to label developmentally or adult-born neurons. At 10 weeks of age rats were injected with either the HM4Di agonist CNO or vehicle, then trained in the water maze (8 trials). One hour after water maze training brains were collected and processed immunohistochemically for BrdU, GFP and c-Fos to identify neurons that were recruited during learning. We found that silencing a subset of adult-born neurons (aged 4 weeks) increased activity levels in the developmentally-born neuron population. However, silencing adult-born neurons did not affect activation in other adult-born neurons within the DG, suggesting limited interaction within the adult-born population. We are currently looking at activation of interneurons (PV+ and SST+) within each treatment group to determine if silencing adult born cells impacts downstream activity in inhibitory interneurons. Our findings indicate there is a modulatory subcircuit between cell populations of different ages within the DG, which has implications for the role of adult neurogenesis on neuron recruitment during learning.

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Poster

166. Animal Cognition and Behavior: Learning and Memory: Dentate Gyrus and Neurogenesis

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

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Topic: H.01. Animal Cognition and Behavior

Support: R37AG036800
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The Evelyn F. McKnight Brain Research Foundation

Title: Investigation of age-related impairment in pattern separation employing modified version of water maze beacon task

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Abstract: Previous studies inspecting transcriptional changes within the brain during aging indicate that cognitive impairments are associated with differentially expressed genes, linked to defined neural systems (e.g. episodic memory-CA1, executive function-prefrontal cortex). These studies found that behavior was not correlated with gene expression in regions that are not the primary locus of the cognitive process (e.g. executive function-CA1). These studies provide information on possible molecular mechanisms for age-related cognitive decline. Aging is associated with a decline in pattern separation (PS), a process often measured by individuals' ability to distinguish whether a given image is identical or merely similar to previously viewed images. The dentate gyrus (DG) is implicated in PS. While a number of changes occur in the DG during aging (decline in neurogenesis, impaired synaptic plasticity, loss for afferent input), it remains unclear what specific changes may occur in the DG with age that lead to a decline in PS performance. We hypothesize that aged animals with impaired PS ability may express a distinct transcriptional profile relative to unimpaired animals, specifically within the DG. In order to examine PS, we have modified the water maze version of the beacon task in an attempt to test PS between similar spatial locations. Two groups of male F344 rats were tested with the target and decoy platforms separated by 45 or 75 cm. Preliminary findings for the first 3 days of training suggest that both young (4 mo, n = 10) and middle-age (12 mo, n = 24) rats make significantly more errors when beacons are 45cm apart compared to 75cm apart (young: $p = 0.009$, middle-age: $p = 0.003$). Middle-age rats sustained a high number of errors over several days of testing, but only when beacons were closer together, whereas young rats made steadily fewer errors over

time regardless of beacon separation. Following the beacon test, rats were tested for spatial reference memory and no age-related differences were observed. These results suggest that the beacon task may be sensitive to an age-related impairment in PS when beacons are sufficiently close together. At the completion of behavioral testing, hippocampal subregions will be isolated and transcriptomic information will be derived from the DG and CA1 in order to investigate possible molecular contributors to a decline of PS with advancing age.

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Poster

166. Animal Cognition and Behavior: Learning and Memory: Dentate Gyrus and Neurogenesis

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

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Topic: H.01. Animal Cognition and Behavior

Support: NIMHD G12MD007599
NIH-NINDS Grant R25NS080686

Title: Dietary curcumin enhances adult hippocampal neurogenesis and two types of neurogenesis-dependent learning

Authors: *H. KHANDAKER[1], A. AUBRY[1], R. RAVENELLE[1], D. GORDIAN[2], C. UBRI[2], S. HANIF[2], G. SCHAFE[1,2], N. BURGHARDT[1,2]
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Abstract: Curcumin is the active ingredient in the turmeric root and has long been known to have anti-inflammatory properties. Interestingly, 5 consecutive days of curcumin treatment has also been shown to promote cell proliferation and cell survival in the dentate gyrus of the hippocampus (Kim et al., 2008). However, it is not known if curcumin-induced increases in adult hippocampal neurogenesis promote neurogenesis-dependent learning. We tested the effects of dietary curcumin on two types of hippocampal-dependent learning that require adult-born granule cells: behavioral pattern separation and cognitive flexibility. The effects of curcumin on behavioral pattern separation were tested by providing adult male mice with chow containing 1.5% curcumin or a control chow for 28 days prior to training in a contextual fear discrimination task. Mice remained on their respective diets throughout the duration of behavioral testing. Mice were therefore tested when newly generated neurons resulting from curcumin treatment were approximately 28-days of age, which is when young neurons are first thought to integrate into hippocampal circuitry. We found that curcumin-treated mice (N=12) consistently discriminated between two similar contexts by the second day of testing, while mice on control chow (N=11)

did not show evidence of discrimination until the eighth day of testing. This improvement in behavioral pattern separation correlated with an increase in the total number of doublecortin-positive cells. We also tested the effects of 5 days of dietary curcumin on contextual fear discrimination (curcumin: N=8; control chow: N=7), a treatment period that is too short for newly born neurons to form functional synapses. We found no effect of curcumin under these conditions. Finally, we tested the effects of 28 days of curcumin treatment on the ability to distinguish between distinct contexts and found no significant group differences (curcumin: N=10; control chow: N=11), indicating a specific role for curcumin in behavioral pattern separation rather than general fear learning. We are currently testing the effects of 28 days of dietary curcumin on cognitive flexibility in the active place avoidance task. Our preliminary results indicate that curcumin does not affect learning to avoid the initial shock zone location but does improve the ability to suppress this previously learned response while learning to avoiding the new shock zone in the opposite side of the room (curcumin: N=7; control chow: N=8). Together, our data suggest that dietary curcumin may be an effective way to stimulate adult hippocampal neurogenesis and reduce interference between similar aversive memories.

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Poster

167. Human Cognition and Behavior: Executive Function

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 167.01/GGG13

Topic: H.02. Human Cognition and Behavior

Support: NIGMS CoBRE P20GM103645
Brown University Department of Neuroscience Connors Fellowship
The Carney Institute for Brain Science at Brown University Innovation Award

Title: The influence of reward on abstract sequential monitoring dynamics in the rostrolateral prefrontal cortex and striatum

Authors: *T. H. MCKIM, T. M. DESROCHERS
Neurosci., Brown Univ., Providence, RI

Abstract: Everyday goals, e.g. picking up groceries to cook dinner, intimately intertwines the reward (tasty meal) and guiding sequential tasks (obtain ingredients, prepare and cook the food). A change in the reward (store out of preferred ingredient) may change your performance of these task sequences. A previous study demonstrated with fMRI and TMS that activation in the rostrolateral prefrontal cortex (RLPFC) is increasingly engaged (ramps up) and necessary during sequence performance (Desrochers et al., 2015). In animals, neural ramping dynamics in the

frontal cortex, and dopamine levels in the striatum, have been shown to be modulated by reward. However, it remains unknown whether reward modulates the RLPFC ramping signal in humans during sequential tasks. We tested the hypothesis that RLPFC ramping underlies progress toward the completion of a goal by manipulating reward value during a sequential control paradigm. We employed a task where participants tracked a repeated series of four item sequences associated with high versus low reward during fMRI scanning. On each trial, participants indicated with one of two button presses whether the image was in or out of a pre-determined order, during two trials types: 1) all stimuli were visible (Vis) or 2) all but the last stimulus in the block was occluded by an irrelevant distractor (Occ), requiring participants to monitor the sequence without external cues. Behavioral results (n=18) demonstrated elevated reaction times (RTs) at the first position within the sequence as evidence of initiation cost (Pos1>Pos2,3,4, $F_{1,17}$ = 48.44, p<0.001) and replicating previous results (Desrochers et al., 2015). Overall, RTs were faster for high value sequences ($F_{1,17}$ = 16.13, p=0.002), and this initiation cost was selectively reduced for high value Occ sequences (Pos1>Pos2,3,4, $F_{1,17}$ = 112.96, p<0.001). Preliminary fMRI analyses also replicated ramping activation in the RLPFC during sequence monitoring. Further, we found increased ramping activation in RLPFC and caudate nucleus of the striatum in Occ high relative to Occ low sequence monitoring. These results suggest that this ramping signal may be modulated by progress through sequence monitoring, and raise the possibility that similar underlying neural computations between species during progress toward a goal. Future studies will use the same sequential task in awake-behaving primates undergoing fMRI to test functional homology of frontostriatal circuits and their neural dynamics during sequential monitoring.

Disclosures: T.H. McKim: None. **T.M. Desrochers:** None.

Poster

167. Human Cognition and Behavior: Executive Function

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 167.02/GGG14

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant F32DA038927

Title: Human habenula tracks task and motivational context

Authors: *D. FURMAN, M. D'ESPOSITO
Helen Wills Neurosci. Inst., Univ. of California Berkeley, Berkeley, CA

Abstract: The habenula, a bilateral epithalamic nucleus, has recently gained attention for its putative role in regulating brainstem monoamine systems. Research in rodents and non-human primates suggests that the nuclei integrate motivational, homeostatic, and contextual information from diverse regions including the basal ganglia and frontal cortex, potentially in the service of

driving both learning and more immediate behavioral flexibility. Of the limited work that has examined the function of the habenula in humans, the majority has focused on its encoding of negative reward expectation and receipt; here, we explored the habenula's response to explicit changes in task goals as well as to task-relevant motivational cues. High-resolution echo planar imaging data (multiband factor=4, partial Fourier=6/8, TR=2s, TE=35.6ms, 1.54mm isometric voxels, 76 slices) were acquired on a 3T Siemens Trio MRI machine as 32 healthy participants (ages 18-30) performed a cued task-switching task. On each trial, a number-letter pair appeared in one quadrant of the screen; stimulus location (top vs. bottom) cued participants to categorize either the letter as vowel/consonant or the number as odd/even with a left/right-handed button press. Task switch trials occurred when a stimulus crossed the horizontal meridian relative to stimulus location on the previous trial. Motivational context was encoded by stimulus color; prior to scanning, participants were trained to associate one of three distinct colors with the threat of losing an endowed bonus for slow or inaccurate behavior. Stimulus colors appeared with equal frequency across the task. Regression analyses were conducted on average blood oxygen-level dependent (BOLD) signal extracted from manually-traced left and right habenula regions-of-interest to examine the effects of task switching and motivational salience. We found that explicit task switching (vs. task maintenance) elicited increased activation in the right habenula, and that motivational cues (vs. neutral cues) evoked activation in the left habenula. For motivationally salient task-maintenance trials, magnitude of left habenula activation was associated with improved performance when a switch in motor response (e.g., left-to-right hand) was required, providing further support for the hypothesized role for the region in promoting behavioral flexibility. Together, these results suggest that the human habenulae receive (potentially asymmetric) input encoding elements of both behavioral and motivational context, and may together bias downstream motor preparatory circuitry, perhaps in part via indirect regulation of nigrostriatal dopamine release.

Disclosures: D. Furman: None. **M. D'Esposito:** None.

Poster

167. Human Cognition and Behavior: Executive Function

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 167.03/GGG15

Topic: H.02. Human Cognition and Behavior

Support: Medical Research Council (UK) intramural program MC-A060-5PQ10

Title: Differential representation of broad context and task details in default mode and executive control networks

Authors: *V. SMITH[1], D. J. MITCHELL[2], J. DUNCAN[2]
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Abstract: It is well known that the default mode network (DMN) is important for internally-focused self-relevant thought processes. However, more recently, the DMN has also been implicated in cognitive transitions. A paper by Smith et al. (in prep) suggests that the DMN might represent broad spatial and temporal context, important during internal mentation but also during cognitive switches, when context may impose constraints on novel behavior. Within this broad context, frontoparietal control regions (multiple-demand or MD system) are proposed to control the specific steps of the current task. We predicted that (a) background context would be strongly represented in the DMN, (b) specific task operations would be strongly represented in the MD system, and (c) in the DMN, broad context representation would diminish as one becomes focused on a task, only to reemerge with substantial change. Using functional magnetic resonance imaging, we scanned participants during alternating periods of rest, sustained performance of a fixed task, and task switching. To provide broad background context, task stimuli appeared against one of two different scene backgrounds. Using MVPA, we examined context and task decoding in DMN regions, MD regions and the whole brain. As predicted, the results show differential representation of context and task details in DMN and MD regions.

Disclosures: V. Smith: None. **D.J. Mitchell:** None. **J. Duncan:** None.

Poster

167. Human Cognition and Behavior: Executive Function

Location: SDCC Halls B-H

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Program #/Poster #: 167.04/GGG16

Topic: H.02. Human Cognition and Behavior

Support: NSF Grant 1734883
UCSD Chancellor's Research Excellence Scholarships

Title: Resting state and task-related brain dynamics supporting creativity

Authors: *S. RAJ[1], A. SCHPERBERG[3], B. TSAI[4], S. BROWN[1], T.-P. JUNG[2], Y. WU[2]
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Abstract: Creativity is likened to fluid thought and defined as the ability to generate original, useful contributions to human endeavors in the form of new ideas, representations, and material objects. This study investigates how creative thinking is modulated by different modes of task engagement. An open-ended and a guided scenario were created in the Unity 3D game engine tasking the player with finding a way to enter a locked house. In the open-ended version, the player discovers alternative entry points and devises methods for accessing them using tools scattered in the environment. In the guided version, the player follows a trail of notes with

instructions on where to look for a hidden key. Twenty-three healthy adults participated in two separate data recording sessions, each featuring either the guided or discovery-based scenario presented in counter-balanced order. Immediately before and after each scenario, resting state EEG was recorded (Emotiv Epoch headset). Additionally, a psychometric test known as the Alternative Uses Task was administered, challenging participants to enumerate as many novel uses for a conventional item (e.g. a paper clip) as possible within a fixed time.
After the guided scenario, participants tended to generate slightly more alternative uses for common objects, but tended to produce fewer original responses. Notably, these behavioral changes were accompanied by a slight decrease in post-task resting state beta (15 – 35 Hz) and gamma (50+ Hz) power. On the other hand, after the discovery-based scenario, neither fluency nor originality of responses changed. However, resting state beta and gamma band power – along with spectral activities in the alpha range (8 – 12 Hz) – all tended to increase after the discovery-based task. This pattern of outcomes reveals that open-ended problem solving tends to modulate resting state EEG to a greater degree than tasks that simply involve following instructions. Further, the observed resting state changes may be related to neurocognitive mechanisms that sustain the originality of creative thought in the wake of resource intensive task engagement.

Disclosures: S. Raj: None. **A. Schperberg:** None. **B. Tsai:** None. **S. Brown:** None. **T. Jung:** None. **Y. Wu:** None.

Poster

167. Human Cognition and Behavior: Executive Function

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Program #/Poster #: 167.05/GGG17

Topic: H.02. Human Cognition and Behavior

Support: NNSFC Grant 31271085

Title: Neural representations of the transfer of proportion congruency effect

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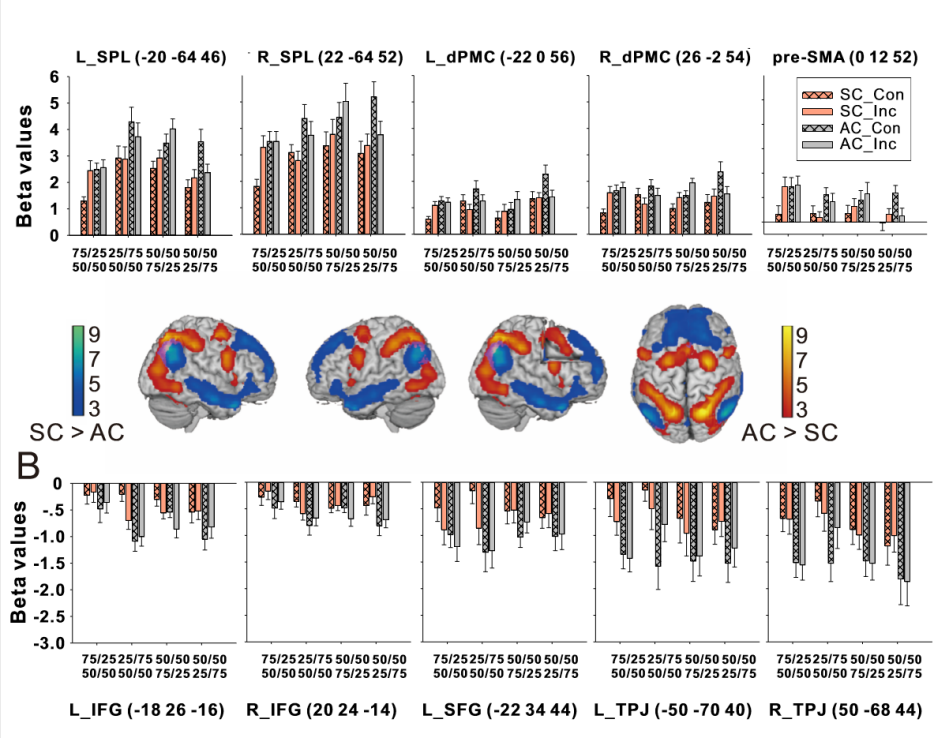
Abstract: The proportion congruency (PC) effect is usually used to investigate the dynamics of cognitive control in experiment. Some studies manipulated the transfer of PC effect to knowledge this cognitive process. However, these studies often ignored the role of stimulus-response associations learning, which recently documented to be important for PC effect. We employed Hedge and Marsh task, and combined it with a between subjects PC manipulation to investigate the neural representations of transfer of PC effect. Six-four neurologically and psychiatrically healthy volunteers (mean age ±SD: 20.8± 1.9 years; 34 females) participated in

the experiment. The pattern of the positive and reversed Simon effect varied across conditions, suggesting that participants used strengthened task-irrelevant S-R associations to predict responses.

What's more, the pattern of transfer of PC effect was consistent with the S-R associations learning accounts. Functional neuroimaging identified PC effects that interacted with task S-R associations, showing greater activity in frontoparietal regions, including bilateral superior parietal lobule (SPL) and dorsal premotor cortex (dPMC), pre-supplementary motor area/anterior midcingulate cortex (Pre-SMA/aMCC), and left dorsolateral prefrontal cortex (DLPFC), see Figure 1. The activity of these regions was also modulated by PC effect in frequency-unbiased context. The aMCC and DLPFC shifted to responding mainly to the conflict induced by the strengthened irrelevant S-R associations. The SPL and dPMC might represent the strengthened irrelevant S-R associations.

Keywords: cognitive control, contingency learning, proportion congruency effect, stimulus-response associations, transfer effect



Disclosures: T. Xia: None. **H. Li:** None. **L. Yang:** None. **L. Wang:** None.

Poster

167. Human Cognition and Behavior: Executive Function

Location: SDCC Halls B-H

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Program #/Poster #: 167.06/GGG18

Topic: H.02. Human Cognition and Behavior

Support: Data collection and sharing for this project was provided by the MGH-USC Human Connectome Project.

Title: Different patterns of multiple network membership underly regions of the rostrocaudal hierarchy

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Abstract: The lateral frontal cortex (LFC) is organized hierarchically along a rostrocaudal (RC) axis such that rostrally maintained, temporally abstract task information and caudally maintained, immediate action options are integrated and controlled by a centrally positioned "apex" area (Badre & Nee, 2018). Different LFC regions are also organized into several different large-scale networks that are also likely linked to distinct cognitive functions (Dosenbach, 2008). At present, it is unclear how the RC hierarchy emerges across functionally disparate networks. Furthermore, most regions of LFC (or "nodes" in network analysis) are usually described as having similar network characteristics (Gratton et al, 2016), despite the differences in their hierarchical roles. LFC nodes are often categorized as "connector hubs" that serve as bridges between networks, but these analyses typically assign nodes to the single network with which that node is most strongly connected. Thus, it is unclear which networks these LFC nodes might bridge, or how this may differ with hierarchical position.
To address this, we employed a new graph theory method (Najafi et al, 2018) that assigns nodes to multiple networks using a mixed-membership algorithm (Gopalan & Blei, 2013) and finds the proportion of connections the nodes have within each of their assigned networks. To relate these data to the RC hierarchy, we defined our network using a parcellation (Schaefer et al, 2018) in which distinct nodes of interest (NOIs) spatially corresponded to each of 5 LFC regions that have previously shown hierarchical structure (Nee & D'Esposito, 2018). We used 15 minutes of resting state fMRI data collected from 100 healthy subjects as part of the Human Connectome Project to define a whole-brain graph comprised of 7 intrinsic networks (Yeo et al, 2011) and assessed the mixed network membership of our 5 NOIs. We found that our NOIs were associated with 3 of the 7 networks, and that each individual NOI was associated with only 2 of those 3 networks. NOIs associated with the caudal regions in the hierarchy preferentially connected with 1 of their 2 associated networks, while more rostral NOIs showed more balanced connections between networks. Furthermore, the NOI associated with the apex of the hierarchy and a second, nearby NOI were members of the same network pairs, but only shared membership with 1 of the 2 networks associated with the most rostral and caudal NOIs. These results suggest that LFC regions along the RC axis have different connectivity patterns bridging multiple intrinsic networks that may support their ability to integrate and process task-related information in a hierarchical way.

Disclosures: S.L. Cookson: None. **D.J. Lurie:** None. **M. D'Esposito:** None.

Poster

167. Human Cognition and Behavior: Executive Function

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Program #/Poster #: 167.07/GGG19

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant F32AG056080
NIH Grant R21AG058111
Marcus and Amelia Wallenberg Foundation

Title: Proactive control in context: Context-cued predictions of control demands facilitate perceptual decisions in virtual environments

Authors: *J. JIANG[1], A. D. WAGNER[2]
[1]Psychology, [2]Dept. of Psychology, Stanford Univ., Stanford, CA

Abstract: Human behavior relies on cognitive control to adaptively adjust mental states and behavior to internal goals. Recent studies have shown that, in addition to passively reacting to current task demands, cognitive control can also be regulated proactively based on the predicted forthcoming need for cognitive control. Of central interest is determining the mechanisms through which forthcoming cognitive control demands (CCD) are predicted. In this behavioral study, we tested the hypotheses that (a) CCD can be associated with spatial contexts, such that (b) subsequent encounters with a spatial context can drive the retrieval of its associated CCD and thus enable proactive control. The experiment consisted of 6 runs of 8 blocks each, during which participants needed to draw on selective attention to make perceptual decisions. In each block, participants (N = 47) were cued to navigate to one of four buildings in a virtual 3D environment. They then performed 8 trials of a perceptual decision-making task within a virtual room in the building. Each trial started with the presentation of a task cue, followed by a bivalent image of two overlapping translucent images (one face image and one object image). Based on the cue, participants were required to indicate either the gender of the face or the type of tool. To manipulate the contextual CCD, participants performed mostly face judgments in two buildings and mostly object judgments in the other two buildings. In the analyses, the learning of the association between the spatial contexts (i.e., buildings/rooms) and their corresponding CCD was modeled using reinforcement learners (one per context), which predicted the contextual CCD (i.e., the likelihood of performing the face task vs. the object task) at the trial level. We predicted that proactive retrieval of expectations about the context-associated CCD would facilitate behavioral performance when it matched the actual task demand indicated by the task cue. To control for context-free learning of CCD, an additional reinforcement learner predicting CCD based on trial history (regardless of context) was used as a covariate. Confirming our prediction, a trial-level mixed effect model showed responses were faster when the discrepancy between

contextual and actual CCD was smaller ($P < 0.001$). Future studies will combine this design with functional magnetic resonance imaging to investigate hippocampal computations supporting the learning of the context-CCD associations and interactions with frontoparietal networks of control.

Disclosures: J. Jiang: None. **A.D. Wagner:** None.

Poster

167. Human Cognition and Behavior: Executive Function

Location: SDCC Halls B-H

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Program #/Poster #: 167.08/GGG20

Topic: H.02. Human Cognition and Behavior

Support: Swartz Foundation

Title: Oscillatory mechanisms of planning vs. memory

Authors: *R. J. GOUGELET[1,2], M. MIYAKOSHI[2], B. VOYTEK[1], S. MAKEIG[2]
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Abstract: This study investigates neural oscillation dynamics during a dart-throwing task in humans. In one condition, subjects were tasked with waiting a trial-randomized three to nine second delay before throwing a dart at a randomly located target. In another condition, subjects similarly waited randomly determined delays, but were initially only briefly exposed to the target and had to throw from memory. We use these conditions as operationalizations of memory and planning to compare and contrast their underlying neural oscillatory functional mechanisms as inferred via EEG. We suggest that, in both memory and planning, neural oscillations serve to integrate and maintain task-relevant information over time. In both memory and planning, we further suggest that the relative extent of oscillatory integration and maintenance dynamics over time improves performance. In memory, however, oscillatory activity protects against memory decay, whereas in planning, oscillatory activity facilitates online preparatory rehearsal. Despite this functional difference, we suspect the underlying physiological mechanisms, i.e. neural oscillation dynamics, are the same. We found that inter-regional and intra-regional theta-alpha phase-amplitude coupling (PAC) among independent component analysis (ICA)-derived components were indeed associated with improved performance in the memory condition, but, contrary to our expectations, not in the planning condition. We also found no association for inter- or intra-regional theta-alpha amplitude-amplitude coupling (AAC) among independent components. These findings suggest that theta-alpha PAC may only serve a functional role in memory, but not planning, whereas theta-alpha AAC shows no evident role in neither memory nor planning, though further investigation is warranted. Next, we intend to examine the anterior-

posterior spatial distribution of theta-alpha PAC components; component phase and peak frequency modulation dynamics; and other forms of cross-frequency coupling, within and between other frequency bands.

Disclosures: R.J. Gougelet: None. **M. Miyakoshi:** None. **B. Voytek:** None. **S. Makeig:** None.

Poster

167. Human Cognition and Behavior: Executive Function

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Program #/Poster #: 167.09/GGG21

Topic: H.02. Human Cognition and Behavior

Support: MOST 105-2632-H-038-001
MOST 106-2410-H-038-004-MY2

Title: tRSA reveals information embedded in prestimulus affected poststimulus in congruency sequence effect

Authors: *P. Z. CHENG[1], T.-Y. HSU[2]
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Abstract: When encountering sequences of incoming events, some information from preceding events can be carried over to the following event while shaping how we react subsequently. The congruency sequence effect (CSE) is a consistently observed effect which suggests that behavioral responses to conflicting stimuli can be adjusted contingent to the conditions of the previous trial. Such carryover information must be retained by previous trials information and further embedded and preserved in the ongoing neural activity. However, the existence of such information transmission between trials remains to be tested. To approach this question, we adopted a prime-probe arrow task to induce the congruency sequence effect. Meanwhile, EEG was recorded to measure the mechanism underlying the CSE. Integrated spatial correlation coefficient and representational similarity analyses both were used to estimate topographical EEG at each time point. If the information contained in pre-stimulus periods persists over time, a high correlation between topographic EEG during pre- and post-stimulus periods should also be observed and reflected on the similar topographic representation similarity. Representational similarity analyses showed that topographical properties were highly correlated with the conditions in previous trials affect responses to current trials from pre-stimulus period to 500 ms after stimulus onset. Time to time correlation between pre- and post-stimulus periods also indicated information from previous trials affected current trials' pre- and post-stimulus periods. In sum, information from previous trials were carried over to the current trials and embedded in EEG signals, alternating responses to current stimuli.

Disclosures: P.Z. Cheng: None. **T. Hsu:** None.

Poster

167. Human Cognition and Behavior: Executive Function

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Topic: H.02. Human Cognition and Behavior

Support: NIGMS CoBRE P20GM103645
ONR MURI N00014-16-1-2832
James S. McDonnell Foundation
Brown University Department of Neuroscience Connors Fellowship
The Carney Institute for Brain Science

Title: Testing the necessity of the rostrolateral prefrontal cortex for sequence monitoring with continuous theta burst stimulation (cTBS)

Authors: *A. R. SPIRO[1], T. H. MCKIM[2], D. BADRE[1,3], T. M. DESROCHERS[2,4,3]
[1]Cognitive, Linguistic, and Psychological Sci., [2]Neurosci., [3]Carney Inst. for Brain Sci., [4]Psychiatry and Human Behavior, Brown Univ., Providence, RI

Abstract: Everyday goals require performing sequences of tasks. Evidence from fMRI and single pulse transcranial magnetic stimulation (TMS) has demonstrated that the rostrolateral prefrontal cortex (RLPFC) is increasingly activated and necessary when performing sequences of tasks (Desrochers et al., 2015). A recent fMRI experiment observed that RLPFC was again increasingly activated when participants only monitored a repeating sequence of four images without performing a separate subtask at each step (Desrochers & Badre, 2016). However, fMRI cannot establish whether RLPFC is still necessary in this simplified sequential monitoring task. Here, we tested whether continuous theta burst stimulation (cTBS, Huang et al., 2005) to RLPFC changed task performance during this monitoring task compared to RMPFC or sham stimulation. On each trial, participants pressed a button to indicate whether a presented image was in or out of a pre-instructed sequence order. Further, for a block of sequences, all stimuli were either all visible or all but the last stimulus in the block was "occluded" by an irrelevant, placeholder image. When the sequence was occluded, it had to be monitored from memory. Participants were able to change their responses within a set time window to correct any response errors. Using a within-subjects design, stimulation order was counterbalanced across brain regions on separate days: left RLPFC, RMPFC, and right RLPFC (sham). Results demonstrated sequence initiation costs as elevated reaction times at the first sequence position, providing evidence that participants were tracking the repeating sequence. CTBS stimulation changed the proportion of trials on which participants changed an initial incorrect response to a correct response. This change in corrections was evident for out-of-sequence items, relative to in-sequence items, in the

occluded condition. Specifically, cTBS to RLPFC increased the proportion of corrected responses, while cTBS to RMPFC decreased this proportion. This change in corrected response rates was not apparent in visible trials. We also observed a trend that the effects of RLPFC stimulation varied across sequence position, such that earlier positions were more vulnerable to disruption. These results suggest that RLPFC may be necessary when accurately detecting and responding to errors while only monitoring a sequence of state transitions. We are currently running a replication study focused on response changes during the occluded condition.

Disclosures: A.R. Spiro: None. **T.H. McKim:** None. **D. Badre:** None. **T.M. Desrochers:** None.

Poster

167. Human Cognition and Behavior: Executive Function

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Topic: H.02. Human Cognition and Behavior

Support: Australian Research Council Discovery Project 170101840
Australian Research Council Future Fellowship FT170100105
MRC intramural funding SUAG/035/RG91365

Title: Multivoxel coding on error trials links neural activity in frontoparietal cortex to behaviour

Authors: *A. WOOLGAR[1,2], N. DERMODY[3], S. AFSHAR[2], M. A. WILLIAMS[2], A. N. RICH, 2109[2]
[1]MRC Cognition and Brain Sci. Unit, Univ. of Cambridge, Cambridge, United Kingdom; [2]Macquarie Univ., Sydney, Australia; [3]Max Planck Inst. for Human Cognitive Brain Sci., Leipzig, Germany

Abstract: Understanding what happens in the brain when we make errors may help us link neural representations and behaviour. Multivoxel pattern analyses of fMRI activity have demonstrated coding of a range of task features when participants perform tasks successfully. Here, we used a novel application of these methods to discover the source of errors in a challenging stimulus-response task. On the basis of participant responses, we separated errors where participants applied the wrong stimulus-response mapping rule (rule errors) from other (unspecified) errors. We trained a pattern classifier on correct trials to identify the neural signature of correctly encoded rule and stimulus information, and then used this classifier to examine what information is held in frontoparietal "multiple-demand" (MD) regions, and visual cortex, on error trials. The result was a striking double dissociation: When participants made rule errors, frontoparietal MD regions held information about the correct *stimulus* but consistently represented the incorrect *rule*. Under these conditions, there was strong coding of the correct

stimulus in the visual cortex. In contrast, for unspecified errors, frontoparietal activity patterns represented the correct *rule* information, but the incorrect *stimulus* information. When this was the case, the visual cortex lacked coding of the stimulus. These neural data therefore suggest that the unspecified errors may have been due to incorrect perception of the visual information. Interestingly, the visual cortex did not represent the incorrect stimulus under these conditions; rather there was no information about the stimulus at all, suggesting that the incorrect stimulus information in frontoparietal MD cortex may have been internally generated. Rather than reducing to noise, frontoparietal patterns of activation on errors systematically encode incorrect stimulus or rule information, diagnostic of the particular error the participant will make. This demonstrates a crucial role for these regions in determining success or failure, and provides strong evidence for the link between multivoxel patterns in these brain regions and behaviour.

Disclosures: A. Woolgar: None. **N. Dermody:** None. **S. Afshar:** None. **M.A. Williams:** None. **A.N. Rich:** None.

Poster

167. Human Cognition and Behavior: Executive Function

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Program #/Poster #: 167.12/GGG24

Topic: H.02. Human Cognition and Behavior

Support: ONR MURI N00014-16-1-2832
McDonnell Foundation Grant

Title: Two distinct processes underlie control policy learning

Authors: *A. BHANDARI[1], D. BADRE[1,2]
[1]Cognitive Linguistics and Psychological Sci., Brown Univ., Providence, RI; [2]Carney Inst. for Brain Sci., Providence, RI

Abstract: Performing complex, temporally extended tasks requires working memory control policies adapted to the sequential dynamics of the task environment. Computational models have posited ‘gating’ policies that control the updating of information into working memory (input gate), and its influence on behavior (output gate). The cognitive and neural processes that support the learning of such gating policies for novel tasks remain poorly understood. To examine these processes, we asked subjects undergoing fMRI scanning to perform 20 novel working memory control tasks, each for 12 trials. Each task had a unique sequential trial structure but all tasks shared the same stimulus-response rules. We find evidence that implementing gating policies relies on two distinct learning processes. One process is reflected in the rapid speeding of responses over the first few trials with a given sequential trial structure. This early trial RT effect is best correlated with activity changes in the caudate nucleus. A

second, slower process is reflected in the diminishing size of this early RT effect as subjects are exposed to different sequential task structures. This 'learning-to-learn' like effect is best correlated with diminishing engagement of frontoparietal cortex on the early trials of each task and with changes in the geometry of task representations in these regions. Collectively, our results suggest that two distinct, interacting processes underlie the learning of efficient gating policies.

Disclosures: A. Bhandari: None. **D. Badre:** None.

Poster

167. Human Cognition and Behavior: Executive Function

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Program #/Poster #: 167.13/GGG25

Topic: H.02. Human Cognition and Behavior

Support: CONACYT
DGAPA project IN224414-2

Title: Regions and laterality of the prefrontal cortex and their participation in creativity

Authors: *L. V. ORTEGA LEONARD[1], E. AGUILAR CASTAÑEDA[2], I. Y. DEL RÍO PORTILLA[1]
[1]Lab. de Sueño Facultad de Psicología, Univ. Nacional Autónoma de México, Mexico city, Mexico; [2]Inst. Nacional de Neurología y Neurocirugía, Mexico city, Mexico

Abstract: Objectives The aim of this study considers the involvement of the prefrontal cortex in creativity and hemispheric asymmetry. Because, are not clear the role of each area (medial, orbital, and dorsolateral) and the hemispheric differences in the creativity. One way to study these characteristics is through pathologies as a tumor, capable of causing structural and functional changes in the brain. Method Were evaluated twelve patients with diagnosis of tumor type meningioma frontal, classified according to the anatomical location (medial, orbital, and dorsolateral) and hemispheric. As well as twelve healthy control participants, matched for age, gender and education. All participants' creative thinking was assessed using the two scales of the Torrance Test of Creative Thinking (TTCT): figural (TTCT-F) and verbal (TTCT-V). Results According to laterality of tumor, in six patients it was in right hemisphere and in six in left hemisphere. For the localization of tumors by prefrontal areas regardless of the laterality, four tumors were found in the medial, four in the orbital and four in the dorsolateral areas. Comparisons among patient and healthy controls groups revealed the following:1) no significant difference was found for age and education and 2) in the assessment of verbal and figural creativity, no significant differences were found. In comparison in creative thinking with laterality of tumors no significant differences in tumor localization hemispheric were found.

Analysis of the differences between the frontal regions (medial, dorsolateral and orbital) revealed significant differences in the properties of figural TTCT; elaboration (p=0.02) and abstraction of titles (p=0.02), patients with tumor in the dorsolateral area have a lower score on elaboration. While patients whose tumor was developed in the medial area scored higher on property of abstraction of titles. Conclusions There are no differences between patients and controls, in verbal and figurative creative thinking, so in addition to the frontal regions, the participation of other cortical and subcortical regions is indispensable. It was not observed that the laterality of the tumor impacted creative thinking, so it is concluded that creativity is not a lateralized function, but is derived from the interaction and integration of information across the left and right hemispheres. Regarding the properties of elaboration and abstraction of titles of the figural scale of the TTCT, the patients had a poor performance when the tumor was found in the dorsolateral region, in comparison with the medial region, in which they had a higher performance.

Disclosures: L.V. Ortega Leonard: None. **E. Aguilar Castañeda:** None. **I.Y. del Río Portilla:** None.

Poster

167. Human Cognition and Behavior: Executive Function

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R01 MH094305
NSF Grant IIS 1718802

Title: Supramodal involvement of the cognitive control network in uncertainty processing

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Abstract: Information processing under conditions of uncertainty requires the involvement of cognitive control. Although we have demonstrated behaviorally that cognitive control is supramodal (e.g., across visual and auditory modalities), direct evidence about whether the underlying neural mechanism is also supramodal is still lacking. In this functional magnetic imaging study, we employed visual and auditory perceptual decision making tasks to examine brain activity increasing as a function of uncertainty in both modalities. A delayed-response approach was adopted to control the potential confounding of motor response. We found that only the cortical regions of the CCN showed a monotonic activation increase as a function of

uncertainty in both visual and auditory tasks, which did not overlap with regions associated with motor response. These findings suggest the supramodal involvement of the CCN in cognitive control for high level uncertainty processing.

Disclosures: T. Wu: None. **A. Spagna:** None. **P.R. Hof:** None. **J. Fan:** None.

Poster

167. Human Cognition and Behavior: Executive Function

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 167.15/GGG27

Topic: H.02. Human Cognition and Behavior

Support: KAKENHI Grant 16H06405
KAKENHI Grant 15H01828
Grant-in-Aid for JSPS Research Fellow 18J10631
Meiji Yasuda Life Foundation of Health and Welfare Grant

Title: Does high-groovy rhythm facilitate the effects of acute mild exercise on executive functions?: Possible role of the subjective sensitivity in exercise with music

Authors: *T. FUKUIE[1], K. SUWABE[2], G. OCHI[2], K. HYODO[3], K. BYUN[2], H. SOYA[2]
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Abstract: Physical exercise has beneficial effects not only on peripheral function, but also on the brain function. Previous studies including ours have revealed an acute exercise improved an inhibitory executive performance with increased cortical activation in the prefrontal cortex, which might be related to exercise-induced emotional changes (Byun et al., 2014; Yanagisawa et al., 2010). Recently, there is increasing attention on the music which elicits positive emotion as a useful tool for enhancing exercise effects. In particular, high groove music which elicits body movement and positive mood, is promising music compatible with the exercise. However, in terms of the combined effect of exercise and music, various personal sensitivities could modulate the effect (Karageorghis & Priest, 2008; 2012). Thus, the purpose of the present study is to reveal the effect of mild exercise combined with a high-groove rhythm on executive performance focusing on a subjective sensitivity in exercise with music. In the preliminary study of forty four young adults, we confirmed that participants felt a high groove and positive valence when they listened to a drum music with the medium syncopation degree (MS-rhythm), which is consistent with the previous study (Witek et al., 2014) . In the main experiment, seventeen participants underwent two experimental conditions: ten-minute of mild exercise with MS-rhythms (MS condition) or without music (Control condition). Before and after an acute mild exercise, participants performed a color-word matching Stroop task. The Stroop task has been used for

evaluating inhibitory executive performance in several studies, and known to involve prefrontal brain activity. Mild exercise with MS-rhythms enhanced valence regardless of a subjective sensitivity in exercise with music, but there was no significant difference of changes of executive performance between two conditions. However, we found that the improvement of executive performance was correlated with a degree of subjective sensitivity in exercise with music. These results suggest that a subjective sensitivity in exercise with music may be important role in the strengthen effects of mild exercise with high groove rhythm on executive function. Future studies will examine the brain neural mechanisms for the combined effect, focusing on individual sensitivity by using neuroimaging method and battery test of rhythmic sensitivity.

Disclosures: K. Suwabe: None. **G. Ochi:** None. **K. Hyodo:** None. **K. Byun:** None. **H. Soya:** None.

Poster

167. Human Cognition and Behavior: Executive Function

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 167.16/HHH1

Topic: H.02. Human Cognition and Behavior

Support: 16IRCCSG002

Title: Effect of fatigue of executive function on its cognitive processes

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Abstract: Executive function (EF) is a domain of cognition involving cognitive processes such as response inhibition (RI), working memory (WM), cognitive flexibility (CF), and planning that are important for goal-directed behavior. Compromised executive control is found to underlie performance deficits due to mental fatigue. Yet, the effect of EF fatigue on its specific cognitive processes is not known. To investigate how fatigue of EF itself affects its processes, namely, RI, WM, and CF, global EF was fatigued during a 2-hour session in 6 healthy adults (25.17 ± 3.54 years; 5 males) using a modified version of Berg's Card Sorting Test (BCST), a well-known task in EF research. We enhanced the novelty and task engagement of BCST by increasing its complexity such that one had to constantly adopt effective strategies for changing task demands. Subjective report of mental fatigue (Visual Analog Scale-VAS) and performance on tasks specific to the processes of EF: Antisaccade - AS (for RI); 2-Back (for WM); Plus-Minus - PM (for CF) were measured before and immediately after the fatigue session. Trail Making Test B (TMTB) and original BCST (oBCST) were also used for assessing fatigue effect on global EF. Simple reaction time (RT) was used as a control measure. Cognitive assessments were randomized in order. Subjects were clearly fatigued as the VAS scores increased (pre: 2.22 ±

0.82 vs. post: 7.85 ± 2.46). EF was indeed fatigued as perseverative errors on oBCST (pre: 5.67 ± 1.37 vs. post: 8.33 ± 3.44) and total time (s) for TMTB were increased (pre: 58.96 ± 14.35 vs. post: 63.68 ± 8.55). The effect of fatigue of global EF was observed on its specific processes as RT (ms) on AS task increased (pre: 683.36 ± 65.58 vs. post: 725.69 ± 84.24) whereas, accuracy (%) decreased (pre: 85 ± 6.55 vs. post: 75.93 ± 5.47), and RT (ms) on 2-Back task increased (pre: 619 ± 245.98 vs. post: 750.29 ± 316.45) whereas, accuracy (%) remained similar (pre: 91.66 ± 10.13 vs. post: 90.2 ± 9.62). There was no effect on the PM task as decrease in shift cost (s) (pre: 13.53 ± 5.96 vs. post: 12.95 ± 5.82) was not due to the effects on task-switching. Finally, a marginal difference in simple RT (ms) was observed (pre: 330.35 ± 34.3 vs. post: 349.57 ± 35.78) indicating that effects of fatigue of global EF on its specific cognitive processes may not be attributed to a basic inability to process information or to an overall lack of compliance due to fatigue. Although results are preliminary, it appears that there was a differential effect of fatigue of global EF on its cognitive processes. It seems that RI was most affected by fatigue, reflecting probably, either a high susceptibility of RI to mental fatigue or that RI may require more cognitive resources per se.

Disclosures: N. Kanekar: None. **A. Chimmili:** None.

Poster

167. Human Cognition and Behavior: Executive Function

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 167.17/HHH2

Topic: H.02. Human Cognition and Behavior

Title: Brain network dynamics during fast strategy shifts and incremental task optimisation

Authors: *F. C. REVERBERI[1], S. SEYED-ALLAEI[1], N. SCHUCK[2], D. AMATI[3], A. LAIO[3], M. ALLEGRA[3]
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Abstract: By engaging long enough in any activity, major improvements are expected. This is true for tasks as complex as playing piano and as mundane as preparing home-made tagliatelle. These improvements may happen through multiple paths. One is an “incremental task optimization”: while following the same algorithm to reach the task goals, one can optimize the algorithm implementation, yielding measurable processing gains. Alternatively, learners may turn to a new strategy achieving the same task goals more efficiently than the original algorithm.We re-analyzed a recent fMRI experiment (Schuck et al., 2015) where subjects had to press one of two buttons based on relative position of the visual stimulus - the instructed strategy - or based on the stimulus color - a more effective but uninstructed strategy. We used a new

analysis technique, CPDC (Allegra et al., 2016), to investigate brain network dynamics and its association with learning. We identify a brain network involving areas in the visual cortex, the parietal cortex, the precuneus, and the prefrontal cortex. For subjects following the instructed strategy, incremental learning reflected into a progressive strengthening of several network links parallel to a reduction in response times. In the sudden passage from the spatial to the color strategy, we observed a weakening of some network links, including those that previously showed the strongest strengthening. Our results contribute to the current debate on network neuroscience and learning. CDPC proved to be highly effective for tracking network dynamics and could be fruitfully applied to other tasks.

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Poster

167. Human Cognition and Behavior: Executive Function

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 167.18/HHH3

Topic: H.02. Human Cognition and Behavior

Support: The John Templeton Foundation Grant 57876
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Title: The cost of cognitive control and the balance of random versus directed exploration

Authors: *L. A. BUSTAMANTE[1], A. R. BURTON[1], A. L. BAKER[2], A. SHENHAV[3], N. D. DAW[1], J. D. COHEN[1]
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Abstract: Evidence suggests exerting cognitive control carries an intrinsic cost and that individual differences in subjective costs may account for differences in everyday control allocation. We present two studies: study 1 develops novel methods for quantifying the cost of cognitive control; study 2 tests the hypothesis that control costs modulate the tradeoff between directed exploration (a cognitive strategy assumed to be more demanding) and random exploration.
In study 1 we assessed the relationship between two independent measures of the cost of control. The first method quantifies control costs using a standard patch foraging paradigm where participants (N=18) had to complete a control-demanding task (N-Back) to travel between patches. In a typical foraging problem, participants choose either to exploit the current patch, yielding diminishing rewards, or to switch to a new patch at the cost of travel time. When the

time cost of travel is high, participants stay longer at the current patch. To quantify control costs, we measure how long participants stay at a patch when more demanding levels of N-Back are required to travel. We predicted that participants would accept diminishing rewards in a patch to avoid control demands and used the Marginal Value Theorem to quantify the amount of reward forfeited. In the second method we estimated how many word-reading Stroop trials participants would be willing to complete to avoid a (control-demanding) color-naming trial. We found that most participants treated control as costly (i.e., made demand-avoidant choices) in both tasks and that there was a significant positive correlation between the cost of control associated with the N-Back task and with the color-naming Stroop task within a participant.
In study 2 we tested the relationship between individual differences in control costs as measured in study 1 and the balance between random versus directed exploration using the Infinite Bandits Task. In the Infinite Bandits Task, participants choose between a bandit of certain value and a bandit of unknown value. Exploring the unknown bandit yields information that may be useful for future choices at the cost of certain rewards in the present. Directed exploration provides an optimal solution to this problem involving estimating the expected value of this information. Random exploration promoted by decision noise is assumed to require less effort but on average is less remunerative. Previous research demonstrated that humans trade off these strategies. We predicted that participants who express a higher subjective cost of control will engage in more random exploration. Results of these experiments will be reported.

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Poster

168. Human Cognition and Behavior: Cognitive Development

Location: SDCC Halls B-H

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Topic: H.02. Human Cognition and Behavior

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Ruth L. Kirschstein National Research Service Award (grant number F31EY027201)

Title: Learning to read increases the informativeness of distributed ventral temporal responses

Authors: *M. NORDT[1,2], J. GOMEZ[1], V. NATU[1], B. JESKA[1], M. BARNETT[1], K. GRILL-SPECTOR[1]
[1]Vision and Perception Neurosci. Lab, Stanford, Stanford, CA; [2]Developmental Neuropsychology, Ruhr-Universität Bochum, Bochum, Germany

Abstract: Reading is a uniquely human ability, which is typically acquired during childhood. Visual processing of words - a core component of reading - is subserved by ventral temporal cortex (VTC). However, it is unknown how distributed patterns of responses across VTC to written words change as children learn how to read. Using fMRI and multivoxel pattern analyses we measured distributed responses to characters (including words and numbers) and four other visual domains in VTC in 12 children (5-9 years old), 13 preteens (10-12 years old) and 26 adults (22-28 years old). We report three main findings. First, our results reveal anatomically-specific and hemisphere-specific development of distributed information to words and characters. Specifically, we found substantial increase of word information from age 5 to adulthood in left, but not right VTC (Figure 1A), and in lateral, but not medial VTC. Second, we show that a subset of discriminative voxels (with either positive or negative preference to words) within lateral VTC contains more word information compared to the remaining lateral VTC voxels (Figure 1B). Third and crucially, we found that development of reading ability was best correlated with distributed information across voxels with positive preference to words (i.e. word-selective) in left lateral VTC (Figure 1C). These data suggest that developmental increases in word information of distributed left lateral VTC responses are linked to improvements in reading skills. Overall, our findings have important implications for theories on lateralization and category-selectivity in VTC and for elucidating neural mechanisms of reading disabilities.

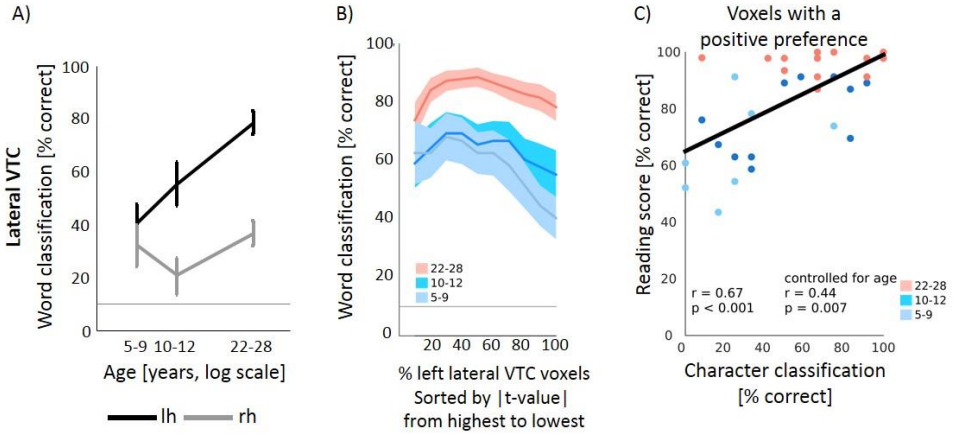

Figure 1. (A) Word classification performance in lateral VTC across age groups. Classification performance was quantified with a winner-take-all (WTA) classifier. Data show mean performance for children (5-9-year-olds, n = 12), preteens (10-12-year-olds, n = 13), and adults (22-28-year-olds, n = 26). *Error bars:* standard error of the mean (SEM). Chance level is 10% (horizontal gray line). (B) Word classification performance as a function of percentage of left lateral VTC voxels sorted by descending absolute t-value (|t-value|) for the contrast pseudowords>non-words. Sample sizes and chance level are as in (A). (C) Scatterplot and correlation between reading ability and character classification from distributed responses in left lateral VTC voxels with a positive preference to words. Only participants who completed the reading test are shown (5-9-year-olds, n = 7; 10-12-year-olds, n = 11; 22-28-year-olds, n = 19).

Disclosures: M. Nordt: None. **J. Gomez:** None. **V. Natu:** None. **B. Jeska:** None. **M. Barnett:** None. **K. Grill-Spector:** None.

Poster

168. Human Cognition and Behavior: Cognitive Development

Location: SDCC Halls B-H

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Program #/Poster #: 168.02/HHH5

Topic: H.02. Human Cognition and Behavior

Support: R01EY021755
R01MH069456

Title: Discovering and aligning cognitive functions during infant fMRI

Authors: *C. ELLIS[1], L. SKALABAN[1], J. S. TUREK[2], V. R. BEJJANKI[3], N. B. TURK-BROWNE[1]
[1]Yale Univ., New Haven, CT; [2]Intel Labs, Intel Corp., Hillsboro, OR; [3]Dept. of Psychology and Neurosci. Program, Hamilton Col., Clinton, NY

Abstract: The brain grows dramatically and heterogeneously during the first couple years of life. This poses a fundamental problem for early developmental fMRI: statistical analyses typically depend on demonstrating reliability across participants and yet it is difficult to know whether there is consistent mapping of cognitive function onto brain anatomy throughout development. This applies longitudinally, where change is most intuitive, but also cross-sectionally, as misalignment in developmental trajectories may lead to more variability than adulthood when everybody is in a mature end-state. The standard solution in adult fMRI, gross anatomical alignment, has become more viable in infants, with the recent publication of age-specific anatomical infant atlases. However, these procedures require high-quality anatomical scans that are difficult to obtain in awake infants. Moreover, anatomical methods are by definition unable to account for variability in the mapping of function to anatomy. This may be especially problematic across development, where the same function may be performed by different regions over time, or the same region may change in functionality. To address these challenges, here we explore the use of functional alignment methods for infant fMRI that are more agnostic to anatomy. We use Shared Response Modeling in the open-source software package BrainIAK, which maps voxels into a lower-dimensional representation of fMRI activity that reflects what is shared across participants while they view a common stimulus. Specifically, we showed 8 infants aged 6 to 36 months, as well as adults, short movies while they were being scanned. We found that functionally aligned participants had more similar representations of a held-out period of the movie than participants that were anatomically aligned. Interestingly, performing functional alignment with adult participants and infant participants together, rather than infant participants alone, resulted in improved performance on this metric. This suggests that functional alignment may be a valuable technique for aggregating data over development and for identifying developmental differences.

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Poster

168. Human Cognition and Behavior: Cognitive Development

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Program #/Poster #: 168.03/HHH6

Topic: H.02. Human Cognition and Behavior

Support: NIMH 2P50MH094258
The Brain Recovery Project (www.brainrecoveryproject.org)

Title: Intrinsic connectivity of the human adult brain after removal of one hemisphere

Authors: *D. KLIEMANN[1], J. M. TYSZKA[1], R. NAIR[1], J. DUBOIS[1,2], R. ADOLPHS[1], L. K. PAUL[1]
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Abstract: A remarkable example of plasticity and reorganization can be seen in patients who recover from hemispherectomy (HS), the surgical removal of one cerebral hemisphere to treat severe epilepsy. Adults who had a hemispherectomy during childhood can often live with high levels of cognitive function. Little is known about the plasticity and functional reorganization that makes such compensation possible. We investigated resting-state functional brain connectivity in 5 adults with HS to address 3 questions: i) does the residual hemisphere show reliable intrinsic functional connectivity?; ii) is this functional architecture consistent across individuals with HS? and iii) how does the connectivity in HS compare to that of healthy individuals?
We measured brain structure and function with MRI in 5 participants with HS (n = 3 right, 4 female, mean age at surgery = 7 years, cause of epilepsy: Rasmussen's encephalitis (n = 3), perinatal stroke (n = 2)). Comparison data was acquired in 61 healthy adults, imaged with identical protocols on the identical scanner (3T Trio, TR/TE= 1000/30 ms, voxel size = 2.5 mm). All participants had largely normal cognitive profiles and intellectual functioning. Measures of resting-state functional connectivity (FC) between brain regions were derived from 2 runs (eyes open, total = 460s). We performed standardized quality control and preprocessing (mriqc, fmriprep), as well as physiological denoising (e.g. white matter signal regression). Functional connectivity matrices were compared i) within-participant across runs, ii) across participants with HS, and iii) across groups (HS and control).
Initial results show that HS participants have a FC structure that is stable across runs, and shows consistency as a group. While large-scale FC structure in HS appears relatively typical (e.g. anti-correlation of default and attention networks), there are atypical features between specific components within networks (e.g., less reliable connectivity between default mode regions) that

may reflect the reorganization expected in these brains. Individuals with HS present a unique opportunity to gain insights into the principles and limits of reorganization in the human brain, its developmental timecourse, and its consequences for the compensation in cognition and behavior.

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Poster

168. Human Cognition and Behavior: Cognitive Development

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Program #/Poster #: 168.04/HHH7

Topic: H.02. Human Cognition and Behavior

Support: Louisiana Board of Regents Graduate Fellowship (2016-20)

Title: Children's learning from distraction varies by selective attention ability

Authors: *J. KING[1,2], J. C. MARKANT[3,2]
[1]Neurosci. Program, [2]Brain Inst., [3]Psychology Dept., Tulane Univ., New Orleans, LA

Abstract: Distraction is typically believed to be detrimental to learning, yet previous research has identified some contexts in which increased distraction can *benefit* learning. Declining selective attention (SA) during aging results in poorer distractor suppression, supporting enhanced memory encoding among older adults when the distracting information is task-relevant. Young children display similar poor distractor suppression due to the protracted development of SA. This study thus examined whether young children's memory can similarly benefit from task-relevant distracting information. In Experiment 1, children ages 4-8 years (N=87) completed a memory-attention task consisting of three phases: encoding, visual search, and retrieval. Children viewed multiple objects during encoding. Next, children searched for a target amongst 0, 5, 10, or 15 distractors. Half of the objects from encoding were included as "relevant" distractors. The remaining half (non-search objects) were not re-presented during the search phase. During retrieval children saw all objects from encoding and an equal number of novel objects and indicated whether they were old or new. We examined response time (RT) to detect the target during search, with slower RT indicating less efficient SA, and the difference in recognition memory sensitivity (d') for the relevant distractors versus the non-search encoding objects. Older children showed more efficient visual search and better memory for relevant distractors compared to non-search encoding images. Search behavior following target detection was the strongest predictor of older children's memory benefit for relevant distractors. Children 6 years and under showed no memory benefit for relevant distractors at a group level. However, within this younger age group, individual differences in the extent of memory benefit for

relevant distractors varied based on age and SA skills. As predicted, the youngest children who also displayed *poor* SA showed a greater memory benefit for relevant distractors. No sex differences were found in visual search or memory performance. In Experiment 2 we tested 7-8-year-old children (N=30) on the same task but prevented them from searching the array following target detection. This constraint abolished children's memory benefit for relevant distractors, suggesting that older children's increased SA abilities may prevent them from learning from distraction while SA is engaged. Thus, children can benefit from relevant distraction during learning; but, the extent of this benefit is influenced by a complex interplay between children's age, selective attention skills, and learning and memory processes.

Disclosures: J. King: None. **J.C. Markant:** None.

Poster

168. Human Cognition and Behavior: Cognitive Development

Location: SDCC Halls B-H

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Program #/Poster #: 168.05/HHH8

Topic: H.02. Human Cognition and Behavior

Title: The role of physical education on academic performance in primary school

Authors: *P. WOLLSEIFFEN[1], S. SCHNEIDER[2]
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Abstract: Introduction: Within a sedentary society sport and exercise have become popular to maintain and restore health throughout the lifespan. In the context of sport and exercise science, in the recent years health is no longer been regarded just from a physical perspective but positive effects of exercise on mental and social health have gained increased attention. Numerous studies reported on positive effects of exercise on academic achievement in school, work-life-balance, satisfaction in the workplace and a decreased risk of age related dementia. Nevertheless, the underlying neurophysiological mechanisms of the effects of an acute bout of exercise on cognitive performance remain unclear. Theories like the transient hypofrontality assume a shift of cortical activity away from frontal cortex regions during exercise, positively influencing cognitive performance post exercise. But it remains unclear if this shift of activity is primarily provoked by exercise per se or if exercise just acts as a distractor to mainly cognitive orientated content in school. This study aimed to evaluate the effects of a regular exercise class and a regular arts class on neuro-cognitive performance, thus allowing to compare between distraction and exercise. **Methods:** Sixteen school children (8-10 years, 8 boys and 8 girls) in grade three in primary school were assessed using electroencephalography before and after (1) a physical education class and (2) an art class. In addition, academic performance was assessed in a standardized assessment of educational attainment (VERA-3) following both classes. **Results:** A significant decrease ($p < .05$) of cortical current density (µV2/mm4) was quantifiable in the

frontal, parietal and temporal lobe after the physical exercise class but not the art class. No changes in cognitive performance assessed after the exercise and the arts class were obtained. **Discussion:** Despite the theory of a transient hypofrontality, activity was reduced after the exercise class in all four lobes. The fact that no straightforward effect on cognitive performance could be shown might be related to the prompt testing post exercise, the children still being in an excited state. We propose that non-major subjects play a major role in school education and are of major relevance to increase pupils concentrativeness and receptivity. The neuro-cognitive effects of exercise should be further evaluated with respect to different kinds of cognitive performance (e.g. creativity, knowledge acquisition) as well as outlasting effects.

Disclosures: P. Wollseiffen: None. **S. Schneider:** None.

Poster

168. Human Cognition and Behavior: Cognitive Development

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Topic: H.02. Human Cognition and Behavior

Support: NIH grants NS085568 (LW), NS091585 (LW), and NS075338 (LW).
National Natural Science Foundation of China 81771235, 81500989 (ZZW), 81771139, 81371199 (AW), 81371202 (HZ).

Title: Desflurane and isoflurane exposure during pregnancy decreased neuronal gene expression and induced functional deficits in juvenile offspring mice

Authors: *S. ZOU[1], H. ZHENG[2], Z. Z. WEI[3], Y. YUE[1], L. WEI[3], A. S. WU[1]
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Abstract: Surgical procedures in pregnant women may cause neurotoxic effects in the hippocampus of unborn babies and affect their brain development. The influence of surgical anesthesia in the pathological change is unclear. The present study tested the hypothesis that anesthetic exposure during pregnancy may impair cognitive and memory functions of the juvenile offspring. Pregnant mice (at the stage of gestational day 14) were treated with 10% desflurane or 1.5% isoflurane for 3 hrs. Hippocampal tissues of both fetal and offspring mice (postnatal day 31) were collected and analyzed by real-time qPCR, Western blot and immunofluorescence. Functional tests were performed to assess cognition, anxiety and memory functions in the offspring mice. The primary culture of neurons, astrocytes, and neural progenitor cells from the cortical region, olfactory bulb, lateral ventricle, dentate gyrus and other hippocampal regions in gestational day 17 and postnatal day 0 - day 3 mice were examined.

Immunostaining was performed with neural markers Nestin, DCX, Tuj1, MAP2, NeuN, GFAP, and Oligo2, cell death marker TUNEL and cell proliferation marker BrdU. In vitro experiments with desflurane and isoflurane exposure significantly increased interleukin (IL)-6 expression and apoptotic gene caspase-3 activation. Anesthetic exposure significantly reduced postsynaptic density (PSD)-95 expression in the primary hippocampal neurons. Similar changes were observed in juvenile offspring mice. Desflurane impaired learning and memory in the offspring mice. These mice showed significantly higher sensitivity to fear conditioning. The affected mice tended to stay less time in the center in the open field test. DCX-positive neuroblasts from the mice with anesthetic exposure showed significantly lower expression levels of Tuj-1, suggesting an impairment in neuronal cell differentiation. These results suggest that anesthetic exposure during surgery to pregnant mom may induce detrimental effects in the juvenile offspring mice via cell death induction and cell differentiation disruption.

Disclosures: S. Zou: None. **H. Zheng:** None. **Z.Z. Wei:** None. **Y. Yue:** None. **L. Wei:** None. **A.S. Wu:** None.

Poster

168. Human Cognition and Behavior: Cognitive Development

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Program #/Poster #: 168.07/HHH10

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant F32HD079169
Alfred P. Sloan Research Fellowship

Title: Adults vs. kids: Changes in connectivity between the amygdala subnuclei and occipitotemporal cortex

Authors: *H. A. HANSEN, Z. M. SAYGIN
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Abstract: The amygdala, a subcortical structure known for social and emotional processing, can be subdivided into multiple nuclei that each have their own unique functions and connectivity patterns. Tracer studies in adult macaques have shown that the lateral, basal, and accessory basal amygdala subnuclei are differentially connected to visual cortical areas, such that connections are stronger to anterior regions and weaker to posterior regions. Other work comparing infant to adult macaques has shown that infants have similar adult-like projections between the inferior temporal areas and the amygdala, but that infants also have additional connections with inferior temporal cortex that are either totally eliminated or refined with development. Can we delineate the connectivity between the amygdala subnuclei and the occipitotemporal cortex using noninvasive methods in humans, and will it show similar developmental differences as

macaques? To address this question, we anatomically defined the lateral, basal, and accessory basal amygdala subnuclei in 20 adult subjects and 27 kids (ages 7-8). We then combined all Freesurfer anatomical regions in the temporal and occipital cortices in each individual's native anatomy, and split this entire region into five equal sections from anterior to posterior. We also defined functional parcellations in the occipitotemporal cortex (e.g., fusiform face area and parahippocampal place area) to further understand amygdala subnuclei connectivity to these regions in kids and adults. Using Diffusion Weighted Imaging data (b-value 700s/mm^2, 60dir), we ran probabilistic tractography with FSL between the amygdala subnuclei as seeds and the occipitotemporal cortical parcellations as targets. Results showed that indeed, the mean connectivity across subjects to the occipitotemporal cortex significantly decreased on a gradient, with anterior sections showing higher connectivity and posterior sections showing little to no connectivity. Further, connectivity was significantly different from all three amygdala subnuclei, supporting previous research that each subnucleus has unique functions and connectivity patterns. Lastly, there was no significant main effect between adults and kids, but the interaction between sample and occipitotemporal region was significant, supporting previous work that connections in kids are adult-like but become more refined with age. Overall, the development of amygdalar connectivity to the occipitotemporal cortex in humans resembles that of macaques, with certain exceptions that may correspond to differences in functional specialization of the occipitotemporal targets.

Disclosures: H.A. Hansen: None. **Z.M. Saygin:** None.

Poster

168. Human Cognition and Behavior: Cognitive Development

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 168.08/HHH11

Topic: H.02. Human Cognition and Behavior

Support: NSF 1640885

Title: Different developmental trajectories for working memory and reinforcement learning contributions to learning in adolescence

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Abstract: Multiple neurocognitive systems contribute simultaneously to human decision making and learning. For example, dopaminergic projections to the striatum contribute to value-based decision making by implementing a form of reinforcement learning that slowly accumulates

reward information to estimate which choices are most valuable. Prefrontal cortex (PFC) executive functions contribute other computations, such as actively maintaining single trial information in working memory or signaling a need to switch strategy. How the systems work together is not well understood. Here, we investigate the developmental trajectory of their contributions to learning across adolescence. Because PFC and striatal regions mature at different rates, we predicted that their contributions to learning would change accordingly, with behaviors dependent on striatal function stabilizing earlier than those dependent on PFC. We collected measures of learning in 160 youth (ages 8-17 years) and 53 adults (ages 25-30) using four different reinforcement learning tasks. The tasks were selected to broadly sample multiple mechanisms contributing to learning, and included a task designed to separate out contributions of working memory from reinforcement learning (Collins&Frank). We used computational model fitting to identify individual markers of working memory (e.g., capacity) and reinforcement learning (e.g., learning rate). Contrary to our prediction, we found no effect of age on working memory. However, we found strong effects of age on reinforcement learning processes: learning rates increased linearly with age. Furthermore, younger participants were also significantly more likely to neglect negative feedback, and had an overall higher lapse rate. These results shed new light on the developmental science of learning in adolescence: children showed adult level of working-memory contributions to learning, and their weaker overall performance was linked purely to reinforcement learning, rather than executive processes.

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Poster

168. Human Cognition and Behavior: Cognitive Development

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 168.09/HHH12

Topic: H.02. Human Cognition and Behavior

Support: CONACYT 828862

Title: Cognitive development in deaf children with cochlear implant and hearing peers, aged 5 to 12 years old: A comparative study

Authors: *M. Y. PULIDO[1], H. MACÍAS-REYES[3], T. VILLASEÑOR-CABRERA[1], G. RIZO-CURIEL[2], E. LÓPEZ-TORRES[4], A. TORRES-VERGARA[3]
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Abstract: Neurodevelopment is the acquisition and maturation process of cognitive abilities that involve the environment and the intrinsic capabilities of the person, and it is affected by hearing loss. The deprivation of this process can lead to a difference in the performance of cognitive tasks between deaf children and their hearing peers. Bibliographic research shows that performance of deaf children on neuropsychological evaluation is significant lower.
The study was a case-control design. We evaluated 38 Mexican children, aged 5 to 12 years. The cases were 19 children diagnosed with profound sensorineural bilateral pre-lingual hearing loss, matched by gender and aged. The AWARD Neuropsychological test for deaf children (Daza et al. 2011) was used to evaluate vocabulary, selective attention, visuospatial abilities, visual and spatial memory, abstract reasoning, sequential process and praxias.
The majority of the evaluated children were boys (53.3%). The average aged was 7.6 years old. For the cases the average time with cochlear implant were 35 months (+-28) and 56.47 months assisting to language therapy. Lastly hearing children had a better cognitive performance on receptive vocabulary $t(38) = -5.05$, $p= 0.003$ and spatial memory $t(38)=-1.70$ tasks, $p= 0.006$. These results suggest that despite having a cochlear implant, spatial memory and receptive vocabulary are less developed in deaf children than their hearing peers. Currently, we are on the process of getting a bigger sample and a third case group formed by deaf children without cochlear implant.
The findings from this study support those previously found by other studies where it has been observed that deaf children had a performance improvement with the use of cochlear implant but without reaching the performance of their hearing peers.
Gratefulness: PHD. Ma. Teresa Daza-González, Universidad de Almeria, Spain.

Disclosures: H. Macías-Reyes: None. **T. Villaseñor-Cabrera:** None. **G. Rizo-Curiel:** None. **E. López-Torres:** None. **A. Torres-Vergara:** None.

Poster

168. Human Cognition and Behavior: Cognitive Development

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 168.10/HHH13

Topic: H.02. Human Cognition and Behavior

Title: Inter-subject dynamic functional connectivity: Tracking functional network fluctuations during movie watching

Authors: *M. D. ROSENBERG[1], D. C. GRUSKIN[1], E. S. FINN[2], A. J. HOLMES[1]
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Abstract: An individual's pattern of functional brain connectivity predicts abilities including attention and fluid intelligence (Rosenberg et al., 2016; Finn et al., 2015). Although work

suggests that these patterns vary over time (Hutchison et al., 2013), the relationship between dynamic connectivity and ongoing cognition remains unclear. To relate fluctuating connectivity, ongoing perception and cognition, and individual differences in behavior, we analyzed data from the Healthy Brain Network (Alexander et al., 2017). Structural and functional MRI data, collected as participants age 5-20 viewed a 10-min clip of the film Despicable Me, were separated into a discovery (n=73) and a replication (n=74) sample. Functional data were preprocessed, divided into 268 brain regions (Shen et al., 2013), and submitted to a sliding window correlation analysis. In every 20-sec (25-TR) time window (step=1 TR), every pair of node-wise fMRI signal timecourses was correlated to generate a whole-brain functional connectivity matrix (723 matrices/participant). Next, for each connection in these matrices, the timecourse of correlation values was averaged across participants to obtain a measure of how connectivity strength fluctuated at the group level across the movie. Although previous work suggests that sliding window correlations are influenced by head motion and sampling variance (Laumann et al., 2017), averaging connection timecourses across individuals should eliminate noise and leave stimulus-related signal (Hasson et al., 2004). Using this novel inter-subject dynamic functional connectivity approach, we found that mean timecourses of intra-occipital connections were highly conserved between the discovery and replication samples (mean cross-sample r=.72). On average, timecourses of connections involving occipital, parietal, and temporal cortex—critical for vision, spatial attention, and audition—were the most similar across samples, whereas timecourses of connections involving prefrontal, motor, and insular regions were less similar. Suggesting that connectivity fluctuations were influenced by distinct features of the film, we observed a dissociation in both the discovery and replication samples such that limbic-parietal connections tracked the mean number of faces on the screen but not low-level stimulus intensity (mean luminance across RGB color values), whereas prefrontal-prefrontal connections tracked color intensity but not faces. Looking ahead, individuals' connectivity fluctuations during naturalistic tasks may inform individual differences in processes such as perception, attention, and memory across development.

Disclosures: M.D. Rosenberg: None. **D.C. Gruskin:** None. **E.S. Finn:** None. **A.J. Holmes:** None.

Poster

168. Human Cognition and Behavior: Cognitive Development

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 168.11/HHH14

Topic: H.02. Human Cognition and Behavior

Support: CONACYT 828882
CONACYT 828875

Title: Cognitive processes of preschool children exposed to neurotoxic substances, living in Agua Caliente, Poncitlan Jal, Mexico

Authors: *A. L. RODRIGUEZ[1], K. A. CASTELLANOS-HUERTA[1], T. VILLASEÑOR-CABRERA[1], J. GARCIA-ESTRADA[1,2], G. RIZO-CURIEL[2], F. LOZANO-KASTEN[2], A. A. P. LUCANO[3]
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Abstract: Genetic, biological, psychological, and environmental factors are essential aspects during cerebral maturation. Early exposure to neurotoxic substances may affect the Central Nervous System (CNS) and consequently affect children's performance in activities involving cognitive processes and executive functions.
Agua Caliente is a small, rural town located next to Lake Chapala, where most of the inhabitants belong to very low-income families, in some cases living in extreme poverty and suffering from malnutrition. The main economic activities in this town are fishery and chayote agriculture, so the use of agrochemicals and pesticides is frequent. In addition, the use of fire wood for cooking and burning trash results in significant environmental pollution in the area. Chronic exposure to low concentrations of neurotoxic substances affects the synaptic connection in the immature brain which may cause deficiencies in attention, orientation, memory, motor coordination, language and executive functions.
The aim of this study is to research the correlation between the cognitive functions of children between 4 and 5 years old and the presence of pesticides in the community.
30 children were evaluated in this study, out of which half were male, and the mean age of the participants was 5 years. Their neuropsychological development was measured using the Neuropsychological Battery –BANPE- for preschoolers (Ostrosky et. al, 2016), and the presence of neurotoxins was assessed through urine samples analyzed by high performance liquid chromatography (HPLC), Quadrupole triple mass spectrometer (Ms-Ms) and Gas chromatography–mass spectrometry (GC-MS).
Significant correlation was observed among the presence of glyphosate and comprehensive language ($p = .021$), 2,4-dichlorophenoxyacetic acid with articulatory language, ($p = .035$) and theory of mind ($p = .010$), dimethoate and memory ($p = .002$), as well as metoxuron ($p = .000$) with the same cognitive process. metoxuron with expressive language ($p = .013$), molinate with working memory ($p = .037$) and abstraction, ($p = .028$).
Prenatal and postnatal exposure to pesticides affected the cognitive development of these children. For this reason, a cognitive intervention program should be implemented in Agua Caliente, as well as nearby communities.

Disclosures: A.L. Rodriguez: None. **K.A. Castellanos-Huerta:** None. **T. Villaseñor-Cabrera:** None. **J. Garcia-Estrada:** None. **G. Rizo-Curiel:** None. **F. Lozano-Kasten:** None. **A.A.P. Lucano:** None.

Poster

168. Human Cognition and Behavior: Cognitive Development

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 168.12/HHH15

Topic: H.02. Human Cognition and Behavior

Title: Implicit and explicit Theory of Mind tasks are related to maturation of gray matter structure in distinct networks in the brain

Authors: *C. GROSSE WIESMANN[1,2], A. D. FRIEDERICI[3], N. STEINBEIS[4], T. SINGER[2]
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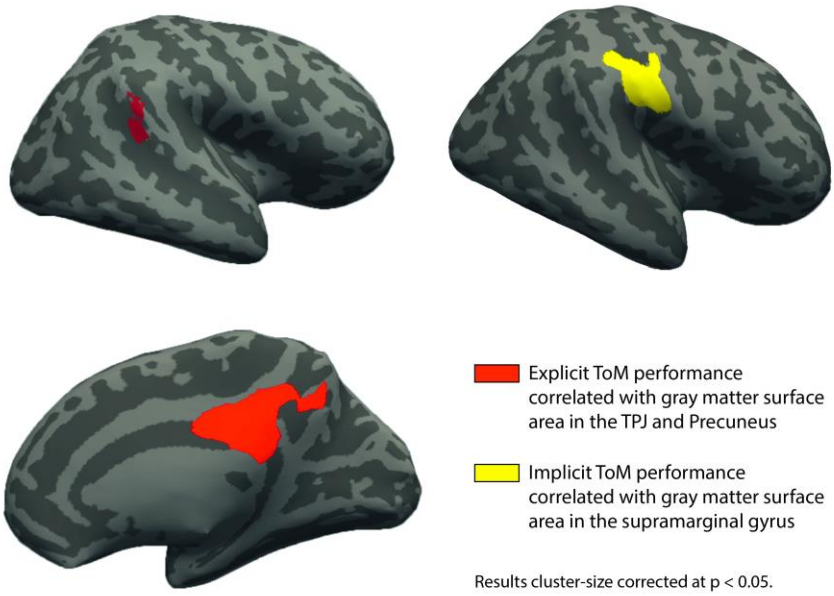
Abstract: Human interaction crucially relies on our ability to infer what is on other people's mind, an ability referred to as having a Theory of Mind (ToM). Decades of research assumed that, in children, this ability emerges around the age of 4 years, when children start passing traditional ToM tasks that ask them to explicitly reason about others' beliefs. Recently, however, novel implicit paradigms have shown that infants younger than 2 years of age already have correct expectations on how another individual will act depending on his or her belief. These findings have created one of the biggest puzzles of current developmental psychology: Why do children consistently fail the traditional explicit ToM tasks, if already infants have correct belief-related expectations in the implicit ToM tasks? Do the implicit and explicit ToM tasks measure the same ability? Or how do infants solve the implicit ToM tasks? The cognitive and neural factors that underlie the different ToM tasks and determine the emergence of a mature ToM in development are not understood to date.
Here, we related cortical thickness and gray matter surface area assessed with brain-structural magnetic resonance imaging (MRI) in 38 children aged 3- and 4-years with their ToM performance in implicit and explicit ToM tasks. This showed that the emergence of explicit ToM reasoning between 3 and 4 years was associated with the maturation of gray matter in regions of the classical ToM network, that is, the precuneus and right temporoparietal junction. The implicit ToM task, in contrast, correlated with gray matter maturation in the supramarginal gyrus. These effects were independent of one another and independent of other co-developing cognitive abilities (i.e., language and executive function). This dissociation on the neural level reflects the behavioral finding of different developmental trajectories and no correlation between implicit and explicit ToM tasks.
Taken together, these results suggest that the processes involved in implicit ToM tasks are different from those required for explicitly reasoning about others' mental states.



Disclosures: C. Grosse Wiesmann: None. **A.D. Friederici:** None. **N. Steinbeis:** None. **T. Singer:** None.

Poster

168. Human Cognition and Behavior: Cognitive Development

Location: SDCC Halls B-H

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Program #/Poster #: 168.13/HHH16

Topic: H.02. Human Cognition and Behavior

Support: NSF Grant DRL-1644540

Title: Neural correlates of phonological processing in dyslexia and comorbid dyslexia-ADHD

Authors: *D. WILMOT[1], A. D'MELLO[1], R. ROMEO[1], C. PEEK[2], O. MEEGODA[3], T. CENTANNI[4], K. HALVERSON[5], J. D. E. GABRIELI[1], J. CHRISTODOULOU[3]
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Abstract: Dyslexia presents as difficulty reading single words accurately and/or fluently in the context of intact cognitive skills. While dyslexia impacts around 10% of the population, up to 42% of individuals with dyslexia also carry a diagnosis of Attention Deficit Hyperactivity Disorder (ADHD). Both dyslexia and ADHD are associated with reading difficulties, yet it is not known whether the neuro-behavioral correlates of reading difficulties in children with dyslexia are similar to those in children with comorbid dyslexia and ADHD. The current research

combined behavioral, clinical, and neuroimaging methods to understand the neural basis of reading difficulty in dyslexia only, or with ADHD.
A total of 44 children (9-12 years old) completed standardized behavioral assessments of reading and related skills, and a neuroimaging session. Groups were dyslexic (n=16, DYS), comorbid (n=15, DYS+ADHD) and typically developing (n=13, TD). During functional magnetic resonance imaging (fMRI), participants completed a reading task during which they indicated via button press whether two words rhymed, or whether two faces matched. Reaction time and accuracy measures were recorded. We examined whether behavioral and/or neuroimaging measures differed between groups. Across all verbal and non-verbal behavioral measures assessed, only measures of non-verbal IQ differentiated DYS and DYS+ADHD groups. Standardized reading measures typically used for diagnostic purposes failed to differentiate DYS and DYS+ADHD groups (e.g., timed and untimed measures of single word reading such as the *Test of Word Reading Efficiency* and *Woodcock Reading Mastery Tests*). Measures of in-scanner reading task performance also failed to differentiate clinical groups; both DYS and DYS+ADHD groups showed reduced accuracy and slower reaction time compared to TD on the word-rhyming condition. However, significant differences were apparent in fMRI results during the reading task such that DYS+ADHD showed greater activation in temporo-parietal regions during word rhyming than the DYS group.
These results reveal unique contributions of neuroimaging to the evaluation of underlying mechanisms in dyslexia with or without ADHD. While behavioral measures did not differentiate reader groups, fMRI findings indicate significant differences in the brain regions recruited for reading. Discussion will address the potential of a multifaceted approach to understanding the variability in reading disabilities.

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Poster

168. Human Cognition and Behavior: Cognitive Development

Location: SDCC Halls B-H

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Program #/Poster #: 168.14/HHH17

Topic: H.02. Human Cognition and Behavior

Support: Solomon Center for Neurodevelopmental Research

Title: Early active non-linguistic acoustic experience supports more efficient syllabic processing in infants

Authors: *S. ORTIZ-MANTILLA, T. REALPE-BONILLA, A. A. BENASICH
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Abstract: Exposure-based plasticity facilitates establishment and ongoing modification of cortical representations as infants become familiar with the distinctive characteristics of the surrounding linguistic environment. Across development, acoustic mapping and oscillatory entrainment allows detailed syllabic encoding, enabling fast, automatic responses to incoming language. Our previous studies have shown (1) that during syllable processing, phase synchrony of theta oscillations in auditory cortices decreases from 6- to 12-months as processing of native syllables becomes more efficient; and (2) that interactive acoustic experience, using temporally modulated non-speech stimuli, enhances both accuracy and speed of discrimination of known and novel key pre-linguistic acoustic cues at 7-months-of age. To explore whether such experience-dependent effects of early non-speech auditory engagement generalizes to syllable processing, infants who had received interactive acoustic experience between 4- and 6- months-of-age were presented at 7-, 9-, 12- and 18-months, with a consonant-vowel contrast in a passive auditory oddball paradigm and compared to age matched cross-sectional naïve controls. Dense-array EEG/ERP (124 sensors) was collected and mapped onto age-appropriate brain templates. Source modeling placed dipoles in both auditory cortices. Temporal-spectral analyses were conducted in source space within the 2-90 Hz frequency range using 1 Hz-wide frequency bins and time resolution of 50ms. Phase synchrony was measured at each age using ITPL (inter-trial phase locking) to examine consistency of phase alignment across trials. When processing the standard syllable /da/, and in line with our previous work, both groups showed less theta ITPL at 12- than at 7-months. However, the specific timing of this maturational process differed as a function of previous acoustic experience. While naïve controls decreased ITPL from 9- to 12-months showing no decline from 7- to 9-months, the interactive group decreased ITPL from 7- to 9-months and showed no decrease between 9- and 12-months. These results suggest that early non-speech acoustic experience modulates theta range phase synchrony, enabling infants who received non-speech acoustic experience to efficiently process syllabic information earlier than naïve controls. As theta closely tracks syllabic information, efficient theta phase synchrony may confer a significant speech processing advantage by facilitating syllable tracking and entrainment, enabling parsing of ongoing speech into meaningful chunks to be decoded, and thus increasing syllable perception and speech intelligibility.

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Poster

168. Human Cognition and Behavior: Cognitive Development

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Program #/Poster #: 168.15/HHH18

Topic: H.02. Human Cognition and Behavior

Support: NSF SL-CN SMA-1640909

Title: Learning to make decisions based on sampling of probabilistic outcomes

Authors: J. ADRIAN[1], J. SNIDER[2], *L. CHUKOSKIE[3]
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Abstract: Every day we make decisions with uncertain outcomes - may it be which ice cream flavor or which life partner to pick. Sampling can help reduce uncertainty by learning the association between decisions and outcomes. The ability to implicitly track probabilistic information is already present in infants, for example during language acquisition. However, we lack a nuanced understanding of how children and adults make explicit use of this information during decision-making under uncertainty. We designed a task to test how implicit probabilistic information is utilized for decision making in a wide range of ages. We also designed the task to offer different amounts of complexity and so that the results are amenable to standard models of probabilistic decision-making behavior.
For choosing between differentially rewarding options under uncertainty, there are two common strategies: matching and maximizing. When matching, options are chosen in proportion to their underlying reward rates, while maximizing is consistently choosing the most rewarding option. Matching behavior is commonly observed in humans and other animals even though it is theoretically less rewarding than maximizing. To shed light on the emergence of these strategies, we used simple (3 arms) and complex (36 arms) multi-armed bandit tasks and matching spatial working memory tasks in children (3-12 year olds) and adults. The multi-armed bandits are baited with the location of the reward determined by a fixed but unknown probability distribution. Participants performed at least 60 trials in a session, with a trial comprising the total search for the target until found.
We determined (1) if and how learning to make decisions from sampling changes across development, (2) if individual differences in decision-making can be explained by spatial working memory capacity, and (3) if decision-making strategies used on a simple 3-armed bandit can be generalized to strategies used on a more complex task with 36 arms. Our results will inform models of decision making during development and how we might best model this type of decision making.

Disclosures: J. Adrian: None. **J. Snider:** None. **L. Chukoskie:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BrainLeap Technologies.

Poster

168. Human Cognition and Behavior: Cognitive Development

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Program #/Poster #: 168.16/HHH19

Topic: H.02. Human Cognition and Behavior

Support: CONACYT 238313
CONACYT 221092
PROMEP NPTC 236855
CONACYT STUDENT GRANT 828865

Title: Assessment of developmental outcome and serum BDNF in young infants exposed to general anesthesia

Authors: *J. C. CUPUL GARCIA[1], Y. RUVALCABA DELGADILLO[2], F. JAUREGUI[2]
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Abstract: Background: Annually millions of children undergo some surgical procedure that requires general anesthesia. The drugs used to induce general anesthesia interfere with the function of N-methyl-Daspartate (NMDA) and Gamma-Aminobutyric Acid (GABA) receptors. The correct functioning of these receptors is crucial during the period of cerebral maturation since them promotes the biosynthesis and secretion of Brain-Derived Neurotrophic Factor (BDNF). The effects of general anesthesia exposures on maturing human brains and the probable alterations in infant development are not yet well described. Aims: In this study, we are using the Bayley Scales of Infant Development to investigate developmental outcomes of early exposure to general anesthesia. We are also correlating these scales with changes in serum levels of the Brain-Derived Neurotrophic Factor to clarify whether the exposure to general anesthetics is a risk factor for Global developmental delay. Methods: We are conducting a cohort study with cases and controls. Children under 3 years of age with a single exposure to general anesthesia has been included and compared with healthy non-exposed controls at the same age and sex. Infant development has been evaluated with the Bayley Scales of Infant and Toddler Development-III. The Human BDNF Colorimetric ELISA Kit will be used to determine the serum levels of BDNF. The study protocol was submitted and approved by the ethics committee of the “Hospital Civil de Guadalajara Fray Antonio Alcalde” registration number 237/17. Results: Until now, the age range of subjects evaluated is 6-24 months. Most of the evaluated subjects are females. The results obtained in the Bayley-III Scales are direct scores that when compared with their respective control evidenced 'risk' of developmental delay in some of the cases. The BDNF analysis will be obtained until the sampling is completed. Conclusions: For the time being, we may assume that general anesthetics may represent at least, a slight risk for developmental delay in some of the children. To confirm or refuse that, we are increasing the number of cases and following them at longer periods.

Disclosures: J.C. Cupul garcia: None. **Y. Ruvalcaba Delgadillo:** None. **F. Jauregui:** None.

Poster

168. Human Cognition and Behavior: Cognitive Development

Location: SDCC Halls B-H

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Program #/Poster #: 168.17/HHH20

Topic: H.02. Human Cognition and Behavior

Support: NSF/BSF BCS #1551330
Washington Research Foundation Funds for Innovation in Neuroengineering

Title: Word selectivity in high-level visual cortex and reading skill

Authors: *E. KUBOTA[1], S. J. JOO[2], E. HUBER[2], J. D. YEATMAN[2]
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Abstract: Word-selective neural responses in human ventral occipito-temporal cortex (VOTC) emerge as children learn to read, creating a visual word form area (VWFA) in the literate brain. It has been suggested that the VWFA arises through competition between pre-existing selectivity for other stimulus categories, changing the topography of VOTC to support rapid word recognition. Here, we hypothesized that competition between words and objects would be resolved as children acquire reading skill. Using functional magnetic resonance imaging (fMRI), we examined the relationship between responses to words and objects in children (N = 24, M = 9.94 years, SD = 1.57) with a wide range of reading skills. We used a one-back task, where subjects were shown images of words, faces, and objects, and asked to respond when an image repeated. Then, we defined regions of interest in individual subjects' native space to examine the relationship between responses to words and objects in two ways. First, we defined the VWFA using a words > objects contrast and found that only skilled readers had this region; for most struggling readers and children with dyslexia there was not a region in VOTC responding more to words than objects. Second, we defined the VWFA using a words > faces contrast and examined selectivity for words over objects within this region. We found that selectivity for words over objects strongly correlated with reading skill (r = 0.81, $p<0.0001$), suggesting reading skill-dependent tuning for words. Furthermore, we found that low word selectivity in struggling readers was not due to a lack of response to words, but due to a high response to objects. Our results suggest that the fine-tuning of word-selective responses in VOTC is a critical component of skilled reading.

Disclosures: E. Kubota: None. **S.J. Joo:** None. **E. Huber:** None. **J.D. Yeatman:** None.

Poster

168. Human Cognition and Behavior: Cognitive Development

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Program #/Poster #: 168.18/HHH21

Topic: H.02. Human Cognition and Behavior

Support: R01 MH085328-12
R01 MH078160-10
R01 MH106564-03

Title: Left inferior parietal connectivity is correlated with motor skill representation in typically developing children

Authors: *N. F. WYMBS[1,2], S. H. MOSTOFSKY[1,2]
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[2]Neurol., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: The neural circuitry of motor skill representation (praxis) is essential for typical child development. In adults, the left inferior parietal lobe (IPL) performs a vital role in the representation of everyday skill irrespective of the hand used, such as pantomime of familiar actions. Currently, less is known of how left IPL connectivity affects praxis in typically developing children. Our aim was to test how whole-brain IPL functional connectivity is related to praxis in children as assessed by action pantomime performance.
We acquired resting state functional magnetic resonance imaging (rfMRI) scans from 193 children (8-12 years). Dual regression and masked independent components analysis (ICA) was used to estimate functional connectivity of the left inferior parietal lobe (IPL). Left IPL subnetworks were first derived from an independent sample (n =60). Using temporally concatenated ICA, a left IPL parcellation was estimated by assigning each IPL voxel to 1 of 4 potential ICs. Four dual regressions were then performed to estimate subject-specific time courses and spatial maps (n = 133). We tested for brain-praxis behavior relationships by including measures of gesture and imitation ability (Florida Apraxia Battery modified for children) and motor coordination (movement assessment battery for children, MABC-2) as covariates.
We observed 4 IPL-based networks: (1) anterior supramarginal gyrus (SMG) - sensorimotor; (2) parietal operculum - perisylvian; (3) posterior SMG - frontoparietal; (4) posterior SMG - default mode. Gesture performance was inversely correlated with anterior SMG - sensorimotor connectivity. Strong gesture ability was associated with reduced connectivity with the bilateral sensorimotor cortex, right DLPFC, superior parietal cortex (SPL), and cerebellum. Further, connectivity between the anterior SMG and left SPL, middle temporal and inferior frontal gyri, and cerebellum was correlated with gesture and throwing and catching ability (MABC -2).

These results suggest that the representation of everyday motor skill in children, or praxis, is associated with reduced connectivity between the left anterior SMG and functionally connected sensorimotor and higher-level cognitive regions. This supports a model of neural efficiency for familiar skills, consistent with models of skill representation during long-term learning. Further, the overlap between pantomime and throwing and catching ability suggests a role for visuospatial ability in the expression of action knowledge through gesture. These findings provide initial evidence towards understanding the role of the left IPL in how children represent motor skills.

Disclosures: N.F. Wymbs: None. **S.H. Mostofsky:** None.

Poster

168. Human Cognition and Behavior: Cognitive Development

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 168.19/HHH22

Topic: H.02. Human Cognition and Behavior

Support: NSF Grant 1649865

Title: Network motifs in the developing brain support multi-domain cognitive function in naturalistic settings

Authors: *C. STAMOULIS[1], F. H. DUFFY[2], P. L. PEARL[2]
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Abstract: To support multisensory processing and the demands of cognitive function across domains in dynamically varying environments, it has long been hypothesized that the brain uses a relatively small set of 'building blocks' (neural primitives or motifs) in theoretically infinite combinations. The emergence, role and variability of these motifs as a function of cognitive domain, sensory modality and/or neural maturation in the developing brain remain elusive. Invasive EEG data from 12 pediatric patients with epilepsy (age 2-22 years; 7 males, 5 females) but otherwise no deficits in sensory processing, collected in an unsupervised and relatively naturalistic setting as patients went about their day, were analyzed over long periods of wakefulness (<5 to 15 hours). Analyzed epochs were far removed from seizure activity (hours before or after seizures). Collectively, the recordings sampled distributed brain areas, including multiple elements of the visual, auditory and language networks. Continuous video was also examined concurrently with the EEG to assess what the patient was doing and who they were interacting with. Functional networks were estimated using probabilistic (information theoretic) measures of directional pairwise association, which are typically robust to the inherent noise in continuously recorded (and thus very high-dimensional) brain signals. A spectral clustering algorithm was used to classify network patterns. Across individuals, brain areas and frequency

bands, a small number (<10) of network motifs was identified, which occurred repetitively over long periods of time, as patients were engaged in social communication and processed multi-modal sensory inputs. The most-frequently occurring motifs were 3-node fully bidirectional, 3-node partially bidirectional and 4-node partially bidirectional motifs. The number of motifs decreased non-significantly as a function of age. The small set dimension of these motifs suggests that despite profound changes in neural circuitry during development, the brain consistently uses a small number of building blocks to maximize efficient and parsimonious processing of myriads of inputs from the outside world and respond to cognitive demands.

Disclosures: C. Stamoulis: None. **F.H. Duffy:** None. **P.L. Pearl:** None.

Poster

168. Human Cognition and Behavior: Cognitive Development

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 168.20/HHH23

Topic: H.02. Human Cognition and Behavior

Support: Brain/MINDS
AMED
CREST

Title: Better understanding but reduced functional integration across brain regions for vocoded speech in autism

Authors: *I.-F. LIN[1], T. ITAHASHI[2], M. KASHINO[4,5], N. KATO[3], R.-I. HASHIMOTO[3]
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Abstract: Previous studies show individuals with autism spectrum disorders (ASD) have worse performance in the speech-in-noise task than neurotypical (NT) individuals, suggesting different auditory processing in ASD. This study was designed to elucidate the correlation between their brain activation patterns and speech understanding under adverse listening conditions.
We recruited 21 ASD and 24 NT adults for the fMRI experiment, and we added another 28 ASD and 24 NT adults for diffusion tensor imaging (DTI). Three kinds of speech materials were presented: clear speech (CS), 8-channel noise-vocoded speech (VS), and spectrally-rotated noise-vocoded speech (RVS). All participants received VS training in the pre-test before the fMRI experiment. In the fMRI experiment, alternating block design for different speech materials was chosen to enhance activation detection, and participants were requested to judge whether the speech materials were intelligible or not. In the post-test, participants listened to a set of sentences and gave the key words. In the pre-test, ASD individuals and NT individuals did not

differ, but ASD individuals showed better performance in the post-test.
The effect of intelligibility was measured by the difference in functional activation between the intelligible speech conditions (CS+VS) and the unintelligible speech condition (RVS). The effect of task difficulty was measured by the difference in functional activation between the easy-to-judge tasks (CS+RVS) and the difficult-to-judge task (VS). For both effects of intelligibility and task difficulty, significant group difference in functional connectivity between left dorsal PMC (dPMC) and left temporal-parietal junction (TPJ) was observed. Furthermore, this effect of task difficulty in functional connectivity was negatively correlated with the ASD participants' autism spectrum quotient scores.
Left dPMC is important for auditory-motor interaction, and left TPJ contains Wernicke's area and the angular gyrus, which are important for speech comprehension. The connection between left dPMC and left TPJ is part of the dorsal auditory pathway that supports input driven auditory-to-motor mapping, which is proposed to facilitate speech comprehension in adverse listening conditions. Nevertheless, we did not observe significant group difference in DTI results between left dPMC and left TPJ. Together, these findings indicate that functional systems that use auditory-motor mapping to facilitate speech comprehension are affected in individuals with ASD, but their structural pathways associated with these functions were not distinguishable from controls.

Disclosures: I. Lin: None. **T. Itahashi:** None. **M. Kashino:** None. **N. Kato:** None. **R. Hashimoto:** None.

Poster

168. Human Cognition and Behavior: Cognitive Development

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 168.21/HHH24

Topic: G.05. Anxiety Disorders

Support: NIH Grant R01 MH094633-01

Title: Functional connectivity during stimulus-driven and goal-directed threat processing in childhood risk for anxiety

Authors: *B. C. TABER-THOMAS[1,2], X. FU[3], E. AUDAY[3], K. PEREZ-EDGAR[3]
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Abstract: Anxiety is associated with altered patterns of attention to threatening information, and it has been suggested that biases to attend to threat in childhood play a role in later anxiety-related outcomes (Pérez-Edgar et al., 2010). Researchers have begun to elucidate the complex frontolimbic neural system involved in threat-biased social information processing, which

includes networks for stimulus-driven (Monk et al., 2008) and goal-directed (Monk et al., 2006) processing (Corbetta and Shulman, 2002). Summarizing across studies, this early work suggests that greater anxiety is related to increased engagement of the stimulus-driven neural system in response to subliminal threat, and increased engagement of the goal-directed system, as well as behavioral attention bias to threat, in response to supraliminal threat. However, relations between anxiety and patterns of stimulus-driven and goal-directed functional connectivity are less well understood and have not been explored in behaviorally inhibited (BI) children who are at heightened risk for anxiety. The present study probed functional connectivity of the amygdala in high versus low BI children ages 9-12 (N=48) during both subliminal and supraliminal threat-related attention (dot-probe) tasks. We predicted that compared to children low in BI, children high in BI would exhibit increased amygdala connectivity with the stimulus-driven neural system for masked stimuli (reflecting heightened stimulus-driven attention to threat), and increased amygdala connectivity with the goal-driven system for unmasked stimuli (reflecting heightened goal-directed attention to threat). Psychophysiological interaction analyses were performed to examine relations between BI and brain connectivity of stimulus-driven and goal-directed attention networks during threat versus neutral conditions. Contrary to expectations, high BI was associated with lower amygdala connectivity during threat-related attention with brain networks involved in both stimulus-driven and goal-directed attention. Specifically, compared to low BI children, high BI children had reduced amygdala connectivity with the neural systems involved in (i) stimulus-driven attention to threat (TPJ, anterior insula, dlPFC), and (ii) visual processing during goal-directed attention to threat (occipital cortex). Taken together, these findings suggest that among children high in BI there may be a disruption in stimulus-driven and goal-directed neural responses to threat. Potential explanatory models of these findings—over-control versus fear disconnection—will be discussed.

Disclosures: B.C. Taber-Thomas: None. **X. Fu:** None. **E. Auday:** None. **K. Perez-Edgar:** None.

Poster

169. Human Cognition and Behavior: Timing and Temporal Processing

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 169.01/HHH25

Topic: H.02. Human Cognition and Behavior

Support: Vanier Canada Graduate Scholarship, NSERC
Canadian Institutes of Health Research

Title: Neural oscillatory mechanisms for interpersonal entrainment in music ensembles

Authors: *A. CHANG[1], P. CHRAPKA[2], D. J. BOSNYAK[1,3], L. J. TRAINOR[1,3,4]
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Abstract: People need to process complex dynamic information when coordinating with others in joint action, conversation and music. Previous EEG studies suggest that neural oscillations entrain to ongoing rhythmic temporal regularities in sensory input and predictively facilitate upcoming sensory perception and motor responses. However, existing studies have several limitations. First, most studies use highly artificial rhythmic stimuli, so it is unclear whether these findings generalize to real-world music and speech, which contain considerable tempo variability. Second, real-world interpersonal entrainment is typically bidirectional, but most neural entrainment studies examine an individual's neural entrainment to a fixed preprogrammed stimulus. We measured body sway and EEG in two professional string quartets (n = 8 musicians) as a real-world example of dynamic sensorimotor interpersonal interaction. We experimentally manipulated leadership, assigning a different musician as leader on each short performance. To investigate the direction and the magnitude of interpersonal entrainment among performers at both the behavioural and neural levels, we used Granger causality, in which the time series of movements or EEG from one musician are used to predict the upcoming movements or EEG from a second musician over and above prediction within a time series. We hypothesized that (1) leaders would influence others more than followers, and (2) the overall magnitude of interpersonal entrainment would predict the quality of the performance on a trial-by-trial basis. Body sway analyses revealed that, as predicted, leaders influenced follower more than vice versa, and the higher the total entrainment strength among the performers, the better the performance quality. For the EEG analyses we used a beamformer to reconstruct the source waveforms generated from auditory cortex, visual cortex, supplementary motor area (SMA) and frontal regions (DLPFC & VLPFC). Initial analyses showed that within-individuals, connectivity was stronger from auditory and visual cortex to SMA and frontal regions than in other directions, which is consistent with the view that sensory information guides executive control (frontal) and motor planning (SMA). Furthermore, across musicians, leader's neural oscillations across all regions better predicted follower's auditory neural oscillations than vice versa. Thus we have shown that interpersonal entrainment can be represented in neural oscillations and reflect directional influences between people.

Disclosures: A. Chang: None. **P. Chrapka:** None. **D.J. Bosnyak:** None. **L.J. Trainor:** None.

Poster

169. Human Cognition and Behavior: Timing and Temporal Processing

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 169.02/HHH26

Topic: H.02. Human Cognition and Behavior

Support: MEXT KAKENHI 16H01513

Title: Perspective-taking interacts with temporal information processing in sentence comprehension

Authors: *S. TOKIMOTO[1], N. TOKIMOTO[2]
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Abstract: This study examines the neural substrate of perspective-taking by analyzing the electroencephalogram (EEG) elicited by the auditory comprehension of sentences for which the comprehender had to adopt the perspective of the person described in them. Recent studies suggest that the ability of perspective-taking can be an integrative function of temporal and spatial information processing. We thus examined the independence and possible interaction of human perspective shifts and temporal perspective-taking by utilizing Japanese subsidiary giving verbs, namely "-ageru" and "-kureru." We manipulated human perspective shifts and temporal perspective-taking independently in experimental sentences by syntactically changing the subject and the object between the speaker and a third person, while we manipulated the tense to be past or non-past tense via sentence-final particles "ru/ta" (non-past/past). The EEG analyses via electrodes indicated the suppression of the beta band for human perspective shifts in sentences in non-past tense and the absence of such suppression in sentences in past tense. The analyses for the clusters of independent components indicated beta suppression for past tense against non-past tense in sentences without a human perspective shift. This response pattern suggested a close relationship between human perspective shift and temporal perspective-taking. The beta suppression for the human perspective shift in our experiment can be understood as a replication of the previous EEG findings observed for perspective-taking in the presentation of visual images. The preceding findings and our result suggest that the ability or the function of perspective-taking is modality-general. Furthermore, the generator of the beta suppression for past tense against non-past tense without human perspective shift was localized in the precuneus, which is consistent with the recent findings indicating that the precuneus is deeply involved in time perception.

Disclosures: S. Tokimoto: None. **N. Tokimoto:** None.

Poster

169. Human Cognition and Behavior: Timing and Temporal Processing

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 169.03/HHH27

Topic: H.02. Human Cognition and Behavior

Support: FWO G.0379.06

Title: Expert musicians keep brain ageing at bay: Neural mechanisms protect movement timing skills from decline

Authors: *R. KRAMPE[1], N. WENDEROTH[2], S. SWINNEN[1]
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Abstract: Expert performance marks the boundaries of the human brain's adaptive capability. Understanding how experts acquire their skills and continue to excel until late adulthood is a major challenge for cognitive neuroscience. Expertise theory predicts that novice performance is constrained by domain-general mechanisms like working memory and cognitive control, while experts rely on domain-specific mechanisms to circumvent normal processing limitations. In laboratory settings experts in their 60s typically perform at young expert levels and outperform young novices in tasks related to their skill. How the expert brain escapes age-related neurocognitive decline is still unknown, partly because the field has yet to achieve a clear understanding of the neural mechanisms underpinning high-level performance resulting from life-long training. Here we show that older expert musicians rely on specialized brain areas (the same relied upon by young musicians) to protect their skill from the negative effects of aging. Brain activity was measured while experts and novices in different age groups performed rhythmic timing tasks. We observed that young and older novices engaged a cognitive control network comprising parieto-frontal regions known for their susceptibility to age-related decline. In contrast, young and older experts activated predominantly sensorimotor areas, even for complex rhythms. While older experts performed at the level of young musicians in motor timing tasks, they showed the same age-related decline as age-matched novices in general processing speed and cognitive control. Older experts can selectively maintain a high skill level, because domain general functions that undergo massive age-related decline play a subordinate role for their performance.

Disclosures: R. Krampe: None. **N. Wenderoth:** None. **S. Swinnen:** None.

Poster

169. Human Cognition and Behavior: Timing and Temporal Processing

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 169.04/HHH28

Topic: H.02. Human Cognition and Behavior

Title: The role of temporal coupling of neural activities in mutual information underlying action and perception

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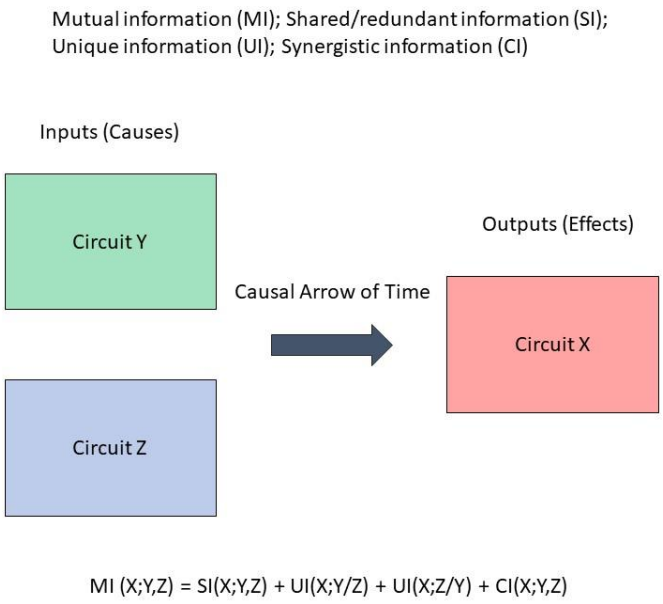
Abstract: Perception and action underlying a particular behavior can be understood as the processing of mutual information, represented by neural spike patterns generated by distributed local circuits across the cortex. However, it is not clear how cortical spike patterns are responsible for conscious behavior underlying action and perception. It has been observed that temporal coupling of neural events is responsible for perceptual functions of the brain. Neural events can be temporally coupled by at least two mechanisms, namely, coincidence detection and synchronization of brain areas by neural oscillations.

In this poster, it will be argued that the mutual information, resulting from the temporal coupling of different regions of brain, can form the basis of communication between regions as well as behavior. The mutual information partly represented by time-series of spike patterns, generated during excitatory phase of local gamma cycles, organized by hierarchical brain oscillations, will enable online behavior.

Moreover, mutual information processed can be decomposed into many components (see the schematic), which include synergistic information, shared or redundant information, unique information. Synergistic information, which will result when a third neuron fires as a result of inputs from two or more neurons, is more likely to play a role in the cause-effect relationship during information processing.



Disclosures: D.S. Gupta: None. **A. Bahmer:** None.

Poster

169. Human Cognition and Behavior: Timing and Temporal Processing

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Title: Neural correlates of resynchronization in a paced finger-tapping task with step-change perturbations

Authors: *L. G. CACERES[1], L. BAVASSI[2], J. KAMIENKOWSKI[3], R. LAJE[1]
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Abstract: Few animal species have the ability of sensorimotor synchronization (SMS), that is keeping pace with an external metronome as in finger tapping to a beat. SMS in humans is a spontaneous and very robust behavior, and it is the basis of music and dance. However, after many decades of research mostly focused in isochronous sequences, the search for neural correlates of the behavior has just begun, e.g. neural signatures of the asynchrony and the stimulus period, and a mechanistic explanation of the behavior in terms of neural processes and brain regions is still missing. The description of neural activity during resynchronization after a perturbation is even scarcer. Here we report an analysis of EEG recordings during a paced finger tapping experiment with tempo perturbations. Ten subjects, all right handed and with musical training, participated in the experiment comprising four sessions. Subjects had to perform a paced finger-tapping task with unexpected step-change perturbations of +10% and -10% of the stimulus period. We simultaneously recorded the tapping asynchronies (time difference between every response and the corresponding stimulus) and the EEG activity. The resulting epochs were arranged in three groups according to the tertiles of the asynchrony distribution at the moment of the perturbation. Our results suggest a novel frontal neural component that spans a few periods while the subject is resynchronizing and that distinguishes among the asynchrony groups. A second finding is that alterations to the time shape of a component of central origin resembling an N1-P2 complex also span several periods after the perturbation and depend on the direction of the perturbation (+10% vs -10%). In addition, a counterintuitive result is that the number of taps it takes to the subject to go back to synchrony after a perturbation only partially correlates with the asynchrony at the time of perturbation (i.e. how far from synchrony the subject is when the

period changes). In summary, we take a step towards finding the neural correlates of resynchronization by showing EEG components that span several periods after a tempo perturbation and whose latencies and/or amplitudes do not correlate with either purely motor or sensory events. The time course of resynchronization at the behavioral level can be predicted by looking at the neural activity at fronto-central locations.

Disclosures: L.G. Caceres: None. **L. Bavassi:** None. **J. Kamienkowski:** None. **R. Laje:** None.

Poster

169. Human Cognition and Behavior: Timing and Temporal Processing

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Program #/Poster #: 169.06/HHH30

Topic: H.02. Human Cognition and Behavior

Support: Swartz Foundation Postdoctoral Fellowship

Title: Finding the beat: A neuro-mechanistic model for rhythmic beat generation

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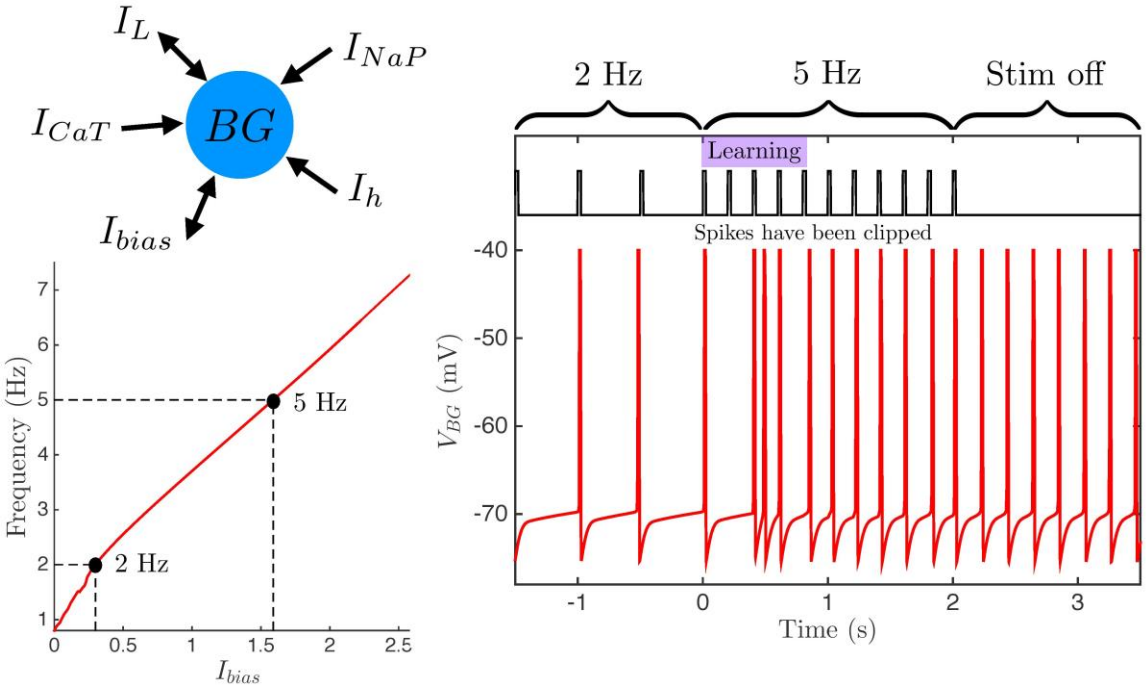
Abstract: When listening to music, we typically lock onto and move to a beat. Behavioral studies on such sensorimotor synchronization (Repp 2005) abound, yet the neural mechanisms remain poorly understood. Time processing has been widely studied in the context of decision making, language, memory and perception (Buonomano 2014). Beat perception may be a special case of interval timing, relying on fast perception and learning of time intervals from 100 to 2000 ms, but differs because of a beat's regularity. Some models of beat perception hypothesize that the brain contains an array of self-sustaining entrainable oscillators, which resonate when forced with periodic stimuli, i.e. musical rhythms (Large et al. 2010). In contrast, our approach does not assume a large ensemble of neurons in which each unit has a specific resonant frequency, but rather, in the simplest case, a single beat generator neuron (BG) which can adapt its frequency and phase to match that of an external rhythm. Our model is a neuronal realization of Mates' dual-process algorithm, for adapting the period and phase (Mates 1994). Using currents, I_{NaP}, I_{CaT}, I_h, I_L, our BG neuron includes the novel use of naturally occurring gamma frequency oscillations to mark time of and time differences between BG firing and stimulus events. A biophysical parameter, I_{bias}, that controls the excitability of the neural system, is iteratively adjusted to alter BG period and firing times. The model quickly learns new rhythms, within a few cycles (see Figure), as found in human behavior. When the stimulus is removed the BG continues to produce the learned rhythm in accordance with a synchronization continuation task.

Our modeling framework proposes neural mechanisms not just for the perception of a beat, but also for an internal neural time-keeper to produce a rhythmic beat.



Disclosures: Á. Byrne: None. **A. Bose:** None. **J.M. Rinzel:** None.

Poster

169. Human Cognition and Behavior: Timing and Temporal Processing

Location: SDCC Halls B-H

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Program #/Poster #: 169.07/HHH31

Topic: H.02. Human Cognition and Behavior

Title: Anticipation of events in time is independent of sensory input modality

Authors: *M. GRABENHORST[1], G. MICHALAREAS[1], L. T. MALONEY[3], D. POEPPEL[2]
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Abstract: The brain exploits continuous sensory input to predict event onsets and generate fast and accurate responses to behaviorally relevant cues. This fundamental operation requires knowledge of both time and, crucially, probability as a function of time. Such computations have

been increasingly investigated, but within a rather narrow scope: research is typically confined to a single sensory modality. Here we argue that if temporal-probabilistic inference is a fundamental cortical operation, it should be observable independent of sensory input modality. Despite the central role of anticipation in behavior, the neural implementations of predictive processes remain debated. To investigate the neural basis of event anticipation, we built on previous behavioral work to include magnetoencephalography (MEG). We recorded MEG in 24 participants who responded to auditory and visual stimuli in two simple reaction time (RT) experiments. The time between 'set' and 'go' signals (the 'go' time) was systematically varied according to different probability density functions (PDF). Participants were asked to react as fast as possible to the 'go' signal. In both auditory and visual experiments, the behavioral results were congruent with the hypothesis of temporal-probabilistic inference as a central process: i) the distribution of participants' RTs was systematically modulated by the presented PDF, ii) differences in average RT and its variance across modalities reflected modality-specific, likely peripheral properties, iii) participants clearly extracted, updated, and used the stochastic information encoded in the 'go' times independent of sensory input modality to anticipate the arrival of the 'go' signal. Finally, we found that a simple nonlinear transformation of the 'go' time PDF provided a good model for the mapping of PDF to RT. We present neural correlates of both modality-specific and modality-independent components manifested in behaviour, focusing on analysis of neural activity during the 'go' time, i.e. processes involved in prediction of 'go' signal onset. Taken together, we suggest that temporal-probabilistic inference is independent of input modality. Based on converging behavioral and neurophysiological evidence, we argue that the anticipation of events in time is a central, elementary operation.

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Poster

169. Human Cognition and Behavior: Timing and Temporal Processing

Location: SDCC Halls B-H

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Program #/Poster #: 169.08/HHH32

Topic: H.02. Human Cognition and Behavior

Support: NIBIB R01 EB022889-02
NIMH R01 MH106147
Carney Institute for Brain Science
Providence VA CfNN

Title: Human neocortical neurosolver (HNN): A new software tool for interpreting the circuit level origin of human MEG/EEG data

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Abstract: MEG/EEG are the leading methods to non-invasively record human neural dynamics with millisecond temporal resolution. However, the underlying cellular and circuit level origins of these macro-scale signals is not well determined for many recording conditions. This fundamental limitation constrains the translation of MEG/EEG findings into novel principles of information processing, or into new treatment modalities for neural pathologies. As such, there is a pressing need, and a unique opportunity, to relate the macro-scale signals to their underlying meso-scale generators. To address this need, we built the Human Neocortical Neurosolver (HNN), an open-source, user-friendly neural modeling tool designed to help researchers/clinicians interpret human brain imaging data. HNN is now available online (http://hnn.brown.edu). HNN presents a convenient GUI to an anatomically and biophysically detailed model of a laminar neocortical circuit, representing a canonical column under thalamocortical and cortico-cortical drive, making it easier to generate and evaluate hypotheses on the mechanistic origin of signals measured with MEG/EEG, or intracranial ECoG. A unique feature of HNN's model is that it accounts for the biophysics generating primary electric currents underlying such data, so simulation results are directly comparable to source localized data (units of measure: nA*m). To facilitate community adoption of HNN, we developed online resources with tutorials on how to use HNN to simulate commonly measured EEG/MEG signals, including sensory evoked responses and low frequency oscillations (alpha, beta, gamma). By learning how to simulate these signals, users gain insight into how to adjust parameters in our model for their hypothesis-testing. In the tutorials, we describe how to use HNN to visualize primary electrical currents (nA*m), individual cell spiking, and frequency domain oscillations (time-frequency analysis, power spectral density). We also show how to load data into HNN and compare simulated electrical sources with recorded source localized data. Ongoing efforts are aimed at integrating HNN's circuit-level modeling with the minimum-norm estimate source localization software (https://www.martinos.org/mne/stable/index.html), so researchers can compute MEG/EEG source estimates and test hypotheses on the circuit origin of their data in one software package. We are also expanding HNN to support simulation of local field potentials, parameter optimization, and integration with NetPyNE (https://pypi.org/project/netpyne) for flexible network specification. These efforts are aimed at expanding use of HNN to a wider community.

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Poster

169. Human Cognition and Behavior: Timing and Temporal Processing

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 169.09/HHH33

Topic: H.02. Human Cognition and Behavior

Support: JSPS KAKENHI Grant JP17K04452
NICT grant
Inogashira hospital research grant
Keio university psychiatry department research grant

Title: Frontopolar cortex activation during far future thinking in depression

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Abstract: Objective: Thinking about the future unhopefully is a core symptomatology, which leads to hopelessness in depression. Prior studies have shown that anterior medial prefrontal cortex (mPFC), especially frontal pole (Brodmann 10: BA10), is associated with future thinking. Patients with depression consistently have presented with dysfunction in anterior mPFC in prior neuroimaging studies. However, the relationship between depressive symptomatology and brain activity related to future thinking is unclear. We examined brain activity during future thinking in patients with major depression compared with healthy controls using task-based fMRI. **Method:** We assessed 23 patients with major depressive disorder and 23 healthy controls (HC). On the basis of Future Thinking Implicit Relations Assessment Procedure, Keio-version future thinking task (KFT) was developed. KFT is composed by 4 different temporal conditions (far future/near future/ far past/ near past) with 16 trials for each. Each trial begins by projecting temporal words (e.g. “in the future”) and its contexts (e.g., “your dream”) on a screen, and participants were asked to image their future or recall their past. This was followed by the second slide which included a full sentence (e.g. “in the future, your dream will come true.”), the participants pushed a response button for yes or no. Reaction time (RT) in seconds and participants’ response was collected. After task-functional fMRI, 10 minutes resting state fMRI was performed to explore brain functional connectivity from BA10. **Result:** The depression group showed a higher ratio of negative meaning response to the far future, the near future and the near past conditions compared to HC. Regarding RT, between-group difference was most pronounced on the positive meaning response of far future condition. Patient groups exhibited increased activation in BA10 when thinking about far future compared with HC. Seed from right BA10 significantly increased the functional connectivity to the posterior cingulate cortex in depression group. **Conclusions:** Current findings suggest that depression patients have

pessimistic future thinking, especially difficult to image their distant future which may associate with abnormal frontal pole activity pattern.

Disclosures: N. Katayama: None. **A. Nakagawa:** None. **S. Umeda:** None. **Y. Terasawa:** None. **H. Tabuchi:** None. **T. Kikuchi:** None. **M. Mimura:** None.

Poster

169. Human Cognition and Behavior: Timing and Temporal Processing

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 169.10/HHH34

Topic: H.02. Human Cognition and Behavior

Support: JSPS Grant-in-Aid for JSPS Fellows 17J02991

Title: Perceptual deterioration for self-produced timing can be caused by common noise between motor and perceptual timing

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Abstract: To achieve accurate motor timing, we are required to accurately perceive timing of our own action. Previous studies have indicated that the perception of self-produced timing is more variable than that of passively presented timing for subsecond visual targets (Gorea et al., 2010) and for suprasecond auditory targets (Mitani & Kashino, 2016). However, it remains unclear how the perceptual deterioration for self-produced timing is caused. Here, we show that common noise between motor and perceptual timing can cause the perceptual deterioration by using mathematical modeling. The current model (Fig. 1) is composed of three independent Gaussian random variables; judgment (Nj), production (Np), common noises (Nc). The judgement criterion (Tj) is the sum of judgment and common noises. The reproduced interval (Tp) is the sum of production and common noises. The judgment (D) is the comparison between the judgment criterion and the reproduced interval. We analyzed the psychometric function p(D='long'|Tp) in this model. The results show that when the variance of the common noise is relatively large to those of the other noises, the perceptual variability calculated from the psychometric function increases (Fig. 2). More interestingly, the increase of the perceptual variability is prominent when the variance of the common noise is relatively large to that of the production noise. In conclusion, the commonality between perception and action can deteriorate the perception for self-produced timing.

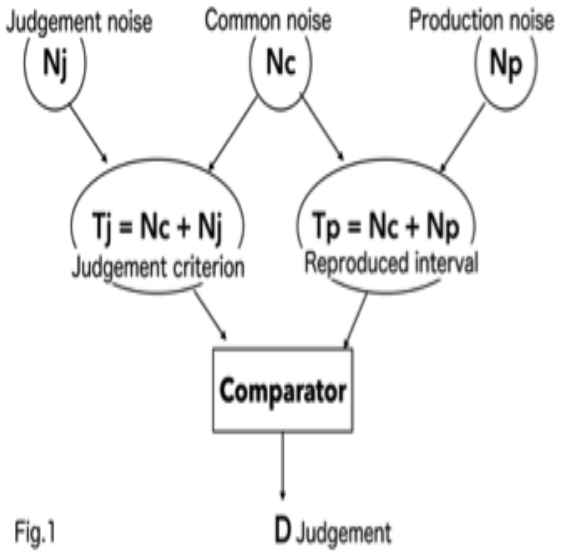

Fig.1

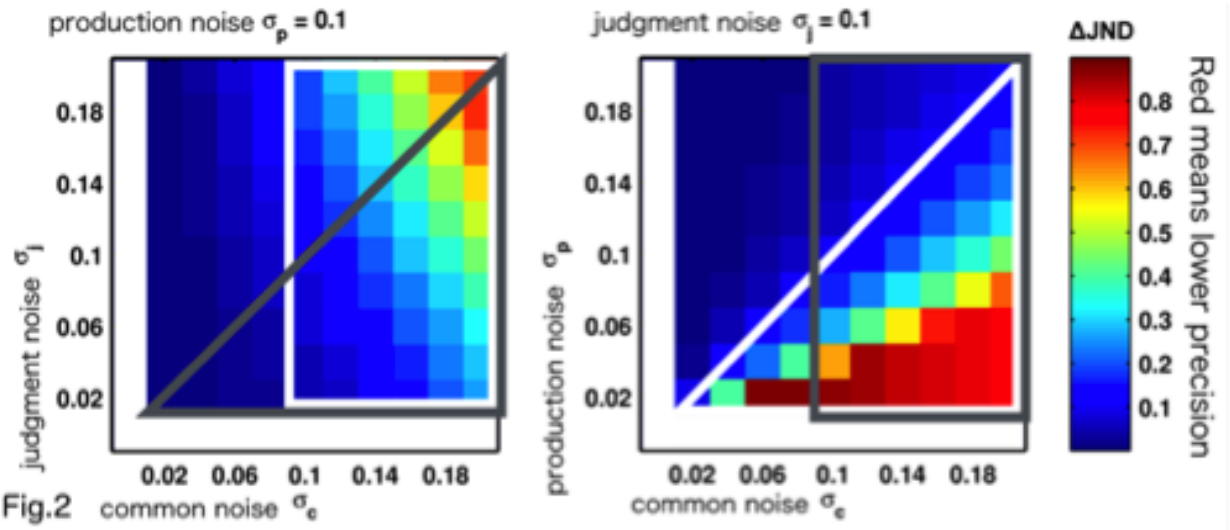

Fig.2

Disclosures: **K. Mitani:** 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.; Japan Society for the Promotion of Science. **M. Kashino:** A. Employment/Salary (full or part-time):; Nippon Telegraph and Telephone Corporation.

Poster

169. Human Cognition and Behavior: Timing and Temporal Processing

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 169.11/HHH35

Topic: H.02. Human Cognition and Behavior

Support: ERC-YStG-263584
ANR10JCJC-1904

Title: Temporal metacognition as the decoding of self-generated brain dynamics

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Abstract: Metacognition, the ability to know about one's thought process, is self-referential. Here, we studied the brain mechanisms underlying metacognitive inferences in a self-generated behavior. Human participants generated a time interval, and evaluated the signed magnitude of their timing (first and second order behavioral judgments, respectively) while being recorded with time-resolved neuroimaging. We show that the first- and second-order judgments relied on the power of beta oscillations (β; 15-40 Hz). The spread of an individual's β power state-space trajectories during timing was indicative of the individual's metacognitive inference. Our results suggest that network inhibition (β power) instantiates a state variable determining future network trajectory; this naturally provides a code for duration and metacognitive inferences would consist in reading out this state variable. Altogether, our study describes oscillatory mechanisms for timing suggesting that temporal metacognition relies on inferential processes of self-generated dynamics.

Disclosures: T.W. Kononowicz: None. **C. Roger:** None. **V. van Wassenhove:** None.

Poster

169. Human Cognition and Behavior: Timing and Temporal Processing

Location: SDCC Halls B-H

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Program #/Poster #: 169.12/HHH36

Topic: H.02. Human Cognition and Behavior

Support: EU Horizon 2020 Grant 641100
NWO Grant 453-16-005

Title: Decoding the influence of context on time perception

Authors: *A. DAMSMA, N. SCHLICHTING, R. EIKE, H. VAN RIJN
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Abstract: Our perception of time is influenced by the context we perceive it in. For example, when we have to reproduce a range of intervals, short intervals are often overestimated whereas

long intervals are underestimated. In most models of interval timing the assumption is that this effect is driven by the weighting of the currently perceived interval with previous memory traces (i.e., the context). This implies that the influence of context should only be observed after the current duration has been perceived. Alternatively, however, it is possible that already the *perception* of the duration is influenced by its context. The goal of the current EEG study is to identify when context shapes interval timing and which ERP components are associated with behavioral context effects. Participants performed a temporal reproduction task in which half of the blocks contained relatively short intervals and the other half relatively long intervals. Crucially, there was one interval that was presented in both conditions. As expected, the behavioral data showed that this middle interval was estimated longer in the long than in the short context blocks. Preliminary ERP analysis shows that the CNV, a slow negative component often observed in timing tasks, was more negative during the perception of the middle interval in the short compared to the long context blocks. In addition, multivariate EEG decoding indicates that it is possible to distinguish whether a particular interval appeared in the short or long context right after the onset of the perception stage, as well as between the perception and the reproduction stage. Together, these results suggest that temporal context already affects the perception of duration, providing additional empirical constraints for computational models of time perception.

Disclosures: A. Damsma: None. **N. Schlichting:** None. **R. Eike:** None. **H. van Rijn:** None.

Poster

169. Human Cognition and Behavior: Timing and Temporal Processing

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Program #/Poster #: 169.13/HHH37

Topic: H.02. Human Cognition and Behavior

Support: EU Horizon 2020 FET Proactive grant TIMESTORM 641100
NWO grant 453-16-005

Title: Quantifying attention and its effect on interval timing

Authors: *N. SCHLICHTING, A. DAMSMA, M. ZIEGLER, R. DE JONG, H. VAN RIJN
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Abstract: The influence of attention on the perception of time has attracted considerable interest among researchers. It is commonly found that directing attention to time prolongs the perceived duration of a given interval, whereas distracting attention from time shortens the perceived duration of the interval. In these studies attention is typically modulated indirectly, for example in dual-task or cueing paradigms. Recently however, steady-state evoked potentials (SSEPs), the EEG responses to rhythmic stimuli, have been identified as a potential biological marker of

attention: stronger EEG responses at the target frequency are associated with more attention being paid to the stimulus. In this study, we aimed to quantify the amount of attention dedicated to a prospective timing task using auditory SSEPs to test the effect of attention on interval reproductions. Participants listened to tones of 1.2, 1.4 and 1.8 s with a carrier frequency of 500, 550 or 600 Hz that were amplitude modulated at either 35 or 45 Hz. These stimuli were embedded in white noise that was phase-aligned with and amplitude modulated at the same frequency as the stimulus. The white noise started earlier than the stimulus to ensure that participants were entrained to the amplitude modulation. After an inter-stimulus-interval of 0.8 s, the amplitude modulated tone started again to mark the onset of the reproduction phase and was terminated by participants with a button press. In the planned analysis, we will quantify attention by extracting power values at the target frequencies on a single-trial basis during both encoding and reproduction phase. These measures are then used to predict behavioral performance, while higher power values (i.e., more attention spent on the stimulus) are hypothesized to be associated with over-reproductions.

Disclosures: N. Schlichting: None. **A. Damsma:** None. **M. Ziegler:** None. **R. de Jong:** None. **H. van Rijn:** None.

Poster

169. Human Cognition and Behavior: Timing and Temporal Processing

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Topic: H.02. Human Cognition and Behavior

Support: DFG HE-7520/1-1 to SKH
MarieCurie EuroTalents to SKH
ANR-16-CE37-0004_04 AutoTime to VvW

Title: One clock: Shared mechanisms for implicit and explicit timing?

Authors: *S. K. HERBST[1,2], J. OBLESER[2], V. VAN WASSENHOVE[1]
[1]Univ. Paris-Sud, Univ. Paris-Saclay, NeuroSpin Ctr., Cognitive Neuroimaging Unit, CEA DRF/Joliot,INSERM, Gif sur Yvette, France; [2]Univ. of Luebeck, Luebeck, Germany

Abstract: Temporal regularities are inherent in our environment, and human observers efficiently extract them to form temporal predictions when processing sensory input. Moreover, time provides the structure to human subjective experience, and can be apprehended devoid of sensory input. Thus, time is both, implicit and essential for cognition, but also explicit, i.e. consciously represented. Here, we asked whether implicit and explicit representations of time rely on shared versus separate cognitive and neural mechanisms. We assessed implicit and explicit timing in an auditory foreperiod paradigm, presenting pure tones in white noise. We

induced an implicit, but deliberately non-rhythmic variation of temporal predictability: Per block, the interval between the standard and target tone, i.e. the foreperiod, was either deterministically predictive of the onset of the target tone (but varying over blocks), or non-predictive (varying within a block). To measure implicit timing, we asked participants to perform a pitch discrimination task on the tones and measured the difference in pitch discrimination sensitivity between the predictive and non-predictive conditions, via the slope of the psychometric function. To measure explicit timing, we used the same stimuli but asked participants to overtly judge the duration of the foreperiod interval, i.e. perform a temporal bisection task, and used the slope of the non-predictive condition as a measure of explicit timing sensitivity. Behavioral data (N=14) revealed that temporal predictability improved pitch discrimination sensitivity, replicating previous results. Critically, here we showed that even different predictive intervals could be learned over blocks. The explicit timing task showed no benefit of temporal predictability. To assess whether the representation of time that serves temporal prediction could also be exploited for explicit timing, we correlated the benefit from temporal prediction with the explicit timing performance ($r = 0.54$). Thus, individuals who efficiently formed temporal predictions also performed better when estimating time explicitly. Combined MEG/EEG data are currently being collected to compare the neural dynamics of implicit and explicit timing. Applying machine learning techniques (decoding) to preliminary data (N=10) revealed a distinction between neural activity during predictive and non-predictive foreperiod intervals, in the implicit and explicit timing task. In sum, the data show that human listeners flexibly exploit implicit temporal contingencies present in sensory input, and that this skill is related to explicit timing performance.

Disclosures: S.K. Herbst: None. **J. Obleser:** None. **V. van Wassenhove:** None.

Poster

169. Human Cognition and Behavior: Timing and Temporal Processing

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 169.15/HHH39

Topic: H.02. Human Cognition and Behavior

Title: Accuracy in chunk retrieval is correlated with the presence of acoustically driven delta brain waves

Authors: *J. M. RIMMELE[1], D. POEPPEL[1], O. GHITZA[2]
[1]Dept. of Neurosci., Max Planck Inst. For Empirical Aesthetics, Frankfurt am Main, Germany; [2]Dept. of Biomed. Engin., Boston Univ., Boston, MA

Abstract: Two MEG studies, Doelling et al. (2014) and Ding et al. (2016), form the basis for the question we ask here. On the one hand, Doelling et al. showed that acoustic landmarks drive delta-theta oscillations to enable speech comprehension by facilitating bottom-up perceptual

segmentation. On the other hand, Ding et al. showed that delta-theta oscillations correspond to multiple levels of linguistic structure (the phrasal and syllable level, respectively) with no acoustic cues at these time scales present. Two alternative, equally valid interpretations can explain the presence of such neurophysiological patterns: (i) they reflect cortical tracking generated by a syntax driven, built-in hierarchical oscillatory mechanism, or (ii) they reflect the information transit at the end of a decoding process (at the syllable and at the phrasal level), guided by acoustic cues; and if neuronal oscillators are involved, the brain waves should exist only when the presentation rate is inside the oscillators' frequency range. In this MEG study we focused on the phrasal level. A recent behavioral study (Ghitza, 2016) showed that digit sequences are accurately perceived only when the 'chunks' are played at a rate within the delta range (~ 0.5 - 2 Hz). Building on that study, the experiment reported here tested (i) whether the appearance of response peaks in the delta range is related to the (acoustic) grouping of digit sequences to larger chunks; and (ii) what is the relative role of acoustically driven delta versus syntax driven delta. The data suggest a correlation between accuracy in performance and the presence of delta spectral peaks in the neural activity. Importantly, these delta peaks are reliable only when the digit sequences carry robust phrasal acoustic cues, i.e., they are acoustically driven.

Disclosures: J.M. Rimmele: None. **D. Poeppel:** None. **O. Ghitza:** None.

Poster

169. Human Cognition and Behavior: Timing and Temporal Processing

Location: SDCC Halls B-H

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Program #/Poster #: 169.16/HHH40

Topic: H.02. Human Cognition and Behavior

Title: The rate of birdsong as a window into auditory aesthetic perception

Authors: *T. C. ROESKE, P. LARROUY-MAESTRI, Y. SAKAMOTO, D. POEPPEL
Neurosci., Max Planck Inst. for Empirical Aesthetics, Frankfurt Am Main, Germany

Abstract: Human listeners admire the beauty of bird song. Do we like bird song because of its intrinsic properties as natural kinds, or does our perceptual apparatus render them into a stimulus optimized for preferences of the human auditory system? While bird song is evolved to entice avian - not human - listeners, it shares a number of characteristics with music and is organized into rhythmic/melodic sequences. But bird song also has tonal and temporal properties that differ from music. For instance, it contains very high note rates rare in music. To test how we handle such rate variability in our preferences, we asked human listeners to manipulate the playback speed of bird song using a continuous dial, to either settle on their preferred tempo or the one that seemed most natural. Next, a click train was added to the stimulus, and listeners were asked to select a speed fitting the selected song tempo. Participants were comfortable selecting bird

song rates faster than typical musical modulation spectra. Interestingly, when listeners were asked to match a click train, they choose considerably slower rates (around 2 Hz) than the mean rate selected for the stimulus. These results suggest that human listeners accept and appreciate non-human note rates in bird song as an integral part of a natural stimulus, while grouping them perceptually into rates more typical for musical beats.

Disclosures: T.C. Roeske: None. **P. Larrouy-Maestri:** None. **Y. Sakamoto:** None. **D. Poeppel:** None.

Poster

169. Human Cognition and Behavior: Timing and Temporal Processing

Location: SDCC Halls B-H

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Program #/Poster #: 169.17/HHH41

Topic: H.02. Human Cognition and Behavior

Support: NSF Grant BCS1358907

Title: Timing of memory induced activations across the parietal and frontal nodes of the default network

Authors: *Y. FANG[1,2], O. RACCAH[1], L. SHI[1], A. ARETI[1], J. PARVIZI[1]
[1]Neurol. and Neurolog. Sci., Stanford Univ., Stanford, CA; [2]Psychology, South China Normal Univ., Guangzhou, China

Abstract: The human default mode network (DMN), a distributed set of inter-connected brain regions, is engaged during internally-directed tasks, while deactivating for conditions that require external attention. To date, virtually all that is known regarding DMN function is based on evidence from neuroimaging modalities with low temporal resolution. By taking advantage of the high temporal resolution and signal-to-noise ratio of intracranial electroencephalography (iEEG), the current study aims to determine the relative timing of task-evoked activations across the posteromedial cortex (PMC) and medial prefrontal cortex (mPFC) - two central nodes of the DMN. We used high-frequency broadband power (HFB; 70-170 Hz) as a measure of local cortical engagement. Five neurosurgical patients performed a behavioral task that probed autobiographical memory retrieval, which induced HFB responses in both PMC and mPFC regions. Across all subjects, we found that memory-induced mPFC activations lagged PMC activations by ~100ms. This relationship was significant based on nonparametric permutation tests ($P = 0.013$, 50,000 reps.). To conclude, the current work reveals a clear temporal order across well-characterized DMN nodes, and provides insight into the temporal dynamics of DMN engagement during autobiographical memory retrieval.

Disclosures: Y. Fang: None. **O. Raccah:** None. **L. Shi:** None. **A. Areti:** None. **J. Parvizi:** None.

Poster

169. Human Cognition and Behavior: Timing and Temporal Processing

Location: SDCC Halls B-H

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Program #/Poster #: 169.18/HHH42

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant NS092079

Title: Role of cerebellar-dependent interval representations in shifting attention to off-beat times of rhythmic streams

Authors: *A. BRESKA[1], R. IVRY[2]
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Abstract: Environmental rhythms are potent cues for allocating attention in time, typically leading to facilitated processing of on-beat, relative to off-beat, events. However, humans can also shift attention to off-beat times, when off-beat times are task relevant and on-beat times are distracting. This could be mediated by adjusting attentional oscillations to a different phase of the rhythm. Alternatively, it could rely on isolated-interval prediction mechanisms that may be time-locked to the beats of the rhythmic signal. To compare these hypotheses, we tested off-beat attention in patients with cerebellar degeneration (CD). Prior work showed that CD patients can use rhythmic cues to orient in time, but are impaired in using isolated-interval predictive timing. Thus, if off-beat timing is interval based, we would expect CD patients to fail to show attention benefits at off-beat relative to on-beat times. Participants provided speeded responses to a visual target that followed a rhythmic stream of visual stimuli (inter stimulus interval, ISI = 1.2 secs). In attend-on-beat blocks, targets appeared frequently on-beat (ISI = 1.2 secs) and rarely off-beat (ISI = 0.7 secs). In attend-off-beat blocks, they appeared frequently off-beat and rarely on-beat. In interval blocks, included to confirm that the patients have difficulty using isolated-interval cues, we cued the target interval (to be either 0.7 or 1.2 secs) in an aperiodic stream. In the interval blocks, the CD group showed reduced reaction time (RT) benefit on validly relative to invalidly cued trials, compared to neurologically healthy controls. Consistently, in this condition the patients did not exhibit EEG signatures of temporal orienting, including the modulation of low frequency potentials or beta-band activity. This indicates that the CD group was impaired in isolated-interval prediction, in line with previous work. In the attend-on-beat condition, the CD group was able to attend to task-relevant on-beat times, expressed in faster RT to on-beat compared to off-beat targets, and both EEG signatures. In contrast, in the attend-off-beat condition, RT did not differ between on-beat and off-beat targets. This was due to slowing of RT to on-beat targets relative to the attend-on-beat condition. The EEG showed that in the attend-

off-beat condition, the pattern of low frequency potentials differed from that in the attend-on-beat condition, but beta-band activity was not affected. These results suggest that off-beat attention entails the dual recruitment of rhythm- and interval-based representations, expressed in distinct frequency bands, for inhibition in on-beat times and enhancement in off-beat times.

Disclosures: A. Breska: None. **R. Ivry:** None.

Poster

169. Human Cognition and Behavior: Timing and Temporal Processing

Location: SDCC Halls B-H

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Program #/Poster #: 169.19/HHH43

Topic: H.02. Human Cognition and Behavior

Support: NIMH R01 MH60046

Title: Neurocognitive function and sensory gating differences in bipolar patients with and without a history of psychosis

Authors: *M. ALI[1], N. AFKHAMI[2], J. V. PATTERSON[2]
[1]Univ. of California, Irvine, Orange, CA, [2]Psychiatry & Human Behavior

Abstract: Sensory gating describes neurological processes that allow the brain to filter out repetitive stimuli. Evoked brain potentials such as P50 and N100 can be measured in response to a series of paired click stimuli. In normal controls a decrease in the response to the second click (S2) compared to the first click (S1) indicates normal sensory gating. Findings regarding differences in sensory gating between bipolar patients with and without psychosis have been inconclusive. Additionally, previous studies have been unable to conclusively establish whether bipolar patients with psychosis are more severely impaired on measures of neurocognition than bipolar patients without psychosis. This study explored sensory gating differences between bipolar I patients with and without a history of psychosis and investigated the relationship between P50 and N100 sensory gating and neurocognitive function. Measures of P50 and N100 gating included latency and amplitude, S2/S1 gating ratios, the difference between S1 and S2 amplitudes, and a novel measure, the S1 and S2 waveform difference. Neurocognitive tests were normalized using z-scores with reference to controls and compiled into domains. ANOVA, correlation and regression were used to compare sensory gating measurements among groups and to study the relationship between sensory gating and neuropsychiatric tests measuring cognitive function. Psychotic bipolar individuals presented with smaller differences between S1 and S2 amplitudes for P50 and N100 measures in comparison to controls and non-psychotic bipolar individuals. The N100 difference waveform amplitude was smallest for bipolar patients with psychosis while the P50 waveform latency was longest. Similar results were obtained using a group by click analysis. These results suggest that, in this study, the S1 and S2 waveform

difference for P50 and N100, and the differences between S1 and S2 amplitudes measures provided more discriminating indices of sensory gating than the S2/S1 gating ratio, which did not show any differences between groups. The cognitive domains of attention, immediate memory, visual memory, executive function, delayed memory, and language were found to correlate significantly with the P50 potential in psychotic bipolar individuals. Attention, visual memory, executive function, delayed memory and short-term memory cognitive domains correlated significantly with the N100 potential in non-psychotic bipolar individuals. Differing correlation patterns indicate that N100 and P50 brain potentials are implicated in separate cognitive domains depending on the presence of psychosis.

Disclosures: M. Ali: None. **N. Afkhami:** None. **J.V. Patterson:** None.

Poster

169. Human Cognition and Behavior: Timing and Temporal Processing

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Program #/Poster #: 169.20/HHH44

Topic: H.02. Human Cognition and Behavior

Title: Contribution of movement trajectories to error correction and timekeeping during sensorimotor synchronization and syncopation to auditory and visual metronomes

Authors: *A. PABST, R. BALASUBRAMANIAM
Univ. of California, Merced, Merced, CA

Abstract: Research on sensorimotor synchronization of finger-tapping has investigated the contribution of movement trajectories to timing accuracy via asymmetry in movement velocities as well as the dynamics of the movement (Balasubramaniam et. al, 2004; Hove et. al 2010). The present study directly compares synchronization (in-phase) and syncopation (anti-phase) to both auditory and visual stimuli at multiple time intervals and examines how individual movement trajectories contribute to error correction under multiple stimulus modalities and task constraints. Using a Vicon Nexus motion capture system, seventeen participants (15 female; aged 18-32 years; all self-reported to be right-handed) were asked to independently synchronize and syncopate with their right index finger to both visual and auditory stimuli at intervals of 500ms, 750ms, and 1000ms. Time spent in both the extension (upward) phase of movement and the holding period (haptic contact) are relatively invariant across both stimulus modalities and tapping styles but differed across metronome frequency. Time spent in the flexion (downward) phase of movement did not significantly differ across stimulus modality, tapping style, or metronome frequency. Extension velocity was found to have significant differences across visual and auditory stimulus modalities, which demonstrates that the upward phase of movement is the main contributor to variability and timing. No correlations were found between relative asynchrony and the length of time spent in the movement phases, indicating that there are

multiple error correction strategies for timing across stimulus modalities and tapping styles. While the upward phase of movement has been found to be the key contributor to variance and timekeeping across visual and auditory modalities, differences between synchronization and syncopation to stimuli were not found – suggesting that strategies for tapping styles to minimize error are mechanically similar, but may have different neural circuitry underlying those timekeeping processes.

References:
Balasubramaniam, R., Wing, A. M., & Daffertshofer, A. (2004). Keeping with the beat: movement trajectories contribute to movement timing. *Experimental Brain Research, 159*(1), 129-134.
Hove, M. J., Spivey, M. J., & Krumhansl, C. L. (2010). Compatibility of motion facilitates visuomotor synchronization. *Journal of Experimental Psychology: Human Perception and Performance, 36*(6), 1525.

Disclosures: A. Pabst: None. **R. Balasubramaniam:** None.

Poster

169. Human Cognition and Behavior: Timing and Temporal Processing

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 169.21/HHH45

Topic: H.02. Human Cognition and Behavior

Support: Kakenhi 25119002
Kakenhi 25119007
Kakenhi 18H05197

Title: A map of time in the medial cerebral cortex: A cross-linguistic fMRI study with speech stimuli

Authors: *L. W. TANG[1], T. TAKAHASHI[2,3], S. KITAZAWA[2,3], T. SHIMADA[4], M. KOMACHI[5], N. IMANISHI[6], Y. NISHIYAMA[7], T. IIDA[8], Y. OTSU[4]
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Abstract: It remains elusive how the human brain distinguishes among the past, present and future. By analogy to a somatotopic map in the primary sensory cortex, we hypothesized that the

human brain has a "map of time" where each of the past, present, and future is represented. We tested this hypothesis by utilizing tense in language. We used fMRI to compare brain activities while 51 participants (18 Japanese, 15 English, and 18 Mandarin native speakers) listened to speech stimuli that referred to the past (e.g., 'I read a book yesterday'), present ('I started a meeting now'), or future ('I will read a book tomorrow').
In addition to 144 grammatical sentences that consisted of an adverb of time, an object, and a verb in the past, present, or future form, we prepared 36 ungrammatical sentences, like 'I read a mountain today'. The participants were asked to listen to each sentence in an MRI scanner, and to press a button only when they felt the sentence was unnatural. After MRI measurements, the participants were asked to rate the time that each sentence referred to by using a nine-point scale (1: the far past, ..., 5: present, ..., 9: far future). Brain activations in response to grammatical sentences were compared by using SPM12, across the 'Past' (1 to 4), 'Present' (5), and 'Future' (6 to 9) sentence groups.
The bilateral precuneus and the posterior cingulate cortex were activated more strongly in response to the 'Present', or to the 'Future' stimuli, as compared to the 'Past' stimuli ($p < 0.05$, cluster-level FWE correction). The region revealed by the 'Present > Past' contrast was located in the posterior part of the precuneus, whereas the region revealed by the 'Future > Past' contrast was located in the anterior part of the precuneus and extended to the posterior cingulate cortex. We found no significant across-language differences in the level of the contrasts in either activated region. The results suggest that the medial regions involving the precuneus and the posterior cingulate cortex constitute a 'map of time' that is shared across native speakers of different languages.

Disclosures: T. Takahashi: None. **S. Kitazawa:** None. **T. Shimada:** None. **M. Komachi:** None. **N. Imanishi:** None. **Y. Nishiyama:** None. **T. Iida:** None. **Y. Otsu:** None.

Poster

169. Human Cognition and Behavior: Timing and Temporal Processing

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 169.22/HHH46

Topic: H.02. Human Cognition and Behavior

Support: DFG TRR 169/B1
SFB 936/A3

Title: Enhanced delta band phase alignment reflects temporal prediction processes during cross-modal time perception

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Abstract: The brain constantly predicts the nature and timing of upcoming events. Such predictive processes might be governed by neural oscillatory activity. During temporal prediction processes, for instance, it has been shown that the phase of oscillations in the delta band (1-4 Hz) aligns to the onset of rhythmic stimuli. However, whether such phase alignment reflects predictive processes of endogenous oscillations or simply event-related stimulation activity is still under debate. Here, we aimed at investigating neural oscillatory activity during temporal expectation processes and at dissociating them from activity related to external stimulation. We recorded magnetoencephalography from healthy participants involved in a uni- and a cross-modal temporal prediction task. In the unimodal condition, a visual stimulus moved across the screen until it disappeared behind an occluder. After a variable time, the stimulus reappeared on the other side of the occluder. Participants' task was to judge whether the stimulus re-appeared *too early* or *too late* based on the velocity of the stimulus. In the cross-modal condition, a tactile instead of a visual stimulus was presented to the index finger at the time of stimulus reappearance. We contrasted these conditions to a working memory control task, where physical stimulation was equal to the temporal prediction condition, but the task was to correctly remember stimulus luminance. During temporal prediction, we observed decreased beta band power (15-30 Hz) in visual (unimodal condition) and in visual and tactile regions (cross-modal condition) contralateral to the expected stimulus as compared to the working memory task. Moreover, we found enhanced inter-trial phase coherence in the delta band with strongest effects in prefrontal regions, primary and supplementary motor cortex. In the cross-modal condition, strong inter-trial coherence effects were additionally apparent in somatosensory cortex and superior temporal regions. These results suggest an important role for the phase of delta oscillations in temporal predictive processes. Since physical stimulation was equal between the conditions, the observed phase alignment to the offset of the stimulus could not simply been driven by external stimulation. It might rather reflect internal prediction processes that are inherent to the phase of an endogenous delta oscillation. These results provide new insights into the relationship between neural oscillatory activity and processes related to predictive coding and temporal expectation.

Disclosures: J. Daume: None. **A.K. Engel:** None.

Poster

169. Human Cognition and Behavior: Timing and Temporal Processing

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 169.23/HHH47

Topic: H.02. Human Cognition and Behavior

Support: ERC Grant DYSTRUCTURE n.295129
HBP SGA1 n.720270
ERC Grant CAREGIVING n.615539

Title: The timescale of brain processing in the human cortex

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Abstract: In the brain, as in any complex system, dynamical events occur across many different timescales. Thus, the most relevant timescale is strongly dependent on the question at hand. Here, we investigate which is the optimal timescale of cortical processing yielding a rich repertoire of spatiotemporal dynamics across the brain. In particular, we characterize the richness of the dynamical repertoire by the novel concept of "*hierarchy*" (Deco and Kringelbach 2017), which expresses the heterogeneity of the relevancy of each brain region for the broadcasting of information across the whole brain. Furthermore, we investigate how the timescale and the hierarchy changes under different brain states (i.e. awake versus deep sleep).
Previous work using fMRI have revealed spatial correlation maps, i.e. resting state networks (RSNs) at an ultraslow timescale (< 0.1 Hz) (Biswal, Yetkin et al. 1995, Smith, Fox et al. 2009), assuming that they are a good quantitative account of information processing. However, it is not clear that the restricted spatial information of RSNs provides a full description of the richness of spatiotemporal dynamics across the brain. Evidence from other studies using EEG and MEG have shown that other timescales may be more relevant when considering spatiotemporal dynamic features beyond static spatial maps (de Pasquale, Della Penna et al. 2010, Brookes, Woolrich et al. 2011, Hipp, Hawellek et al. 2012).
To address this question, we use human ECoG data to determine the spatiotemporal dynamical complexity at a given timescale by the novel method 'Brain Songs' recently introduced by Deco & Kringelbach. This method, inspired in the cell-assembly method used in neurophysiology (Abeles and Gerstein 1988, Luczak, Bartho et al. 2009), captures low-dimensional features that extract meaningful spatiotemporal dynamics, i.e. repeated temporal patterns.
The measure of the richness of the dynamical repertoire at a given timescale goes beyond the existing static spatial RSNs and consider the most important spatiotemporal dynamical features and the hierarchical functional structure of the brain. A rich hierarchical functional structure implies diversity and heterogeneity across brain regions which is highly consistent with recent observations (Chaudhuri, Knoblauch et al. 2015). We show that using the independent measure of hierarchy, there is an optimum at the relevant timescale of 200 ms. This is consistent with the emerging behavioral evidence for a similar critical timescale for signals to be broadcast across the brain (Del Cul, Baillet et al. 2007, Dehaene and Changeux 2011).

Disclosures: J. Cruzat: None. **M.L. Kringelbach:** None. **G. Deco:** None.

Poster

169. Human Cognition and Behavior: Timing and Temporal Processing

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 169.24/HHH48

Topic: H.02. Human Cognition and Behavior

Support: CONACYT Grant 236836
CONACYT Grant 196
PAPIIT Grant IN202317
CONACYT Scholar Ship 479910

Title: Human neuroanatomical and neurophysiological markers for timing variability during a synchronization-continuation task

Authors: *G. PAMELA[1], C. I. DE LEÓN-ANDREZ[2], R. RODRÍGUEZ-CRUCES[3], Y. AYALA[3], L. CONCHA[3], H. MERCHANT[4]
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Abstract: The ability to detect the regular pulse in music and to synchronously respond to this pulse is a fundamental trait of musical and dance cognition. Human psychophysics has revealed that the perception and production of rhythmic intervals follow the scalar property, a form of Weber's law that defines a linear increase in temporal variability (SD) as a function of the timed interval. Little is known about the anatomofunctional substrates of the scalar property during rhythmic tapping. Therefore, here we correlated the performance variability of human subjects during a synchronization-continuation tapping task (SCT) with two neurometric markers: the cortical gray matter thickness (CT) and the frequency following response (FFR). The SCT consisted in tapping in synchrony with an auditory or visual metronome (synchronization phase), and then to reproduce the interval without a sensory guide (continuation phase). The tempos ranged from 550 to 950 ms. Structural resonance image (T1) and scalp-auditory evoked potential (Cz site) were obtained in 32 healthy human subjects (21-31 years) without musical training. We found that the CT of the right ventral premotor and auditory cortex was positively correlated with the scalar property during the continuation phase. Thus, subjects who exhibits a larger time-dependent component in his/her internally-guided production of time intervals showed greater cortical thickness in these areas. Additionally, we found that the inter-trial consistency of the FFR response was correlated with the SD of the produced intervals ($r = -0.48$, $p = 0.009$), during the synchronization phase. This result indicates that subjects with a more stable representation of the regular temporal structure of sound over time exhibited less variable rhythmic entrainment.

Our findings suggest that different neuroanatomical and physiological markers can account for distinct aspects of tapping performance during SCT.

Disclosures: G. Pamela: None. **C.I. De León-Andrez:** None. **R. Rodríguez-Cruces:** None. **Y. Ayala:** None. **L. Concha:** None. **H. Merchant:** None.

Poster

169. Human Cognition and Behavior: Timing and Temporal Processing

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 169.25/HHH49

Topic: H.02. Human Cognition and Behavior

Support: CUNY Doctoral Student Research Grant
CUNY Psychology Travel Award

Title: Occipital-parietal alpha frequency correlates with visual-temporal acuity but is not entrained by visual stimulation

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Abstract: Perception is better at expected moments in time and at certain phases of ongoing neural oscillations in the alpha-band (8-13hz). Studies employing flickering stimuli have further shown that alpha flicker can rhythmically modulate near-threshold target detection for a short period of time after stimulation offset. Activity synchronized to the flicker persists in visual areas for a similar amount of time after stimulation, supporting the recent proposal that endogenous brain rhythms are malleable, particularly alpha oscillations. However, it is still not entirely clear whether post-stimulation fluctuations in target detection are due to entrainment of endogenous alpha oscillations or to temporal expectation effects that have been found across a range of stimulation frequencies and thus are not unique to the alpha-band. In the current study, we addressed this question by examining whether rhythmic stimulation can entrain thresholds in the two flash-fusion task, a measure of temporal resolution which has been previously found to correlate with individual alpha frequency and is not sensitive to rhythmic temporal expectation. Participants were asked to discriminate whether one or two circles appeared on a parafoveal location while the interval between the two flashes varied (10-50 ms). In a no-stimulation control condition, we replicated the finding that individual alpha frequency correlates with two-flash fusion thresholds. However, we found no change in two-flash fusion thresholds after 960ms of bilateral visual stimulation at either 8.3hz or 12.5hz despite strong stimulus-evoked synchronization in scalp-recorded EEG. Taken together, these findings suggest that while endogenous alpha activity in occipital-parietal areas is consistently involved in the temporal resolution of perceptual sampling, this activity does not appear to be entrained by external

stimulation. In light of our null finding, future research exploring the malleability of endogenous oscillations should be careful to dissociate temporal expectation from entrainment effects.

Disclosures: M.J. Gray: None. **T. Emmanouil:** None.

Poster

169. Human Cognition and Behavior: Timing and Temporal Processing

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 169.26/HHH50

Topic: H.02. Human Cognition and Behavior

Title: Time perception and satiety: Does hunger dilate subjective time?

Authors: *L. MARK[1], J. C. J. LIU[2]
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Abstract: Extant research suggests that various homeostatic states affect our perception of time, consistent with observations that there are common neural substrates underlying both time estimation and self-consciousness. For example, being subject to altered temperature or pain have both been found to dilate time perception, resulting in the experience of time 'standing still'.
In this research, we extended previous findings by exploring how another physiological state - hunger - modulates the subjective experience of time. Because hunger varies throughout the day, we used an ecological momentary assessment (EMA) paradigm to track participants' hunger levels and time perception within their natural environments. We hypothesized that time would dilate in states of fasting, and that the magnitude of temporal dilation would increase as participants report higher levels of subjective hunger.
Participants were 53 healthy young adults who downloaded a phone-based app written for the study. The app was designed to contact participants 7 times a day for a week (for a total of 49 measurement points). At each notification, participants were shown a red screen for five durations (2000, 4000, 6000, 8000, and 10000 ms) and were asked to focus on the fixation cross in the middle of the screen. Participants then used a keypress to reproduce the duration that the screen was red. Between trials, there was an inter-stimulus interval of 1000-3000ms where a black screen was shown. At the end of the 5 reproduction trials, participants reported their hunger levels using a sliding scale ranging from "0: not hungry at all" to "10: very hungry". Participants also reported how alert, fearful, and happy they felt, and whether they had eaten in the past 30 minutes.
To accommodate the nested within-subject design of EMA, we ran a multilevel regression analysis to explore the influence of hunger levels on time estimates. Reproduced intervals increased with actual stimulus duration ($p < 0.001$), highlighting participants' ability to track time using a phone-based platform (as they do in laboratory studies). This effect was moderated

by participants' hunger levels ($p = 0.003$), such that the slope was steeper when participants reported greater levels of hunger; that is, at higher stimulus durations, participants tended to over-estimate time intervals in the fasted than fed state.
To our knowledge, our study represents the first use of an experience sampling method to explore time perception. Using this paradigm, our results suggest that everyday variations in hunger levels predict the subjective experience of time. Thus, as has been found with other physiological states, time dilates when one is hungry.

Disclosures: J.C.J. Liu: None.

Poster

169. Human Cognition and Behavior: Timing and Temporal Processing

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 169.27/HHH51

Topic: H.02. Human Cognition and Behavior

Support: European Research Council (Grant Agreement No 682117 BiT-ERC-2015-CoG to D.B.)

Title: Topographical representation of auditory stimulus durations

Authors: *S. KULASHEKHAR[1], S. MAASS[2], O. REYNAUD[3], H. VAN RIJN[4], D. BUETI[1]
[1]Dept. of Cognitive Neurosci., Intl. Sch. for Advanced Studies (SISSA), Trieste, Italy; [2]Fac. of Behavioural and Social Sci., Groningen, Netherlands; [3]Ctr. for Biomed. Imaging (CIBM) EPFL, Lausanne, Switzerland; [4]Univ. of Groningen, Groningen, Netherlands

Abstract: Studies using single-neuron recordings in animals suggest that the encoding of temporal information in the sub/supra-second range is achieved via duration tuning of neurons in the visual, auditory, medial premotor and prefrontal cortices. However, whether duration selective neurons have an ordered topographical arrangement in timing brain areas still remains unclear. In a recent study we addressed this issue using ultra-high-field functional magnetic resonance imaging (7T fMRI) and visual temporal discrimination paradigms. The data revealed a clear topographical organization of stimulus durations in the supplementary motor area (SMA) along the rostro-caudal direction, with shorter duration represented in the rostral SMA.
Here we first asked if the topographical organization of durations in SMA was independent from the sensory domain of the presented stimuli, and second, if this topography was influenced by changes in stimulus perception. If the topographical organization was independent from the nature of sensory stimulus, a similar organization would be observed in the SMA for both visual and auditory stimuli. Further, if the durations are represented in an absolute, rather than in a relative topographical manner, changes in the perceived durations would have no influence on the layout of the underlying neuronal tuning.

To test these hypotheses, we acquired fMRI (7T) data from 12 subjects using an auditory temporal reproduction task of 3 distinct durations. The task required the subjects to estimate the duration of a sound and then reproduce the estimated duration. In order to manipulate time perception the durations were presented in two distinct temporal contexts: 0.32s, 450s, 0.650 and 0.65s, 0.85s, 1.1s, with 0.65s being the shared duration. The fMRI data were analyzed using a general linear model approach. The encoding of durations was associated with activity in the brain regions known to be involved in the temporal processing of auditory stimuli, including the SMA. The results show a topographic organization of durations in the SMA similar to that of the visual domain with an influence of the temporal context on the organization of the SMA temporal maps. The present study further adds to the evidence that neuronal tuning mechanism could support the representation of sub-/supra-second durations.

Disclosures: S. Kulashekhar: None. **S. Maass:** None. **O. Reynaud:** None. **H. Van Rijn:** None. **D. Bueti:** None.

Poster

169. Human Cognition and Behavior: Timing and Temporal Processing

Location: SDCC Halls B-H

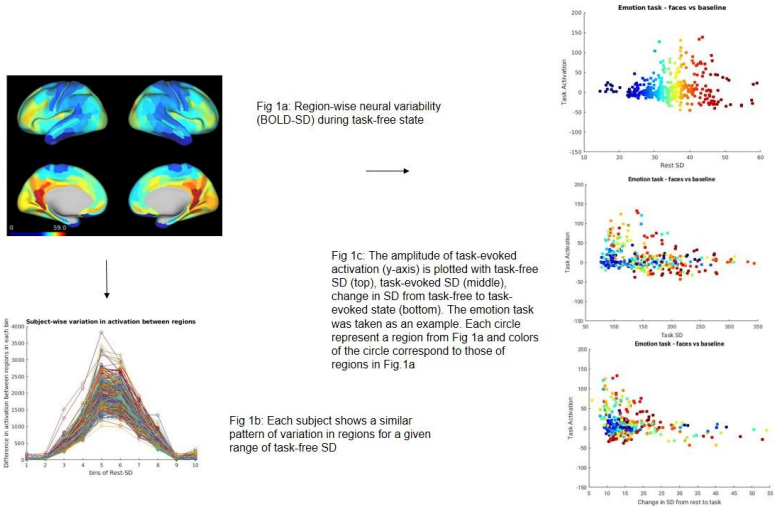
Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 169.28/HHH52

Topic: H.02. Human Cognition and Behavior

Title: Topology of signal variability of ongoing spontaneous brain activity and its impact on task-induced neural activation

Authors: *S. TUMATI, M. GOLESORKHI, G. NORTHOFF
Univ. of Ottawa, Ottawa, ON, Canada



Abstract:

Spontaneous (task-free) activity predicts task-evoked activity and is altered in various disorders. Neural variability of ongoing spontaneous and task-evoked states is a central feature of brain function, but it is unknown if the topography of task-free neural variability impacts the task-evoked state. Neural variability (operationalized as standard deviation (SD) of the BOLD signal) in the task-free and task-evoked state in 1084 subjects from the Human Connectome Project dataset, was related to task-evoked activation (amplitude of BOLD signal from general linear models) in seven tasks. First, the topography of task-free SD (fig 1a) differed from that of regional homogeneity and the power law exponent, though these measures were correlated at the whole brain level. Moreover, subject-level topographical organization of task-free SD was highly correlated with the group average topography, suggesting that it may be functionally relevant. Second, task-induced activation was more likely in regions with medium task-free SD, while regions with high or low task-free SD show low task-evoked activation, indicating that optimal values of task-free SD allow for greater differentiation between regions during task-evoked activation within and across tasks (fig 1b,c). Third, regions with low to medium values of task-evoked SD (i.e. SD of the BOLD signal during task) were more likely to show high task-evoked activation, consistent with past studies reporting reduction of variability (SD) in response to a task. Finally, in most regions the difference or change in SD from task-free to task-evoked state was small, including those that exhibited high task-evoked activation. Regions with a large reduction in SD were unlikely to show task-evoked activation, indicating that optimum task-free SD allows larger differentiation between regions during tasks. Thus, we show that the topography of task-free neural variability shapes the degree of task-evoked activation. This approach may prompt new investigations into the ubiquitous changes in spontaneous activity in various disorders.

Disclosures: S. Tumati: None. **M. Golesorkhi:** None. **G. Northoff:** None.

Poster

169. Human Cognition and Behavior: Timing and Temporal Processing

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 169.29/HHH53

Topic: H.02. Human Cognition and Behavior

Support: NSF BCS143 9267
NIA R01 AG034613

Title: The effect of aging on fMRI activity during a sequence memory task

Authors: *A. GUDMUNDSON[1], S. M. STARK[2], C. E. STARK[1]
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Abstract: A fundamental part of memory for events is the ability to organize and order events in time. Our lab has collaborated in the development of a cross-species, non-spatial memory task that would allow us to investigate the neurobiological basis of sequence memory using an integrative approach that capitalizes on the strengths of simultaneous human and animal model research. In the task, participants are shown a sequence of trials (4 odors for rats and 6 fractal images for humans) and must indicate on each trial whether an item is “in sequence” or “out of sequence” by releasing or continuing to hold a response key. Previous work demonstrated that rats and humans performed similarly for both in sequence and a variety of out of sequence probe trials, suggesting that there are likely to be comparable cognitive processes and underlying neural mechanisms between the species (Allen et al., 2014). Likewise, in both species, we have observed evidence for hippocampal and prefrontal contributions and interactions in the task (Allen et al., 2016; Elias et al. 2016; Boucquey et al., under review). We have now extended our analysis to compare young and aged adults to identify age-related changes. To provide the most valid age-related comparison, we matched behavior across aging by allowing for a slightly longer response window (Allen et al., 2015). Functional MRI results showed that similar brain regions, including the Medial Temporal Lobe and Prefrontal Cortex, was active for all participants when performing the Sequence Memory task. However, activity was still somewhat greater for young participants than aged participants. These results importantly replicate previous findings in young and extend these neural signatures into aging. Additionally, Multi-voxel Pattern Analysis (MVPA) demonstrates that the Medial Temporal Lobe could sufficiently decode items presented In Sequence. Further analysis will be important in determining which specific subregions are contributing to this pattern of activity. More importantly, these findings will provide new insight into how the brain codes for temporal information.

Disclosures: A. Gudmundson: None. **S.M. Stark:** None. **C.E. Stark:** None.

Poster

169. Human Cognition and Behavior: Timing and Temporal Processing

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 169.30/HHH54

Topic: H.02. Human Cognition and Behavior

Support: NSERC

Title: Differential generation of saccade, fixation and image onset event-related potentials in the human mesial temporal lobe

Authors: *C. KATZ[1], K. PATEL[1], O. TALAKOUB[2], K. L. HOFFMAN[3], T. A. VALIANTE[4]
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Abstract: Encoding and recollection of our visual world depends on how such information interacts with ongoing activity within our visual and memory systems. Electrophysiological responses in memory related structures during performance of mnemonic tasks that use visual stimuli, speak directly to these interactions. Indeed electrophysiology responses of medial temporal lobe (MTL) structures have identified event related potentials (ERPs) in response to presentation of visual stimuli (image-onset) and eye movements (saccades and fixations). Such responses provide mechanistic insights to how these responses are generated, and when explored in the human, fundamental insights to how the human visual system interacts with memory related structures. We hypothesized that since eye movements and image-onset both provide visual centers with salient information, perhaps they engage the same mechanisms. To explore this question, we used intracranial electroencephalography (iEEG) data from the MTLs of 11 patients with medically refractory epilepsy who participated in a novel visual search task. We sought to characterize electrophysiological responses of MTL structures to saccades, fixations and image onset. We demonstrated that the image-onset response is a stimulus specific evoked/additive response with a low-frequency power increase and post-stimulus phase clustering. Conversely, as previously reported, ERPs following eye movements were found to be a pure phase reset mechanism. Furthermore, this reset was associated with saccade onset and not saccade termination (fixation), suggesting it likely represents a supramodal signal, rather than a visual response - in stark contrast to the image onset response. Our findings are congruent with the suggestion that there may exist fast and slow theta, and further suggests how they differ as externally versus internally generated electrophysiological signatures.

Disclosures: C. Katz: None. **K. Patel:** None. **O. Talakoub:** None. **K.L. Hoffman:** None. **T.A. Valiante:** None.

Poster

170. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Sample Preparation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 170.01/HHH55

Topic: I.03. Anatomical Methods

Support: Grants-in-Aid for Science Research on Innovative Areas "Brain Information Dynamics" (18H05114)

Title: Optical clearing technique for quick reconstruction of whole-cell recorded neurons

Authors: *Y. SATO[1], T. MIYAWAKI[1], A. OUCHI[1], A. NOGUCHI[1], S. YAMAGUCHI[2], Y. IKEGAYA[1]
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Abstract: Neurons are morphologically diverse in branching patterns of dendrites, projecting targets of the axons, and the positions of the somata. Recent studies suggest that these morphological characteristics of neurons are functionally associated with their physiological properties, including firing patterns and the responses to neuromodulators. Therefore, to obtain deeper understanding of neuronal circuit operation, it is important to visualize the morphology of electrophysiologically recorded neurons. One of the most popular methods for this purpose is the neuronal reconstruction of biocytin injected by whole-cell patch clamp pipettes. However, the current protocol requires as long as 24 hours to complete the fixation and reconstruction, preventing us to confirm the results on the same day. Here, we developed a quick reconstruction method by optimizing Sca*l*eSQ, a tissue clearing protocol. To prevent tissues from swelling, which may nonlinearly distort the shapes of neurons, we adjusted osmolality by adding NaCl to the Sca*l*eSQ solution, termed this solution IsoSca*l*eSQ. Addition of 200 mM NaCl almost completely suppressed tissue expansions without affecting the optical clearing capability of the gray matter in the fixed brains. Moreover, no refractive index-matching process was needed for optical clearing for up to 500-µm-thick slices. As a result, IsoSca*l*eSQ solution reduced the total time spent for the reconstruction to 1 hr for *in vitro* patch-clamped neurons and 1.5 hr for *in vivo* patch-clamped neurons. The distortion of subcellular neuronal structures was virtually negligible, and this protocol was suitable for the morphological classification of the recorded neurons. Our novel method accelerates the reconstruction protocol, helping investigate the relationships between the neuronal structure and function.

Disclosures: Y. Sato: None. **T. Miyawaki:** None. **A. Ouchi:** None. **A. Noguchi:** None. **S. Yamaguchi:** None. **Y. Ikegaya:** None.

Poster

170. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Sample Preparation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 170.02/HHH56

Topic: I.03. Anatomical Methods

Support: DFG Collaborative Research Center 889

Title: Comparative imaging allows for an easy estimation of protein copy numbers

Authors: *K. N. RICHTER[1], H. WILDHAGEN[3], S. O. RIZZOLI[2]
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Abstract: When investigating a cellular process, it is of major importance to have a detailed picture of said process, including the proteins involved. Knowing the copy numbers of proteins

contributing to certain molecular pathways, enables us to identify potential bottlenecks within these pathways (see Wilhelm et al., Science, 2014 and Takamori et al., Cell, 2006) and to obtain quantative importance of certain proteins within a process. Furthermore, the information about copy numbers of proteins makes it possible to draw conclusions about potential functions, whenever these are unclear and difficult to address by other methods, such as gene knockout studies. However, the determination of protein copy numbers within a cell or compartment is not always trivial. Traditionally used methods, such as purification and subsequent mass spectrometry analysis cannot be applied to every sample. Therefore, we established an alternative approach to estimate protein copy numbers by using simple immunolabeling and imaging techniques. This comparative imaging approach is based on the knowledge about the copy numbers of synaptic proteins in synaptosome preparations (purified synaptic boutons) from previous studies (Wilhelm et al, 2014). These synaptosomes were obtained from rat brains and were characterized in detail, providing the copy numbers per synaptosome of over 60 synaptic proteins. For a proof of principle, we applied the approach to a well-known sample in neurobiology, the primary hippocampal neuron culture. We immunostained in parallel synaptosome samples and hippocampal neurons for the same target proteins and compared the derived signal intensities by use of a custom written Matlab routine. This enabled us to interpolate the copy numbers for 10 different synaptic proteins in the cultured hippocampal neurons. The comparative imaging approach is especially important for the estimation of protein numbers in samples that are difficult to characterize biochemically, like the inner hair cells (the sensory cells of the hearing system). These cells are very difficult to purify in sufficient numbers to apply biochemical methods for protein quantification. Therefore, we also applied the comparative imaging approach to those cells, in order to test the validity of the approach on a more complex sample.

Takamori, S. et al. Cell 127, 831 - 46 (2006)
Wilhelm, B.G. et al. Science 344, 1023 - 27 (2014)

Disclosures: K.N. Richter: None. **H. Wildhagen:** None. **S.O. Rizzoli:** None.

Poster

170. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Sample Preparation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 170.03/HHH57

Topic: I.03. Anatomical Methods

Support: NRF Grant 2015M3C7A1029032

Title: The effects of DeepLabel^{TM} antibody staining solution for antibody penetration in diverse organs after X-clarity tissue clearing process

Authors: *H. SHIN[1], D. KIM[2], J. JANG[2], S. PARK[1], N. JUNG[1], Y. CHOE[2]
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Abstract: The antibody penetration is an important issue in thick tissues such as whole brain or other organs. Several researchers have difficulty with elucidating the target protein expression in their own samples. In our study, we suggest that DeepLabel**™** (DL) Antibody staining solution can increase the effect of antibody labeling penetration into the deep part of the whole brain after using X-CLARITY tissue clearing system (Logos Biosystems). We started X-CLARITY tissue clearing process for tissue-hydrogel polymerization and electrophoresis in adult mouse (ICR) brain. The CLARITY is a method developed by the scientist at Stanford University that produces whole transparent tissues ready for multiple rounds of antibody labeling and imaging. The method has opened up a world of possibilities, from tracing neural circuitry to exploring the relationship between structure and function with a global perspective. After clearing, we used the DL solution pretreatment while using serum blocking. We also use both primary and secondary antibodies in DL solution. We examined several antibodies such as Coll IV (Collagen type IV) or a-SMA (alpha smooth muscle actin) not only tested in adult brain tissues but also other organs. The deep structure of Coll IV immunolabeled tissue in the half brain became apparent by imaging with a confocal microscope and light sheet microscopy. We can also observe the similar results in using different antibodies. These results suggest that using the DL solution can enhance the effect of antibody penetration in thick tissues such as brain or different organs.

Disclosures: H. Shin: None. **D. Kim:** None. **J. Jang:** None. **S. Park:** None. **N. Jung:** None. **Y. Choe:** None.

Poster

170. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Sample Preparation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 170.04/HHH58

Topic: I.03. Anatomical Methods

Support: Korea Health Technology R&D Project (KHIDI) HI17C1501

Title: Preliminary study to find conditions for efficient generations of cerebral ischemic brain damages using optical thrombosis

Authors: *J.-R. CHOI, H. PARK, S. OH, J. KIM, R.-H. RYU, S. AHN, S. KIM, H. MIN, D. KIM, S. KIM, S.-J. LEE, B. KANG, J.-W. SOHN
DGMIF, Daegu, Korea, Republic of

Abstract: An artificial thrombosis generation is well-established method of preclinical animal models to study mechanisms of thrombosis-caused diseases, for instance, a cerebral stroke and a cardiac infarction. In addition, it is used to investigate diagnostic and therapeutic modalities. Among various artificial thrombosis generation methods, an optical thrombosis using photo-chemical reactions of Rose Bengal has been generally employed for inducing cerebral ischemic brain damages. In this study, a generation of localized brain damages in small animals (rats) was achieved by an optical platform to generate cerebral ischemic damages. Collimated and focused light illuminated from a 532 nm LASER source was accurately positioned by three-dimensional translation stages. Also, a non-magnetic compartment to hold the small animal was integrated in the platform. Therefore, measurements using magnetic resonance imaging can be taken by moving the compartment directly with minimal movements. The localized ischemic brain damage was confirmed and compared with various illumination conditions by both time-varying magnetic resonance (T2) imaging and histological analysis using 2,3,5-triphenyltetrazolium chloride, one of the staining chemicals to represent damaged tissues. Also, we acquired transient thermal effects during optical thrombosis generations by a fiber-coupled thrombosis generation platform that can illuminate the light in a coil of the magnetic resonance imaging system. A preliminary study using the fiber-coupled platform suggested conditions for efficient investigations of brain injury animal models with minimizing thermal brain damages such as burning. We believe that the result of the preliminary study to find conditions will help for the efficient generations of cerebral brain damages to establish small animal models.

Disclosures: J. Choi: None. **H. Park:** None. **S. Oh:** None. **J. Kim:** None. **R. Ryu:** None. **S. Ahn:** None. **S. Kim:** None. **H. Min:** None. **D. Kim:** None. **S. Kim:** None. **S. Lee:** None. **B. Kang:** None. **J. Sohn:** None.

Poster

170. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Sample Preparation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 170.05/HHH59

Topic: I.03. Anatomical Methods

Support: Innovative and Technology Fund ITS/381/15

Title: Developing a novel tissue clearing protocol for visualizing the rat brain

Authors: *K. LEE[1], H. M. LAI[2], C. C. C. PANG[1,3], R. C. C. CHANG[1,4]
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Basic and Clin. Neuroscience, King's Col. London, London, United Kingdom; [4]State Key Lab. of Brain and Cognitive Sciences, The Univ. of Hong Kong, Hong Kong, China

Abstract: Among all different tissue clearing techniques that have been developed recently, delipidation-based clearing techniques such as passive CLARITY technique have been widely applied because of its simplicity and the ability to achieve long-term fluorescence signal preservation. However, these techniques require long clearing time, which causes problems such as protein loss, tissue expansion and deformation. While it is possible to reduce clearing time by performing partial delipidation only, it is difficult to estimate the tissue clearing time since clearing efficiency varies depending on the region and size of tissues. In this study, we established partial delipidation by combining sodium dodecyl sulfate (SDS) with Optical Properties-adjusting Tissue-Clearing agent (OPTICLEAR), a refractive index matching solution recently developed, during the clearing step. To access the clearing efficiency and tissue morphological preservation, 4mm^3 coronal brain cubics were cut in half and cleared with SDS solution only or SDS + OPTICLEAR solution at 37°C. All conditions were performed at 37°C. The transparency level was quantified by measuring absorbance level at 750 nm, with the more transparent the tissues were, the lower the absorbance level would be. Sections were imaged before and after clearing such that their sizes were outlined and calculated by ImageJ software. It was found that the addition of OPTICLEAR into SDS solution enhanced the clearing speed and minimized tissue expansion compared with that in SDS solution only. The image of stainfree gel analysis further proved that the combination of SDS-OPTICLEAR exhibited less protein loss compared with that in SDS solution only. We further accessed the compatibility of novel clearing solution with immunofluorescence staining by staining nucleus basalis of Meynert tissues with choline acetyltransferase (ChAT). The staining in 4% SDS-OPTICLEAR group showed less background and better resolution of ChAT-positive cell morphologies. We further implemented the clearing technique in demonstrating dopaminergic neuronal loss in classical 6-hydroxydopamine model. Taken together, with an improved and faster protocol of making the brain to be transparent, we will be able to speed up immunohistochemical staining to obtain a better resolution for cell-cell interactions and even protein-protein interactions in neurodegenerative diseases.

Disclosures: K. Lee: None. **H.M. Lai:** None. **C.C.C. Pang:** None. **R.C.C. Chang:** None.

Poster

170. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Sample Preparation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 170.06/HHH60

Topic: I.03. Anatomical Methods

Support: Hungarian Academy of Sciences Momentum Program LP-54/2013
NRDI K124972
US NIH NS099457

Title: STORM imaging of synaptic proteins in super-resolved cellular and subcellular contexts

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Abstract: Mapping the nanoscale arrangement of synaptic proteins is a key to understand the molecular logic of neural transmission. In the past, immunogold electron microscopy was the most widely used technique to uncover the precise localization of certain proteins at the nanoscale level. While it serves invaluable information about the subcellular context harboring the target protein, it is very laborious and the low labeling density together with the difficulty to label multiple targets limits its utility. As a potential alternative approach, STochastic Optical Reconstruction Microscopy (STORM), a recently developed fluorescence-based super-resolution imaging method, makes it possible to study the nanoscale distribution of multiple proteins. However, one of the major drawbacks of STORM imaging compared to electron microscopy is that cellular membranes are not visible and interpreting data in a super-resolved cellular and subcellular context still remains a difficult task. Therefore, in the present study, we aimed to improve the nanoscale labeling and imaging methodology of synaptic proteins and the plasma membrane in a cell-specific manner. Deep and superficial CA1 pyramidal cells can be differentiated by their time of birth. Thus, we *in utero* electroporated mice at embryonic day E13.5-14 and E16.5-17, respectively, with a plasmid expressing Green Fluorescent Protein (GFP) fused to Channelrhodopsin-2 (ChR2). Here we show that ChR2-GFP incorporates into the plasma membrane, and STORM imaging outlines the plasma membrane of single electroporated cells, leaving the far-red channel free to label synaptic proteins. This approach could more reliably define whether a given localization point belongs to the investigated cell or subcellular compartment. Moreover, by performing a systematic analysis of several existing approaches, we found the CF568/AF647 dye pair as the most suitable for dual-color STORM imaging and identified the most optimal use of the buffer conditions in tissue preparations. Taken together, these efforts highlight the importance of interpreting STORM super-resolution imaging data in well-defined cellular and subcellular contexts. Importantly, the present methodology can be extended to explore the nanoscale molecular architecture of most cell types in the brain.

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Poster

170. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Sample Preparation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 170.07/HHH61

Topic: I.03. Anatomical Methods

Support: NWO VIDI Grant 14637
ERC Starting Grant 639938 MULTICONNECT

Title: A scalable processing pipeline for high-throughput optical clearing and labelling of very large formalin-fixed human brain samples

Authors: *S. HILDEBRAND, A. SCHUETH, S. SENGUPTA, A. ROEBROECK
Maastricht Univ., Maastricht, Netherlands

Abstract: With the introduction of optical clearing in neuroscience, considerable advances in tissue clearing and large volume microscopy have been made[1-4]. However, volume imaging and cytoarchitectonic characterization of large human brain samples, scalable in terms of time and cost to cover a significant portion of a cortical area, has so far remained challenging. This is especially true for adult formalin-fixed tissue. We recently reported on MASH (Multiscale Architectonic Staining of Human cortex)[5]: a scalable nuclear and cytoplasmic labeling and optical clearing approach suitable for 4 - 5 mm thick archival adult human cortex samples. Here we show a simple, economic solution for consistent and rapid histological processing of an entire human occipital lobe (Fig. 1). To this end we build a custom-made cutting device to acquire consistent 5 mm thick coronal slices of an agarose-embedded occipital lobe. Clearing and labelling could be robustly performed in a glass jar with Teflon spacing elements under constant stirring. This is an important step for mapping and cytoarchitectural characterization of entire sub-systems of the human brain in 3D.

References:

1 Liebmann, T. *et al.* Three-Dimensional Study of Alzheimer's Disease Hallmarks Using the iDISCO Clearing Method. *Cell reports* **16**, 1138-1152, doi:10.1016/j.celrep.2016.06.060 (2016).
2 Murakami, T. C. *et al.* A three-dimensional single-cell-resolution whole-brain atlas using CUBIC-X expansion microscopy and tissue clearing. *Nature neuroscience* **21**, 625-637, doi:10.1038/s41593-018-0109-1 (2018).
3 Renier, N. *et al.* Mapping of Brain Activity by Automated Volume Analysis of Immediate Early Genes. *Cell* **165**, 1789-1802, doi:10.1016/j.cell.2016.05.007 (2016).
4 Ye, L. *et al.* Wiring and Molecular Features of Prefrontal Ensembles Representing Distinct Experiences. *Cell* **165**, 1776-1788, doi:10.1016/j.cell.2016.05.010 (2016).

5 Hildebrand, S., Schueth, A., Herrler, A., Galuske, R. & Roebroeck, A. Scalable cytoarchitectonic characterization of large intact human neocortex samples. *bioRxiv* (2018).

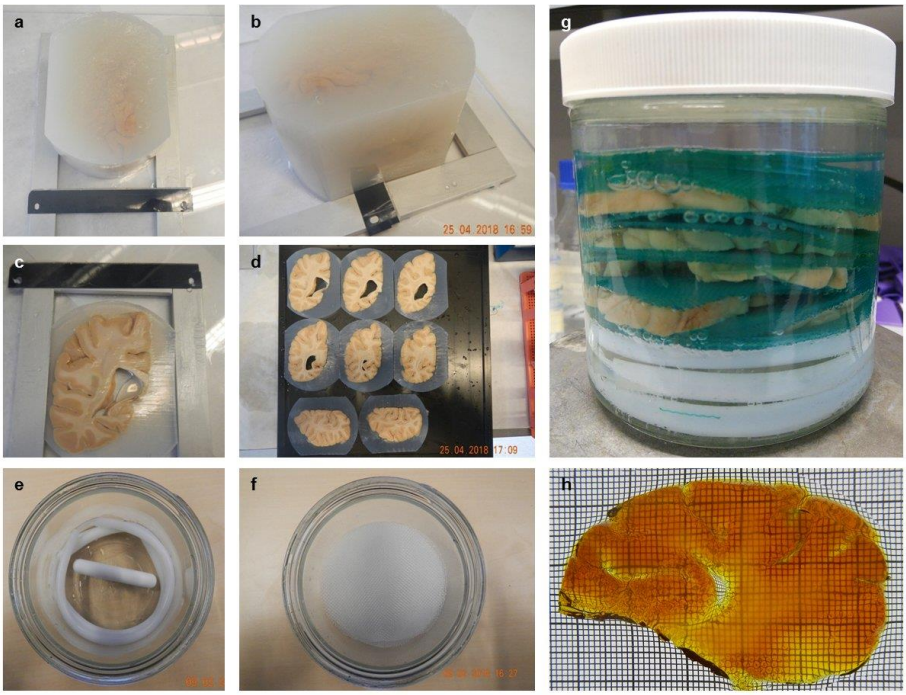

Figure 1: Processing pipeline for optical clearing and labelling of an entire human occipital lobe. The sample is first embedded in 4% agarose in PBS in the desired orientation and manually cut in a custom made cutting device (**a – d**). Brain slices can then be incubated in glass jar for all steps of the clearing and labelling procedure. Care should be taken, that all plastic parts are compatible with the applied chemicals (**e – g**). (**h**) shows a complete 5 mm thick coronal slice of a human occipital lobe after labelling and clearing (grid size: 1 mm x 1 mm).

Disclosures: S. Hildebrand: None. **A. Schueth:** None. **S. Sengupta:** None. **A. Roebroeck:** None.

Poster

170. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Sample Preparation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 170.08/III1

Topic: I.03. Anatomical Methods

Support: KIST 0409-20180017
NRF 2016R1C1B2007319
NRF 2016R1A4A1010796

Title: Volumetric mGRASP: Large-scale 3D mapping of mammalian synaptic connectivity with light microscopy

Authors: *D.-J. KOO[1], M. AN[1], J. KWEON[1], H.-E. PARK[1], H. LEE[2], J. KIM[2], S.-Y. KIM[1]
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Abstract: The mammalian GFP reconstitution across synaptic partners (mGRASP) technique, based on functional complementation between two non-fluorescent GFP fragments, enables accurate detection of synaptic contacts between two defined neural populations with light microscopy. Due to the limited imaging depth in the opaque brain sections, however, obtaining the structural information about the presynaptic and postsynaptic neurons and their synaptic contacts from a large tissue volume remains challenging. Here we address this challenge by integrating mGRASP with tissue clearing techniques to establish a method (termed volumetric mGRASP) for large-scale 3D mapping of mammalian synaptic connectivity with light microscopy. We systematically compared several latest clearing methods, and optimized the most suitable method for the best clearing performance and mGRASP fluorescence preservation. Applying our method to the hippocampal CA3-CA1 synapse, we show large-scale imaging, reliable identification and fine three-dimensional (3D) reconstruction of synapses and participating neurons, and analysis of their structural, spatial and compositional relationships. We are currently investigating the NAc-VTA synapses with our method, to map the detailed sub-circuitry in a subregion- and cell type-specific manner, and correlate this result with functions in reward processing. Our method expands the utility of both mGRASP and clearing techniques, and is broadly applicable to the mapping of synapses between two defined neural populations over a large scale.

Disclosures: D. Koo: None. **M. An:** None. **J. Kweon:** None. **H. Park:** None. **H. Lee:** None. **J. Kim:** None. **S. Kim:** None.

Poster

170. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Sample Preparation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 170.09/III2

Topic: I.03. Anatomical Methods

Support: Vascular Dementia Research Foundation
Synergy Excellence Cluster Munich (SyNergy)
ERA-Net Neuron 01EW1501A
NIH

Title: vDISCO panoptic imaging reveals remote CNS injury effects in intact transparent mice

Authors: *R. CAI[1,2,3], C. PAN[1,2,3], A. GHASEMIGHARAGOZ[1,3], M. I. TODOROV[1,2,3], B. FÖRSTERA[1,3], S. ZHAO[1,3], D. THEODOROU[3,4,5], M. KERSCHENSTEINER[3,4,5,6], A. ERTÜRK[1,2,3,6]

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Abstract: Traumatic injuries of central nervous system (CNS) i.e. traumatic brain injury (TBI) and spinal cord injury (SCI), are one of the main causes of disability and death in people under 40 years old. They often lead to chronic complications including epilepsy, dementia, progressive motor decline, neuropsychiatric disorders and metabolic disorders[1,2]. However, little is known about how CNS trauma impacts the body as a whole and leads to such diverse neuronal and non-neuronal disorders. A major difficulty has been the lack of methods to image intact neuronal connectivity in whole mouse bodies.

Recently developed tissue clearing methods render intact rodent organs and bodies transparent enabling the imaging of unsectioned neuronal connections[3-5]. Here, we developed vDISCO panoptic imaging to visualize sub-cellular details in adult mice through intact bones and highly autofluorescent tissues. First, we constructed the complete neuronal connectome of adult *Thy1*-GFPM mice, in which motor and sensory neurons express EGFP[6]. Applying a closed-head TBI model on *Thy1*-GFPM mice, we discovered a reduction of the complexity of the axonal terminals in the upper torso, implying a partial degeneration of these nerve terminals. Next, we applied SCI in CD68-EGFP mice expressing EGFP in monocytes and macrophages[7]. We found that, as expected, SCI triggered an invasion of CD68 GFP+ cells at the spinal lesion site. Surprisingly, we also discovered a massive accumulation of these immune cells in the surrounding muscles, spinal cord roots and meningeal vessels. These results suggest that the study of CNS trauma requires a broader view- whole animal body in perspective.

In conclusion, vDISCO panoptic imaging provides a holistic view on intact organisms resulting in discovery of previously underestimated critical biological information.

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1. Smith *et al*. Chronic neuropathologies of single and repetitive TBI: substrates of dementia? *Nat. rev. Neurol.* **9**, (2013).

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3. Erturk *et al.* Three-dimensional imaging of solvent-cleared organs using 3DISCO. *Nat. protoc.* **7**, (2012).

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Disclosures: R. Cai: None. **C. Pan:** None. **A. Ghasemigharagoz:** None. **M.I. Todorov:** None. **B. Förstera:** None. **S. Zhao:** None. **D. Theodorou:** None. **M. Kerschensteiner:** None. **A. Ertürk:** None.

Poster

170. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Sample Preparation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 170.10/III3

Topic: I.03. Anatomical Methods

Title: DISCO-Vascular: A complete 3D mouse brain vascular atlas at micrometer scale

Authors: *M. I. TODOROV[1], J. C. PAETZOLD[2], O. SCHOPPE[2], B. MENZE[2], A. ERTURK[1]
[1]Inst. for Stroke and Dementia Res., Klinikum der Univ. München, Munich, Germany; [2]Image-based Biomed. Modeling Group, Dept. of Informatics, Tech. Univ. of Munich, Munich, Germany

Abstract: The vascular pathology is a common feature of numerous neurodegenerative diseases and ageing. While mouse models are often used for the study of vascular diseases, there is still no publicly available complete vascular atlas of mouse brain at the capillary level. A major bottleneck has been the technical difficulty of assembling vascular maps via traditional histology at micrometer scale. Tissue clearing technologies now provide substantial advantages over conventional histology by allowing micrometer resolution scans of large intact tissues. Imaging transparent specimens with single plane illumination (light-sheet) microscopy has become a gold standard for systems biology level analysis including intact rodent brains.
Here, we generated the highest resolution complete vascular map of the mouse brain using DISCO tissue clearing[1,2] approach, called DISCO-Vascular. To provide anatomical reference, we registered the DISCO-Vascular data to the Allen Mouse Brain Atlas.
However, quantitative analysis of the vasculature requires a 3D reconstruction of the vessels (i.e., a semantic segmentation of the imaging data). To this end, we introduce a novel methodology to analyze the details of the mouse brain vasculature using Fast Convolutional Neural Networks (FCNN). The proposed advanced deep learning methods enable a significantly faster and more precise 3D segmentation of vasculature down to the capillary level than traditional approaches. The combination of DISCO-Vascular with this 3D reconstruction thus is capable of providing vascular information registered to all annotated brain regions of the Allen Mouse Brain Atlas.
In summary, we present the first complete mouse brain vascular atlas in 3D. This atlas can contribute as a benchmark for vascular pathology and enable the study of vascular involvement in a range of healthy and pathological states. The proposed DISCO-Vascular atlas is publicly

available for further investigations.
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2. Pan, C. et al. Shrinkage-mediated imaging of entire organs and organisms using uDISCO. Nat. Met. 13, 859-867 (2016)

Disclosures: M.I. Todorov: None. **J.C. Paetzold:** None. **O. Schoppe:** None. **B. Menze:** None. **A. Erturk:** None.

Poster

170. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Sample Preparation

Location: SDCC Halls B-H

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Program #/Poster #: 170.11/III4

Topic: I.03. Anatomical Methods

Support: 2012YQ030260
2015CB755602

Title: A plastic embedding method for preservation of multi color fluorescent protein

Authors: *M. REN, J. TIAN, X. LI, H. GONG
Huazhong Univ. of Sci. and Technol., Hubei, China

Abstract: Resin embedding has been widely used in fluorescent protein labelling tissues especially for high-throughput imaging to acquire fine structure of neural circuits. Meanwhile, multicolor fluorescent protein labeling was beneficial to comprehensive analysis of the fine structure of the brain.
To acquire multiple information from individual sample at the cellular level, we optimized the resin embedding methods to keep the red fluorescent protein (RFP). Which preserved 90% of fluorescence intensity of RFP and reduced the auto-fluorescence effectually by adding fluorescent protein protection reagent and reducing the polymerization temperature. Not only the cell body but the tiny axon can be fine detected. Our optimized method is not only suitable for the classical RFP such as tdTomato/mCherry/DsRed, but also for YFP /GFP / BFP labelling samples. The antigen activity still exists after embedding.
These results suggest that modified GMA embedding method provide a novel approach for high resolution microscopic imaging at a single-cell resolution to study the complex neural circuits. This method could provide better samples for correlated fluorescence microscopy and electron microscopy study.

Disclosures: M. Ren: None. **J. Tian:** None. **X. Li:** None. **H. Gong:** None.

Poster

170. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Sample Preparation

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Program #/Poster #: 170.12/III5

Topic: I.03. Anatomical Methods

Support: 973 project: 2015CB755602
The Science Fund for Creative Research Group of China: 61421064
NSFC projects: 91432105, 91432111, 81527901, and 31625013

Title: Scalable embedding method with a modified hydrogel for optical imaging of fluorescent sample

Authors: *C. ZHOU, T. LUO, X. LI, H. GONG
Huazhong Univ. of Sci. and Technol., Hubei, China

Abstract: The optical imaging methods for whole brain imaging developed recently make reconstruction of the neural circuits and the complete neuron morphology possible. However, the low fidelity of tissue morphology and fluorescence signal during the samples preparing limits the higher level application. We developed a new embedding method with a modified hydrogel for fluorescent biological-tissue. This method is simple and compatible with kinds of fluorescent proteins and fluorescein while preserves cellular anatomical structure and morphology in detail. Moreover, the fluorescence intensity can maintain several months. After embedding, biological tissue is a part of transparent, which can increase the image depth and achieve more detail signal. With this new embedding strategy, we can embed a whole mouse brain in two days and imaged it with kinds of imaging systems, including normal fluorescent system, laser scanning confocal microscope, Series two-photon (STP) and fluorescence micro-optical sectioning tomography system (fMOST) etc. The results indicate that this embedding method is effective and universal for big volume tissue embedding such as macaque brain, while can be extended to other biological tissue embedding, especially for organization structure with plentiful biological tissue clearance or poor uniformity, such as heart or liver.

Disclosures: C. Zhou: None. **T. Luo:** None. **X. Li:** None. **H. Gong:** None.

Poster

170. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Sample Preparation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 170.13/III6

Topic: I.03. Anatomical Methods

Title: Hybrid of magnetic particle imaging and optical multimodality imaging system for brain imaging

Authors: *H. HUI[1], X. YANG[1], J. TIAN[2]
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Abstract: In the last decade, a novel in vivo imaging modality called Magnetic particle imaging (MPI) has been growing fast because of its extremely high contrast and sensitivity. It has the ability to detection of nanomolar concentrations of super-paramagnetic iron oxide (SPIO) nanoparticles as contrast agent, sub-mm spatial resolution, and without tissue signal attenuation. For quantitative tomographic brain imaging and early diagnosis of brain cancer, it is necessary to combine MPI and other molecular imaging modalities, such as CT and optical imaging modalities. Here, we present our design of a hybrid of MPI with our previous developed optical multimodality imaging system. All the optical molecular imaging devices are mounted on a gantry. Therefore, all the imaging devices can rotate during the experiments. The X-ray source and X-ray flat panel detector are used for (Computed tomography) CT imaging. The Charge-coupled Device (CCD) camera is used for Cerenkov luminescence tomography (CLT), Bioluminescence tomography (BLT), Fluorescence molecular tomography (FMT) imaging. MPI module is placed behind the gantry for optical multimodality. At the beginning of the experiment, optical multimodality imaging probes are injected into the mouse via tail intravenous injection. Then, the anaesthetized mouse is placed on the holder. Then the system starts to acquire data of optical molecular imaging. After the optical molecular imaging data acquisition, MPI imaging is performed with i.v. injection of SPIO nanoparticles. Finally, three-dimensional reconstruction of optical and MPI data is performed by our developed software 3DMed. In conclusion, hybrid of MPI and optical multimodality imaging system will be a new in vivo imaging method for brain imaging of small animal.

Disclosures: H. Hui: None. **X. Yang:** None. **J. Tian:** None.

Poster

170. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Sample Preparation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 170.14/III7

Topic: I.03. Anatomical Methods

Title: Improved axial dimension resolution in 3D fluorescence imaging by refractive index matching and specimen clearing

Authors: *D. W. BEACHAM[1], D. D. CASH[2], A. W. YORK[2], B. BOAL[2], O. GOLUB[2], M. WICKMAN[2], E. J. WELCH[2]
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Abstract: Fluorescence microscopy enables researchers to observe biological targets within both cultured cells and animal tissue specimens. Its utility is generally limited to cultured cell monolayers and 5-10 micron (μm) thick sections, as thicker samples begin to lose transparency. Loss in transparency arises from the refraction of light as it travels through thicker (greater than 10μm) sections making acquisition of three-dimensional (3D) fluorescent images problematic. The refraction of light is due to differences in the refractive indices (RI) of tissue components (e.g. lipids and proteins) and the surrounding media. Light emitted by fluorophores within a biological specimen travels through the specimen/imaging media, glass, and air/immersion fluid before photons are collected at the microscope objective. Any RI mismatch along the light path limits both the axial (z-dimension) resolution and achievable focal depth. Immersion oil (RI=1.52) eliminates RI mismatch between the microscope objectives and cover glass, but commonly used mounting media and imaging solutions have RI values less than or equal to 1.47. In practice, the thickness of biological specimens that can be imaged in a medium with an RI=1.47 is ~20 μm due to deterioration in axial resolution beyond 20μm. To fully understand biological significance, imaging of labeled targets within biological specimens thicker than 20 μm is necessary. To enable deep tissue 3D fluorescence imaging, ProLong™ Glass Antifade Mounting Media and CytoVista™ Tissue Clearing Reagents are presented. ProLong™ Glass is recommended for use with specimens up to 200 μm thick and exhibits a cured RI of 1.52. Point-spread-functions (PSF) of sub-resolution microspheres were measured using scanning confocal microscopy in media with RI=1.52 and 1.47. A quantitative improvement in the axial resolution of microspheres at focal depths beyond 100 μm is observed for samples prepared in ProLong™ Glass. To demonstrate improved image quality, maximum intensity projections are presented showing greater focal depth within thick biological specimens mounted in ProLong™ Glass. Imaging specimens greater than 200 μm will often require additional clearing to render the sample optically transparent. To address this need, we demonstrate the use of CytoVista™

Tissue Clearing Reagents for clearing biological specimens up to 1 mm and also discuss considerations for the optimization of immunostaining.

Disclosures: D.W. Beacham: A. Employment/Salary (full or part-time):; Thermo Fisher Scientific. **D.D. Cash:** A. Employment/Salary (full or part-time):; Thermo Fisher Scientific. **A.W. York:** A. Employment/Salary (full or part-time):; Thermo Fisher Scientific. **B. Boal:** A. Employment/Salary (full or part-time):; Thermo Fisher Scientific. **O. Golub:** A. Employment/Salary (full or part-time):; Thermo Fisher Scientific. **M. Wickman:** A. Employment/Salary (full or part-time):; Thermo Fisher Scientific. **E.J. Welch:** A. Employment/Salary (full or part-time):; Thermo Fisher Scientific.

Poster

170. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Sample Preparation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 170.15/III8

Topic: I.03. Anatomical Methods

Support: NIH Project: Molecular Mechanisms Underlying Glioma Invasion of the Human Subventricular Zone
American Cancer Society: Targeting a Migratory Route for Human Glioma Stem Cell Dissemination

Title: Pressurized Immunohistochemistry (pIHC) of cleared human and mouse brain tissue

Authors: *R. FIORELLI[1], G. SIDHU[1], A. CEBRIAN-SILLA[2], E. MENDELEZ[1], S. V. MEHTA[1], J. GARCIA-VERDUGO[2], N. SANAI[1]
[1]Barrow Neurolog. Inst., St. Joseph's Hosp. and Med. Ctr., Phoenix, AZ; [2]Lab. of Comparative Neurobio., Inst. Cavanilles, Univ. of Valencia, Valencia, Spain

Abstract: Imaging of brain tissue in 3D is an essential tool to understand cytoarchitecture and spatial relationship of cell populations. Recent development of clarification techniques has advanced substantially the potential for 3D imaging of thick (i.e. 100 - 2000+ µm) tissue samples of both human and mouse brain tissue. However, immunohistochemistry (IHC) of such samples has limited applications since labeling antibodies have limited diffusion in thick tissue.
Here, we have developed a custom air-tight device (i.e. Pressure Box) of simple manufacture, in which atmospheric pressure could be increased using N_2 gas. When the Pressure Box was used in experiments of indirect IHC, we found that the speed and depth of penetration of antibodies was greatly increased by pressurization. This technique, denominated pressurized IHC (pIHC), enabled us to achieved intense and uniform immunostainings to a depth greater than 2-fold compared to non-pressurized control.

We compared the performance of pIHC in human brain tissue passively clarified with two published techniques, i.e. PACT and CUBIC, showing compatibility with minor differences between the two. pIHC increased the depth and intensity of several antibodies, including Laminin, α-SMA, Iba -1, GFAP, Map-2 as well as of the dyes DAPI and Lectin. TEM analysis showed that pressurization does not induce tissue artifacts. We show that pIHC is compatible with co-localization studies, quadruple channel immunostainings and heat-mediated antigen retrieval in 1-mm thick sections of an endogenously fluorescent mouse model of brain tumor. Finally, we demonstrate that pressurization enables staining of free floating 40-µm mouse brain sections in half of the time compared to standard protocols (i.e. 8 vs. 19 hrs).
Thus, pIHC not only has the potential to be included in the pipeline of most of the clarification methods, but also be employed in any histology lab to speed up IHC protocols.
Application of pressure on molecular labeling techniques will provide the basis for further technique development, including increasing the potential for more efficient processing in translational and diagnostic methods on human specimens.
Disclosure: Elements of this project are subject to a pending U.S. patent application.

Disclosures: **R. Fiorelli:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Dignity Health. **G. Sidhu:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Dignity Health. **A. Cebrian-Silla:** None. **E. Mendelez:** None. **S.V. Mehta:** None. **J. Garcia-Verdugo:** None. **N. Sanai:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Dignity Health.

Poster

170. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Sample Preparation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 170.16/III9

Topic: I.03. Anatomical Methods

Support: NIH T32 DE014318

Title: A modified iDISCO+ protocol for clearing and visualization of intact human teeth

Authors: P. M. LOCOCO[1], M. WIDBILLER[2], A. R. DIOGENES[1], *K. M. HARGREAVES[1]
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Abstract: Advancements in microscopy and data processing have fueled a resurgence in whole-tissue clearing techniques. Several novel solvent- and aqueous-based tissue clearing protocols

that have been developed to permit large-volume section-free imaging of intact tissue specimens have generated exciting new insight into three-dimensional tissue architecture. Although tremendous progress has been made clearing various soft tissues and even bone, studies with teeth have yet to be conducted. Teeth are uniquely composed of both hard and soft tissues, including three distinct mineralized tissues (i.e., enamel, cementum, and dentin) that encapsulate a highly vascularized and innervated soft connective tissue (i.e., dental pulp). Though histological assessments are feasible, current preparation protocols are extremely time-consuming and often produce widespread distortions and processing artifacts that complicate visualization and analysis. Thus, the purpose of this study was to determine whether tissue clearing was amenable with decalcified human teeth. Here, we report on the ongoing development of a reproducible protocol to successfully clear an intact human tooth via modification of the solvent-based clearing technique, iDISCO+. Following decalcification with EDTA (10%) and Morse's solution (25% formic acid), our modified iDISCO-protocol rendered whole teeth almost completely transparent. Immunolabeled structures were easily visualized within cleared samples using confocal microscopy, including CD31-labeled vasculature and NFH-labeled neurons. Automated filament tracing algorithms were applied to reconstruct and quantify tooth innervation. Collectively, we determined that whole teeth indeed can be cleared to visualize native morphology without sectioning. With continued optimization and improvements in imaging and analysis, this approach may provide unprecedented insight into tooth anatomy that could improve the current understanding of dental pathologies and aid in development of better interventions.

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Poster

170. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Sample Preparation

Location: SDCC Halls B-H

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Topic: I.03. Anatomical Methods

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Searle Scholars Program
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NCSOFT Cultural Foundation

Title: eTANGO: A technology platform for rapid, uniform, cost-effective staining of intact brains

Authors: *D. YUN[1,2], Y.-G. PARK[1,2], J. H. CHO[3], G. DRUMMOND[1,2], Y. TIAN[3], H. CHOI[1,2], T. KU[1,2], L. RUELAS[3], K. CHUNG[1,2,3,4,5]
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Abstract: Rapid advances in volumetric imaging and tissue-clearing technologies combined with an ever-expanding library of molecular probes provide new opportunities for holistic interrogation of the brain. However, molecular phenotyping of intact tissues has long been limited by the lack of a reliable tissue staining method. We have developed a rapid and reliable labeling technique termed eTANGO (**e**lectrophoretic **T**ransport of **A**ctivity-modulated molecules in a **N**anoporous **G**el **O**rgan hybrid) that allows complete and uniform labeling of large-scale intact tissues within 2 days, compared to weeks to months with conventional methods. eTANGO combines the SWITCH framework and stochastic electrotransport to modulate reaction kinetics and increase transport speed of the molecular probes. SWITCH (Murray, Cell, 2015) inhibits antibody reactions during transport into the tissue. Stochastic electrotransport (Kim, PNAS, 2015) rapidly transports antibodies into tissue without damaging its structure. Combining these two processes allows for uniform and robust labeling of a delipidated mouse hemisphere in 2 days at a fraction of the cost of passive staining approaches. eTANGO is compatible with many cell-type and structural markers, and it also works for carbohydrate-binding proteins and nucleus dyes. We have successfully performed multiple rounds of labeling of the same sample using eTANGO and have visualized various cell-type markers in large-scale brain tissues. In addition, we developed a fully automated system to carry out the process. We envision that eTANGO will accelerate discoveries in a broad range of biological research by enabling rapid structural and molecular phenotyping of large-scale biological systems.

Disclosures: D. Yun: None. **Y. Park:** None. **J.H. Cho:** None. **G. Drummond:** None. **Y. Tian:** None. **H. Choi:** None. **T. Ku:** None. **L. Ruelas:** None. **K. Chung:** None.

Poster

170. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Sample Preparation

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Topic: I.03. Anatomical Methods

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McKnight Foundation Technology Award
JPB Foundation (PIIF and PNDRF)
NCSOFT Cultural Foundation

Title: Immersion media, custom tools, and protocols for single-cell resolution mapping of intact brains using lightsheet microscopy

Authors: *N. EVANS[1], Y.-G. PARK[1], D. YUN[1], V. LILASCHAROEN[2], B. LIM[2], K. CHUNG[1]
[1]MIT, Cambridge, MA; [2]UCSD, San Diego, CA

Abstract: Tissue transformation techniques such as CLARITY, SHIELD, MAP, CUBIC, and DISCO allow deep and detailed investigation of intact biological systems without the need for traditional histological sectioning. By rendering whole organs and large tissues optically clear, these techniques can be paired with imaging modalities such as lightsheet fluorescence microscopy (LSFM) to characterize individual cells deep within intact biological structures. However, the resulting imaging results are often limited by the quality of the refractive index matching between the immersion medium and the tissue. Sample variability, degree of delipidation, and even intra-organ tissue density variations can cause spherical aberrations and scattering which prevent accurate, high-resolution visualization and analysis of cells deep within a tissue.
To facilitate multidimensional interrogation of individual cells in intact organs, we have developed immersion media, custom tools, and protocols that are optimized for LSFM imaging of SHIELD- and MAP-processed samples. Our immersion media maximally preserve protein fluorescence and are compatible with immunostaining, fluorescence in situ hybridization, and nuclei labeling. We have demonstrated multicolor single-cell resolution imaging of up to three intact mouse brain hemispheres within one day. The MAP-optimized immersion media enable rapid super-resolution LSFM imaging, up to the working distance of the objectives. We have combined these tools with viral labeling techniques to map neural circuits at single-cell resolution. We anticipate that future advances in this approach will allow high-throughput interrogation of neural circuits at single-cell resolution.

Disclosures: N. Evans: None. **Y. Park:** None. **D. Yun:** None. **V. Lilascharoen:** None. **B. Lim:** None. **K. Chung:** None.

Poster

170. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Sample Preparation

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

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Topic: I.03. Anatomical Methods

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NCSOFT Cultural Foundation

Title: SHIELD: Simultaneous global protection of biomolecules, protein fluorescence, and tissue architecture via polyfunctional crosslinkers

Authors: *Y.-G. PARK[1,2], C. SOHN[1,2], R. CHEN[1,2], M. MCCUE[1,2], G. T. DRUMMOND[1,2], T. KU[1,2], D. YUN[1,2], N. B. EVANS[1,2], H. C. OAK[3], W. TRIEU[4], H. CHOI[1,2], X. JIN[7], V. LILASCHAROEN[8], J. WANG[9], M. C. TRUTTMANN[10], H. W. QI[5,6], H. L. PLOEGH[11], T. R. GOLUB[7], S.-C. CHEN[9], M. P. FROSCH[12], H. J. KULIK[5], B. LIM[8], K. CHUNG[1,2,5,3,7]
[1]Inst. for Med. Engin. and Sci., [2]Picower Inst. for Learning and Memory, [3]Dept. of Brain and Cognitive Sci., [4]Dept. of Biol. Engin., [5]Dept. of Chem. Engin., [6]Dept. of Chem., MIT, Cambridge, MA; [7]Broad Inst. of Harvard Univ. and MIT, Cambridge, MA; [8]Neurobio. Section, Div. of Biol. Sci., UCSD, La Jolla, CA; [9]Dept. of Mechanical and Automation Engin., The Chinese Univ. of Hong Kong, Shatin, Hong Kong; [10]Program in Cell. and Mol. Med., [11]Boston Children's Hosp. and Harvard Med. Sch., Boston, MA; [12]Massachusetts Gen. Inst. for Neurodegenerative Dis., Massachusetts Gen. Hosp. and Harvard Med. Sch., Charlestown, MA

Abstract: Understanding complex biological systems requires integrating molecular and structural information across multiple dimensions. Three-dimensional (3D) tissue-processing techniques provide new opportunities to interrogate different classes of molecules across entire organs. However, existing methods suffer from information loss owing to molecular degradation and tissue damage. Currently, there is no universal method that simultaneously and globally preserves transcripts, proteins, and their key molecular properties while also allowing multiscale interrogation. Here we introduce SHIELD (Stabilization to Harsh conditions via Intramolecular Epoxide Linkages to prevent Degradation), a versatile tissue-processing method that simultaneously preserves key molecular information in cleared intact tissues by tightly controlling the reaction kinetics of a polyfunctional flexible epoxide crosslinker. This chemical modifier renders individual biomolecules highly resistant to denaturation, protects their physicochemical properties (such as protein fluorescence) while minimally altering interactions with molecular probes (including antibodies) and secures them to their physiological location. By screening a library of polyepoxides, we identified a structurally unique polyfunctional epoxide that shields the activity of fluorescent proteins (FPs) against harsh environmental stressors. Controlling the epoxy reaction using SWITCH enables uniform, organ-wide preservation of FP activity, proteins, transcripts, and their probe-binding properties without loss of tissue architecture. SHIELD complements other methods and enables rapid, multiscale, integrated molecular phenotyping of both animal and clinical tissues. For example, SHIELD combined with stochastic electrotransport (SE) enables ultrafast clearing and immunolabeling of

intact core needle biopsies within only 4 hr. SHIELD combined with magnified analysis of the proteome (MAP) allows integrated phenotyping of parvalbumin (PV)-positive neurons in the globus pallidus externa (GPe) and their downstream targets at single-cell resolution. SHIELD is a highly versatile platform that should find wide application in the study of complex biological systems.

Disclosures: Y. Park: None. **C. Sohn:** None. **R. Chen:** None. **M. McCue:** None. **G.T. Drummond:** None. **T. Ku:** None. **D. Yun:** None. **N.B. Evans:** None. **H.C. Oak:** None. **W. Trieu:** None. **H. Choi:** None. **X. Jin:** None. **V. Lilascharoen:** None. **J. Wang:** None. **M.C. Truttmann:** None. **H.W. Qi:** None. **H.L. Ploegh:** None. **T.R. Golub:** None. **S. Chen:** None. **M.P. Frosch:** None. **H.J. Kulik:** None. **B. Lim:** None. **K. Chung:** None.

Poster

170. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Sample Preparation

Location: SDCC Halls B-H

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NARSAD Young Investigator Award
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JPB Foundation (PIIF)
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Title: A high-throughput platform for large-scale mapping of the human brain at subcellular resolution

Authors: *T. KU[1,2], H. CHOI[1], J. WANG[5], D. H. YUN[1], N. B. EVANS[1], S.-C. CHEN[5], M. P. FROSCH[6], K. CHUNG[1,2,3,4,7]
[1]Inst. for Med. Engin. and Sci., [2]Picower Inst. for Learning and Memory, [3]Dept. of Chem. Engin., [4]Dept. of Brain and Cognitive Sci., MIT, Cambridge, MA; [5]Dept. of Mechanical and Automation Engin., The Chinese Univ. of Hong Kong, N.T., Hong Kong; [6]Dept. of Pathology, Massachusetts Gen. Hosp., Boston, MA; [7]Broad Inst. of Harvard Univ. and MIT, Cambridge, MA

Abstract: A detailed understanding of the anatomical and molecular architecture of brain cells and their brain-wide organization is essential for interrogating human brain function and dysfunction. Extensive efforts have been made toward mapping brain cells through various

lenses, which have established invaluable databases yielding new insights. However, integrative extraction of the multimodal properties of various cell-types brain-wide within the same brain, crucial to elucidating complex intercellular relationships, remains nearly impossible. We have developed a high-throughput, cost-effective technology platform for creating a fully integrated three-dimensional (3D) human brain cell atlas by simultaneously mapping the high-dimensional features (e.g., spatial, molecular, and morphological information) of all cells acquired from the same whole brain. We apply a novel technology to transform a large human brain tissue into robust hydrogel-tissue hybrids that are resistant to mechanical damage resulting from repeated molecular labeling and volume imaging. We then apply scalable labeling and imaging technologies suited for a large human brain tissue to map the 3D distribution of various cell-type and structural markers at subcellular resolution within the same brain. We use a host of rapid and highly automated algorithms to perform unbiased, integrative high-dimensional phenotyping of all cells based on their spatial location, molecular expression, and morphology. Our platform may enable the establishment of the most comprehensive 3D human brain map to date, with unprecedented speed, resolution, and completeness. We envision that such a comprehensive atlas will facilitate the integration of a broad range of studies and allow the research community to interrogate human brain structure and function at multiple levels.

Disclosures: T. Ku: None. **H. Choi:** None. **J. Wang:** None. **D.H. Yun:** None. **N.B. Evans:** None. **S. Chen:** None. **M.P. Frosch:** None. **K. Chung:** None.

Poster

170. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Sample Preparation

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Topic: I.03. Anatomical Methods

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Searle Scholars Program
Packard award in Science and Engineering
NARSAD Young Investigator Award
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JPB Foundation (PIIF and PNDRF)
NCSOFT Cultural Foundation

Title: An open-source image-processing library for quantitative multidimensional analysis of volumetric microscopy images

Authors: *J. SWANEY[1], L. D. KAMENTSKY[1], K. CHUNG[2]
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Abstract: Tissue transformation techniques, such as CLARITY, SWITCH, and SHIELD, have enabled structural and molecular phenotyping of the brain with unprecedented resolution and completeness. These methods, when paired with stochastic electrotransport and high-speed fluorescence microscopy, allow rapid volumetric imaging of large numbers of samples, posing computational challenges in data storage and image processing. Here we present an open-source image-processing library suitable for scalable multidimensional analysis of whole-brain images. The library provides a convenient set of tools for registering whole-brain images, segmenting individual nuclei, and classifying cell types. For registration, we first approximately align images by maximizing the normalized cross-correlation between downsampled nuclei images. Then we perform nonrigid registration at full resolution using nuclei centroids as keypoints, which allows for direct measurement of image misalignment in terms of nuclei displacements. After registering two mouse brain images, the average distance between corresponding nuclei was 4 μm. For nuclei segmentation, we use a curvature-based seeded watershed algorithm that achieves an 80% F_1 score in the dentate gyrus. For cell type classification, a Gaussian mixture model based on the fluorescence intensity of all channels near each nucleus is used. The library provides tools for quantitative analysis of cleared tissues and can be easily incorporated into existing image-processing pipelines.

Disclosures: J. Swaney: None. **L.D. Kamentsky:** None. **K. Chung:** None.

Poster

171. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Circuit Tracing

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Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 171.01/III15

Topic: I.03. Anatomical Methods

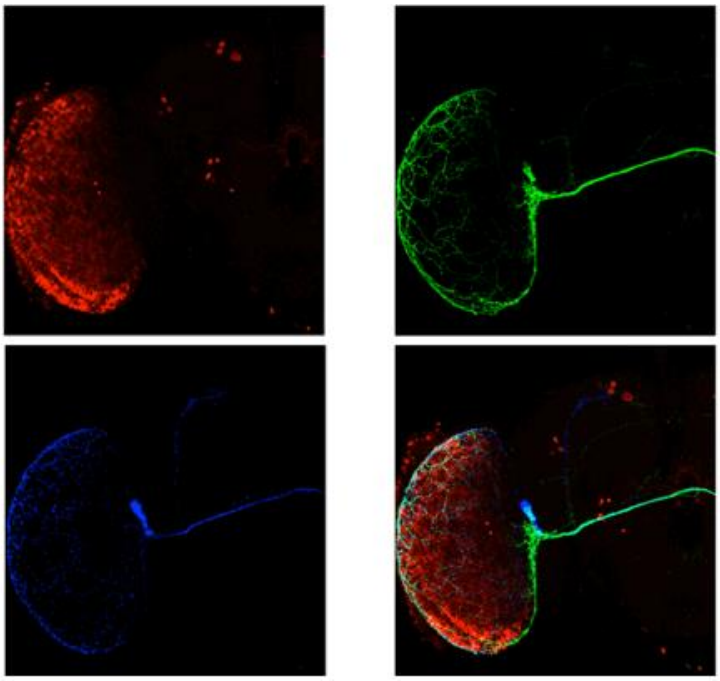
Support: NIH Grant U01MH109147

Title: TRACT: A transsynaptic tool to investigate brain connectivity and to genetically manipulate neurons connected by synapses

Authors: *C. LOIS, T.-H. HUANG, A. DE LA CRUZ, A. CALLEJAS
Div. of Biol. and Biol. Engin., Caltech, Pasadena, CA

Abstract: Understanding the computations that take place in brain circuits requires identifying how neurons in those circuits are connected to each other. In recent years several new approaches have been designed to map the wiring diagrams of brain circuits. We have recently developed a new genetic system called TRACT (*TRA*nsneuronal *C*ontrol of *T*ranscription) with the potential to provide new capabilities towards investigating the relationship between circuit connectivity and function that are not feasible with currently existing methods. TRACT is based on ligand-mediated intramembrane proteolysis, and it is inspired by the molecular mechanisms

of Notch. In this system, neurons expressing an artificial ligand (“donor” neurons) activate a genetically-modified receptor on their synaptic partners (“receiver” neurons). Upon ligand-receptor interaction in synaptic sites, the engineered receptor is cleaved in its transmembrane domain and releases a protein fragment that regulates transcription in the synaptic partners. Our initial experiments have confirmed the feasibility of this strategy to map functional synaptic connectivity in *Drosophila*. Importantly, the method is easily combined with existing technologies that allow for the collection of data about cell identity and physiology in live animals. Thus, the technique allows for simultaneously revealing anatomical connectivity and functional properties of the neurons in the circuit, a critical step in understanding the key computations that are occurring as information is transformed through a circuit. Here we report on advances on the capabilities of the original implementation of TRACT to perform transneuronal tracing and to genetically manipulate neurons connected by synapses, which will enable experiments that are not possible with currently available methods, and will open new avenues towards understanding brain circuit function.



Disclosures: C. Lois: None. **T. huang:** None. **A. de la cruz:** None. **A. callejas:** None.

Poster

171. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Circuit Tracing

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Topic: I.03. Anatomical Methods

Support: SFB889

Title: Mapping brain-wide inputs to parvalbumin-expressing interneurons with intersectional rabies virus tracing

Authors: *G. HAFNER[1], M. WITTE[1], J. GUY[1], E. M. CALLAWAY[2], K. DEISSEROTH[3], K.-K. CONZELMANN[4], J. F. STAIGER[1]
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Abstract: Parvalbumin (PV)-expressing interneurons are the largest subpopulation of inhibitory neurons in the mouse neocortex and are indispensable for controlling the activity level of principal cells. To investigate what areas and cell types can activate PV neurons, we visualized brain-wide inputs to PV neurons in the barrel cortex using retrograde rabies virus tracing. This technique relies on adeno-associated viruses (AAVs) to express proteins for rabies virus entry and spread, together with a modified rabies virus to label monosynaptic inputs. To achieve optimal cell type specificity, we employed an intersectional strategy. We generated Vgat-Cre/PV-Flp transgenic mice, which co-express Cre and Flp in PV interneurons. We combined this line with Cre- and Flp-dependent AAVs, which express their proteins only in the presence of both recombinases. This strategy allowed us to exclude the fraction of Cre-expressing excitatory cells in the PV-Cre line from our starter cell population. We thoroughly tested this new viral construct for Cre/Flp-independent leak expression and found it to be very specific for mapping long-range inputs, while local inputs were slightly confounded by leak expression of the construct for rabies virus entry. We mapped the brain-wide long-range inputs to PV interneurons in the barrel cortex and found input cells in several distant cortical areas and thalamic nuclei. Surprisingly, layer IV sent an equal number of projections from other cortical areas to PV neurons as supra- or infragranular layers; in visual cortex layer IV even sent the most projections, questioning its mere role as an input layer. We mapped local inputs within the barrel cortex, too, and analyzed them with respect to layer distribution and molecular markers. Local inputs were mainly from layer IV and excitatory. A small number of inputs originated from layer I, a previously unknown source of input. Using glutamate uncaging we confirmed this connection and attributed it to pairs of LI interneurons and LII PV neurons. In conclusion, this study provides a fine-grained analysis of the inputs PV neurons integrate and hints that cross-modal integration of sensory information takes place at the level of primary sensory areas involving the action of inhibitory neurons.

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Poster

171. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Circuit Tracing

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 171.03/III17

Topic: I.03. Anatomical Methods

Support: NIH Grant 5R01NS089770

Title: A novel anterograde trans-synaptic tracer

Authors: *R. KERY[1,2], L. CHEN[1], Q. XIONG[1], S. GE[1]
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Abstract: There is a high demand for trans-synaptic labeling and transgene delivery for both anatomical and functional mapping of brain circuits. To achieve anterograde trans-synaptic transgene delivery, I successfully engineered an expression vector encoding a novel fusion protein to be secreted by neurons and up-taken by cells surrounding. To guide and facilitate the fusion at axonal terminals, I included (as part of the fusion cassette) *wheat germ agglutinin* (WGA), which promotes protein trafficking to axonal terminals. To achieve the highest expression efficiency in anterograde targets, I have fused *Cre recombinase* into this cassette so that the recombinase could amplify the expression via recombination in the target cells. After verifying the secretion and uptake capability in cultured neurons, I inserted this fusion cassette into an adeno-associated viral (AAV) vector, and packaged it into AAV serotype 9. After injecting the virus into a variety of locations in the mouse brain *in vivo*, I detected the anterograde synapse targets of the infected neurons. This tool box provides an important option for anterograde trans-synaptic transgene delivery in adult brain circuit.

Disclosures: R. Kery: None. **L. Chen:** None. **Q. Xiong:** None. **S. Ge:** None.

Poster

171. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Circuit Tracing

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 171.04/III18

Topic: I.03. Anatomical Methods

Support: NSFC grant 91632303

Title: Rabies virus pseudotyped with CVS-N2C glycoprotein as a powerful tool for retrograde tracing input neural networks

Authors: *X. ZHU[1], Z. ZHANG[2], H. MI[2], X. HUANG[2], X. YUE[2], K. LIN[2], X. HE[2], F. XU[2]
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Abstract: Development of efficient viral vectors for mapping and manipulating neural circuit is crucial to brain function exploring. Here, we report a modified RABV (SAD-RV-N2C (G)) enveloped with N2C (G), which showed an enhanced retrograde infection efficiency comparing with the widely used viral tracers SAD-RV-B19 (G) and a much more generalized tropism for upstream efferent neurons comparing with the RetroAAV2 in vivo. The SAD-RV-N2C (G) may be served as an effective medium for revealing the structure and function of brain circuit.

Disclosures: X. Zhu: None. **Z. Zhang:** None. **H. Mi:** None. **X. Huang:** None. **X. Yue:** None. **K. Lin:** None. **X. He:** None. **F. Xu:** None.

Poster

171. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Circuit Tracing

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 171.05/III19

Topic: I.03. Anatomical Methods

Support: MOST104-2311-B-010-010-MY3
MOST106-2321-B-010-012
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Title: Stereotaxic surgery for genetic manipulation in neuronal cells in specific regions of neonatal mouse brains

Authors: S.-Y. CHEN, H.-Y. KUO, *F.-C. LIU
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Abstract: Many genes are expressed in embryonic brains, and some of them are continuously expressed in the brain after birth. For such persistently expressed genes, they may function to regulate the developmental process and/or physiological function in neonatal brains. To investigate neurobiological functions of specific genes in the brain, it is essential to inactivate genes in the brain. Here, we describe a simple stereotaxic method to inactivate gene expression

in the striatum of transgenic mice at neonatal time windows. AAV-eGFP-Cre viruses were microinjected into the striatum of Ai14 reporter gene mice at postnatal day (P) 2 by stereotaxic brain surgery. The tdTomato reporter gene expression was detected in P14 striatum, suggesting a successful Cre-loxP mediated DNA recombination in AAV-transduced striatal cells. We further validated this technique by microinjecting AAV-eGFP-Cre viruses into P2 *Foxp2*$^{fl/fl}$ mice. Double labeling of GFP and Foxp2 showed that GFP-positive cells lacked Foxp2 immunoreactivity in P9 striatum, suggesting the loss of Foxp2 protein in AAV-eGFP-Cre transduced striatal cells. Taken together, these results demonstrate an effective genetic deletion by stereotaxically microinjected AAV-eGFP-Cre viruses in specific neuronal populations in the neonatal brains of floxed transgenic mice. In conclusion, our stereotaxic technique provides an easy and simple platform for genetic manipulation in neonatal mouse brains. The technique can not only be used to delete genes in specific regions of neonatal brains, but it also can be used to inject pharmacological drugs, neuronal tracers, genetically modified optogenetics and chemogenetics proteins, neuronal activity indicators and other reagents into the striatum of neonatal mouse brains.

Disclosures: S. Chen: None. **H. Kuo:** None. **F. Liu:** None.

Poster

171. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Circuit Tracing

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 171.06/III20

Topic: I.03. Anatomical Methods

Support: NIH R01 NS034700
NIH R37 NS077908
NIH R01 NS086364

Title: All-optical, large scale mapping of synaptic connectivity in living tissue using patterned illumination

Authors: *K. P. LILLIS, K. J. STALEY
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Abstract: The rapidly developing field of connectomics in neuronal microcircuits can be broadly divided into two categories: anatomical connectomics and functional connectomics. Anatomical synapses can be mapped at a high spatial resolution, but throughput is low and tissue must be fixed. Furthermore, although maps of anatomical synapses can be created with a high degree of certainty, they do not necessarily predict network function: Other factors (e.g. ion channel densities and posttranslational modifications, neurotransmitter release and binding) also influence neuronal and network activity.

Functional connectivity has been measured in living tissue by analyzing correlated activity in populations of neurons. In previous work, we have demonstrated that correlation-based functional connectivity changes dynamically with network state. For example, pre-ictal disinhibition unveils scale-free functional network connectivity in seizing brain slices. Thus, functional connectivity does not necessarily directly reflect anatomical connections – rather, it is a level of abstraction for quantifying network output.
Here, we describe a method by which maps of synaptic connectivity are measured by repeatedly optically stimulating individual neurons while simultaneously imaging the evoked network response. We designed a microscope with a field-of-view (FOV) large enough to capture an entire organotypic hippocampal slice culture. Individual neurons are stimulated by using a digital micromirror device, in the image plane of the excitation light pathway, to pattern blue light onto selected channelrhodopsin-expressing neurons. To accomplish this over such a large area requires high N.A., low-magnification optics, a very bright LED, and high-sensitivity variants of channelrhodopsin (CoChr or CheRiff). In preliminary experiments, we have used red-shifted genetically encoded calcium indicators to record activity and identify the strongest synaptic connections. However, advances in voltage-sensitive fluorophores are rapidly increasing the feasibility of imaging sub-threshold and inhibitory post-synaptic responses.
In this presentation, we will describe in detail the optical system used and how we are addressing technical challenges encountered (particularly off-target stimulation and spontaneous background activity). We demonstrate this technique in a brain slice model of post-traumatic epileptogenesis, but anticipate that it will be broadly useful for understanding how synaptic connectivity architecture relates to the network output of neural circuits in vitro and in vivo.

Disclosures: K.P. Lillis: None. **K.J. Staley:** None.

Poster

171. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Circuit Tracing

Location: SDCC Halls B-H

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Title: High-throughput mapping of corticocortical connectivity of single mouse brains at single neuron resolution

Authors: *L. HUANG[1], J. M. KEBSCHULL[1], S. MUSALL[1], M. T. KAUFMAN[2], A. K. CHURCHLAND[1], A. M. ZADOR[1]
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Abstract: Long-range neuronal projections determine how information flows among various brain areas to generate behavior, but the logic of information flow in the brain remains poorly understood. Disruption of long-range projection is implicated in neuropsychiatric disorders including autism and schizophrenia. A central barrier to deciphering this logic is methodological. Conventional anatomical methods have low throughput and usually lack single cell resolution. Mapping the connectivity of an experimental model system is thus very expensive and labor-intensive.
The Zador laboratory has recently developed a high-throughput method, MAPseq (Multiplexed Analysis of Projections by sequencing), for mapping long-range projections at single cell resolution. The key to MAPseq is that, instead of treating neuroanatomy as a problem of microscopy, it is recast into a form that can exploit the tremendous efficiencies of high-throughput DNA sequencing. In first generation MAPseq, a viral library encoding random RNA barcodes is injected into a single brain area, enabling the multiplexed mapping of ~1000 neurons in a single experiment.
Here we describe a method, Multisource Multiplexed Analysis of Projections by sequencing (MMAPseq), which can be used to map the whole cortico-cortical connectivity rapidly, in a single brain. By optimizing viral vectors, injections, dissections, molecular experiments and the bioinformatics pipeline, with MMAPseq we have been able to map the projections—at single neuron resolution—of more than 50,000 neurons spanning the whole cortex in one individual animal within two weeks, at a cost of about $10K. By contrast, conventional methods require years, thousands of animals, and millions of dollars, and do not achieve single neuron resolution. The results of multi-source MAPseq not only agreed closely with the Allen Connectome, within the noise floor determined by animal-to-animal variation in the Allen dataset, but also were consistent with whole-brain functional imaging data. Analysis of the full corticocortical projection network revealed a modular organization, and non-random connection motifs. Analysis of connections with a mouse model of autism (BTBR) revealed strongly disrupted connections. In summary, multiplexed MAPseq for the first time enables the systematic study of the whole corticocortical projectome in individual animals, at single neuron resolution. These methods open up the possibility of a new era of quantitative comparative connectomics, probing the effects on neuronal wiring of age, sex, environment, genetics, and species.

Disclosures: L. Huang: None. **J.M. Kebschull:** None. **S. Musall:** None. **M.T. Kaufman:** None. **A.K. Churchland:** None. **A.M. Zador:** None.

Poster

171. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Circuit Tracing

Location: SDCC Halls B-H

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Program #/Poster #: 171.08/III22

Topic: I.03. Anatomical Methods

Support: 4R00H099153
1R01NS104828

Title: Directed stepwise tracing and controlling of long-range polysynaptic memory circuits

Authors: E. LI[1], W. DU[1], J. GUO[1], Y.-T. CHEN[1], S. OH[1], A. SAMUEL[1], Y. LI[1], H. K. OYIBO[2], *W. XU[1]
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Abstract: Brain functions rely on the whole brain network formed by multiple orders of synaptic connections. Sensory information, after a series of integration, is bound together in the hippocampus to construct a representation of the world and encode it into memories, which impact various aspects of our behavior later through poly-synaptic circuits. The seemingly homogeneous principal neurons in the hippocampus are highly diverse in gene expression profiles and physiological properties. However, it is not clear to which degree these neurons are differentially wired into the brain circuits. We hypothesize that the principle neurons at CA1 and CA3 subregions of the hippocampus are heterogeneous in long-range poly-synaptic connectivity and this heterogeneity divides the neurons into specific groups which funnel different aspects of the sensory information to the regulation of different aspects of behavior. The poly-synaptic connectivity has been difficult to examine due to a lack of methods which should allow us to continuously follow the pathways in a controlled and directed manner. Here we demonstrate that directed stepwise poly-synaptic tracing can be realized through in vivo reconstitution of the replication of a replication-deficient viral tracer, pseudorabies virus lacking the immediate early gene IE180. By restricting viral replication to a short time window, we minimized the neuronal toxicity which would be severe with uncontrolled viral replication. This makes it possible to both trace and functionally control the circuits of interest. We first validated this technique by tracing the well-established di- or tri- synaptic hippocampal projections. We then examined the indirect synaptic projections from the hippocampus to the striatum and revealed a wiring diagram that different components of the hippocampus are linked to the distinct compartments of the dorsal or ventral striatum through distinct intermediate brain regions. We are currently utilizing this method to selectively control the specific hippocampus-striatum pathways to examine their specific contributions to contextual memory-guided behaviors. *(E. Li, W. Du and J. Guo contributed equally to this work)*

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Poster

171. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Circuit Tracing

Location: SDCC Halls B-H

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Program #/Poster #: 171.09/III23

Topic: I.03. Anatomical Methods

Support: NIAAA U01AA025932
NIAAA RO1AA021505

Title: Retrograde mapping of afferent inputs to direct and indirect pathway neurons in the dorsomedial striatum

Authors: *Y. CHENG[1], J. LU[2], B. BARBEE[1], X. WANG[1], J. WANG[3]
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Abstract: The dorsomedial striatum (DMS) is crucial for goal-directed behavior and heavily implicated in drug and alcohol addiction. Our recent study indicated an opposite role of DMS dopamine D1 receptor (D1R)- and D2R-expressing medium spiny neurons (MSNs) on alcohol consumption (Cheng et al. 2016). However, the cell type of presynaptic neurons that project to DMS D1- versus D2-MSNs remains unknown. To assess whether these presynaptic neurons also contain D1Rs or D2Rs, we used a state-of-the-art rabies viral-based monosynaptic retrograde tracing technology together with D1-/D2-Cre;Ai14 transgenic mice to map these neurons brain-wide. We found abundant DMS-projecting neurons in the different cortical regions, amygdala, thalamus, and midbrain. Interestingly, we found that most D1-MSN-projecting neurons did not express D1Rs, and similarly, most D2-MSN-projecting neurons did not express D2Rs. We only observed a few D1-MSN-projecting neurons in the cortex and thalamus that contained D1Rs; a few D2-MSN-projecting neurons in the same brain regions that expressed D2Rs. In addition, using optogenetic slice electrophysiology and D1-/D2-Cre;Ai32 mice, we found that DMS D1-MSNs received glutamatergic inputs mainly from D2-neurons outside of the striatum, whereas glutamatergic inputs onto DMS D2-MSNs were predominately from D1-neurons. These results suggest that the D1-D2 and D2-D1 projections are the dominant connections in brain-wide, which is an important question in the addiction field that has not been addressed before. Since drugs of abuse and alcohol affect dopaminergic system brain-wide, understanding these connections will improve our knowledge of the DMS circuits in drug addiction.

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Poster

171. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Circuit Tracing

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 171.10/III24

Topic: I.03. Anatomical Methods

Support: NIH/NIMH 2R01MH094360-06

Title: The mouse connectome project at USC: Progress toward assembling neural networks of the mammalian brain

Authors: *H. HINTIRYAN, M. S. BIENKOWSKI, I. BOWMAN, N. N. FOSTER, L. GOU, M. BECERRA, M. ZHU, M. Y. SONG, N. L. BENAVIDEZ, K. COTTER, S. YAMASHITA, D. LO, D. L. JOHNSON, N. S. KHANJANI, S. S. AQUINO, H.-W. DONG
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Abstract: The long term objective of the Mouse Connectome Project at USC (MCP; www.MouseConnectome.org) is to map the interconnections among all regions of the C57Bl/6 mouse brain, to generate a corresponding comprehensive connectome map that represents the interconnections in a common neuroanatomic frame, and to understand how the different brain regions assemble into functional networks based on these connections. The biological significance of assembling a brain-wide wiring diagram is tantamount to that of the Human Genome Project. However, just as knowing the sequence of three billion base pairs in the human genome reveals little about how our bodies are regulated by genes, constructing the connectome will not directly reveal its functional purpose. The ensuing challenge is to analyze the vast connectivity information in a way that is most conducive to generating novel behavioral hypotheses for experimental testing. Here, we report our recent progress in constructing the first and the most detailed wiring diagram of the mouse hippocampus. Our future direction includes constructing these global networks at the cellular level and the further customization of our existing informatics tools to facilitate data visualization and analysis.

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Poster

171. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Circuit Tracing

Location: SDCC Halls B-H

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Topic: I.03. Anatomical Methods

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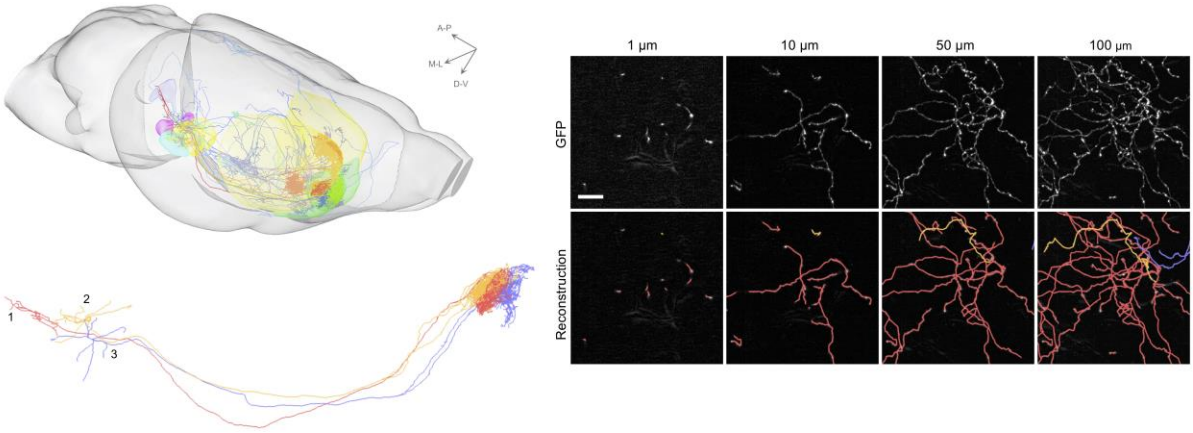
NNSFC 91632302
China MOST 2012YQ03026005
China MOST 2013ZX0950910
China MOST 2015BAI08B02

Title: Reconstructing single neurons from defined cell types at the whole-brain level

Authors: *R. LIN[1], R. WANG[1], J. YUAN[2], Q. FENG[1], H. GONG[2], M. LUO[1]
[1]Natl. Inst. of Biol. Sci., Beijing, China; [2]Wuhan Natl. Lab. for Optoelectronics, Huazhong Univ. of Sci. and Technol., Wuhan, China

Abstract: The morphology, interconnections, and chemical profile together define the function(s) of a given neuron. In the time since the founding of modern neuroscience, huge efforts have been invested in developing tools for probing the structures and functions of the nervous system at the single-neuron level. However, it is still very challenging to visualize the complete morphology of individual neurons. Here we report the development of a pipeline for single-neuron reconstruction at the whole-brain level. We first developed an AAV virus system for sparsely but brightly labeling genetically- or projection-defined neurons with tunable labeling density. Combining our sparse labeling system with the fluorescence micro-optical sectioning tomography (fMOST) imaging platform, we established and optimized a pipeline for whole-brain imaging and reconstruction of single neurons. As a proof-of-principle study, we successfully reconstructed the full morphology of individual dopaminergic neurons and cortical projection neurons. We also combined our labeling system with tissue clearing techniques to achieve rapid reconstruction of interneurons in thick brain sections. Using this approach, we reconstructed the dendritic structures of striatal interneurons. These novel methods will facilitate the studies of the nervous system at the single-neuron level.



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Poster

171. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Circuit Tracing

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 171.12/III26

Topic: I.03. Anatomical Methods

Support: NIH Grant U24MH114827

Title: Investigator

Authors: L. NG[1], D. FENG[1], P. LEAHY[1], T. TICKLE[2], L. BECKER[1], R. YOUNG[1], T. GILLESPIE[3], J. S. GRETHE[4], A. E. BANDROWSKI[4], S. MUFTI[1], A. BERNARD[5], J. C. GEE[7], A. PHILIPPAKIS[2], M. E. MARTONE[3], *M. J. HAWRYLYCZ[6]
[1]Allen Inst. for Brain Sci., Seattle, WA; [2]Broad Inst., Cambridge, MA; [3]Neurosci., [4]UCSD, La Jolla, CA; [6]Modeling, Analysis, and Theory, [5]Allen Inst. Brain Sci., Seattle, WA; [7]Univ. of Pennsylvania, Philadelphia, PA

Abstract: Brain Cell Data Center: Towards a comprehensive brain cell atlas integrating molecular, anatomical, and physiological properties of cell types
A detailed census of the structure and role of cell type specific data in the brain is recognized to be one of the most promising avenues for advancing our understanding of the human brain in health and disease. The Allen Institute for Brain Science has been selected to develop and house the BRAIN Cell Data Center (BCDC) to support the goals of the BRAIN Initiative Cell Census Network (BICCN) by providing a foundational community resource for housing single-cell centered data content in the brain. The BICCN as a 5-year initiative will create a comprehensive 3D common reference brain cell atlas that integrates molecular, anatomical and physiological

properties of brain cell types.
A primary goal of the BCDC is to lead, together with BICCN data generation partners, in the development and deployment of fundamental data models, common community standards, data, and scientific results to improve our understanding of the diverse cell types in the mammalian brain and its organizational logic. The BCDC will develop a data collection, quantification and mapping framework for managing data and information across diverse repositories, establish semantic and spatial community standards for describing and managing singe cell data modalities and create a unified and integrated web-accessible Cell Registry and Portal to support data retrieval, search, visualization, and analysis of cell specific data and knowledge synthesis. An initial portal will be released in 2018 with increasingly integrated data resources appearing in subsequent years.

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Poster

171. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Circuit Tracing

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 171.13/III27

Topic: I.03. Anatomical Methods

Support: NIH Grant F31DC01518503

Title: Input-output organization of the mouse claustrum

Authors: *B. ZINGG[1], H. DONG[2], H. TAO[2], L. ZHANG, 90033[2]
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Abstract: Progress in determining the precise organization and function of the claustrum has been hindered by the difficulty in reliably targeting these neurons. To overcome this, we used a projection-based targeting strategy to selectively label claustrum principal neurons. Combined with adeno-associated virus (AAV) and monosynaptic rabies tracing techniques, we systematically examined the pre-synaptic input and axonal output of this structure. We found that claustrum neurons projecting to retrosplenial cortex (RSP) collateralize extensively to innervate a variety of higher-order cortical regions. No subcortical labeling was found, with the exception of sparse terminals in the basolateral amygdala (BLA). This pattern of output was similar to cingulate- and visual cortex-projecting claustrum neurons, suggesting a common targeting scheme among these projection-defined populations. Rabies virus tracing directly demonstrated widespread synaptic inputs to RSP-projecting claustrum neurons from both cortical and

subcortical areas. The strongest inputs arose from classically defined limbic regions, including medial prefrontal cortex, anterior cingulate, basolateral amygdala, ventral hippocampus, and neuromodulatory systems such as the dorsal raphe and cholinergic basal forebrain. These results suggest that the claustrum may integrate information related to the emotional salience of stimuli and may globally modulate cortical state by broadcasting its output uniformly across a variety of higher cognitive centers.

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Poster

171. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Circuit Tracing

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Support: Brain Mapping of Integrated Neurotechnologies for Disease Studies (Brain/MINDS) from the Japan Agency for Medical Research and Development, AMED
Crick-Clay Professorship in CSHL
H.N. Mahabala Chair, IIT Madras

Title: A modified rapid silver stain technique in a high-throughput neurohistological pipeline

Authors: *J. NAGASHIMA[1], M. HANADA[1], M. LIN[1], Y. S. TAKAHASHI[1], B.-X. HUO[1], A. S. TOLPYGO[2], J. JAYAKUMAR[3], H. OKANO[4], P. P. MITRA[2]
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Abstract: The Gallyas silver stain for myelin is still a widely used technique in neuroanatomical studies and the staining protocol varies depending on the purpose of study. Our high-throughput pipeline requires rapid sectioning and staining of neural tissue, so we have introduced a modified silver staining technique that not only reduces time but also reduces the instances of discoloration. Furthermore, the examination of these high-resolution fiber details is especially important in myeloarchitecture identification and fundamentally serves to validate segmentation results from the computational component of our pipeline. Here we describe the modification of the Gallyas stain in the common marmoset (Callithrix jacchus) alongside the high-throughput neurohistological pipeline With free floating sections, there is a greater possibility for blotchiness and uneven staining due to handler proficiency, folding, or section degradation. Our proposed modification is more productive due to the use of $20\mu m$ frozen brain sections processed with a tape-transfer assisted cryo-sectioning technique which facilitates better preservation of brain morphology and is a more straightforward implementation. As a result, the tape-transfer eliminates the need for Gallyas' pre-stain fixation step with formol, as well as a formol fixation

step that may be used after silver impregnation. Additionally, the stability of the sections on the slides leads to a less sensitive reaction to contaminants, handling issues, and temperature. Sequential phases include pretreatment, rehydration, wash, silver staining impregnation, clearing unbound silver, development, and termination of development. A goal of our high-neurohistological pipeline is to register a whole marmoset brain by utilizing several histological processes. Combining a rapid and efficient myelin stain with our Nissl, fluorescence, and CTB processes further intensifies the accuracy of area differentiation during analysis and supports a cross-modality registration. Thus, the importance of quality sectioning through tape-transfer and the subsequent efficient staining supports the display of specific high-resolution markers in myelin sheaths which allows for a clear quantitative analysis of the myelinated fibers. The problem of section folding during staining has been corrected, but we still face minor instances of uneven staining and blotchiness. In the future, we plan to modify the staining process further to reduce these errors which will then further support area differentiation during analysis.

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Poster

171. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Circuit Tracing

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 171.15/III29

Topic: I.03. Anatomical Methods

Support: MOST 105-3011-F-213-001

Title: Large-scale quantitative analysis of neurons in *Drosophila* connectome via morphological structures by NeuroRetriever

Authors: *T.-Y. WANG[1], N.-Y. CHEN[2], K.-P. CHEN[2], C.-T. SHIH[3], T.-K. LEE[1]
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Abstract: *Drosophila* connectome is composed of 130,000 neurons. Neuroscientists could randomly sample neurons in *Drosophila* brain and image them with confocal microscope to get 3D volume images at single cell resolution[1]. To reconstruct *Drosophila* connectome at single cell resolution, it is necessary to classify neurons with morphological features because multiple copies of specific neuron in connectome might occur during random sampling. Here we provide a standard workflow to systematically cluster neurons in 3D volume image with characteristic quantities from their morphology, called structure indexes (SIs), including branch length, branch

angle and branch level of neuron, etc. First, we apply *NeuroRetriever*[2] on *FlyCircuit v1.2* (28573 single neuron images) to segment neurons from volume data in an unbiased and automated way. Then, we trace segmented neurons with *Fast Automatically Structural Tracing (FAST)* algorithm to get SIs for each neuron. Next, isomap[3] and modularity[4] methods are applied to classify neurons with adequate SIs. The isomap method can define the similarity between neurons by geodesic paths on a high-dimensional manifold. In addition, the best community structure can be defined by optimizing the modularity, which is intuitively maximizing the intra module connections and minimizing the inter module connections. With this workflow, large-scale structural features of neurons could be generated and the neurons could be classified morphologically. More accurate connectome might be generated with these classified neuron types and the vector composed of SIs could be used in the machine learning approach for identifying the morphological type of neurons.
References:[1] Ann-Shyn Chiang, *et al.*: **Three-Dimensional Reconstruction of Brain-wide Wiring Networks in *Drosophila* at Single-Cell Resolution.** Current Biology 2010, **21**: 1-11.[2] Chi-Tin Shih, *et al.*: ***NeuroRetriever*: Automatic Neuron Segmentation for Connectome Assembly.**(in preparation)[3] Joshua B. Tenenbaum, *et al.*: **A Global Geometric Framework for Nonlinear Dimensionality Reduction.** Science 2000, **290**: 2319-2323.[4] Roger Guimerà, *et al.*: **Functional Cartography of Complex Metabolic Networks.** Nature 2005, **435**: 895-900.

Disclosures: T. Wang: None. **N. Chen:** None. **K. Chen:** None. **C. Shih:** None. **T. Lee:** None.

Poster

171. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Circuit Tracing

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 171.16/III30

Topic: I.03. Anatomical Methods

Support: NIH MH105949
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NIH MH114821
The Mathers Foundation; CSHL, Crick-Clay Professorship;
H. N. Mahabala Chair at IIT Madras

Title: An integrated web based computational framework for terabyte size mesoscopic whole mouse brain data

Authors: *X. LI[1], K. RAM[2], F. XU[1], P. MITRA[1]
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Abstract: Most image analysis algorithm development and execution are done in desktop or cluster mode, while with the increasing number of distributed collaboration on one project, it is

becoming more and more time consuming distributing data to different teams and interchanging intermediate results. Here, a web based computational framework is presented, featuring most functions start from data acquirement to annotation, data process, algorithm development and data visualization. Data and QC: As soon as the image slices are scanned in the lab, it will be ready to view online. The QC process including entering metadata, reject or rescan slices can also be done through the web portal. Registration: The backend of registration process is deployed on CSHL black-n-blue grid. The front end is on the portal, allowing user to remotely control the whole registration process including atlas mapping. Annotation tool: The web portal has a built in online annotation tool, where the user can zoom an image into pixel level, and paint individual pixel. The annotation tool also features other drawing methods, like line or polygon. The annotation result is also accessible on the portal, so it can be used for training other algorithms. Image processing: Common algorithms such as process detection, cell detection and counting, injection detection are already integrated. In addition, the portal provide an interactive Python and Matlab develop environment built on top of Jupyter Notebook, which will allow user to try new algorithm such as machine learning and deep learning. Skeletonization: The DiMorSC skeletonization method can also be run via the web portal and produce a tree summarization from previous image processing result. The resulting 3D skeleton tree will be overlayed with either mouse brain atlas or nissl data. Viewer and Navigation: The raw image and all results, including registration, atlas mapping, image processing, 3D skeleton etc. can be viewed online. The web portal provides four navigation tools: list view, tree view, flat map and 3D cursor to help user navigate through the whole dataset. The list view will have all of the brain listed with user specified filter. The tree view tool provides a visualization for the hierarchy of brain regions using a Javascript based hierarchical diagram. The flat map use SVG image to render the mouse brain and create a interactive user experience. 3D cursor navigation tool reconstruct the whole brain from the 3D atlas and provide section views from coronal, sagittal and transverse plane of the mouse brain. User may find their point of interest by clicking on the three plane and all the injection points we have near that specific point of interest will be listed.

Disclosures: X. Li: None. **K. Ram:** None. **F. Xu:** None. **P. Mitra:** None.

Poster

171. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Circuit Tracing

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 171.17/DP13/III31

Topic: I.03. Anatomical Methods

Support: Texas Institute for Brain Injury and Repair (TIBIR)

Title: An automated image analysis pipeline for registration and quantification of volumetric serial two-photon images in animal models of brain injury and disease

Authors: *D. M. RAMIREZ[1,3], A. D. AJAY[1,3], V. O. TORRES[1,3], A. M. STOWE[1,3], M. P. GOLDBERG[1,3], J. P. MEEKS[2,1,3]
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Abstract: Improvements in volumetric imaging instrumentation as well as automated, cluster-based methods for large scale computational processing and analysis of the resultant images have increased tremendously in recent years. In combination, these methods can now enable neuroscientists to investigate brain circuitry in both healthy and diseased states across large numbers of experimental animals. The UT Southwestern Whole Brain Microscopy Facility (WBMF) uses serial two-photon tomography (STPT), a technique that produces high resolution, three-dimensional images of intact, uncleared brain tissue, to explore brain-wide circuit mechanisms associated with neurological and psychiatric disorders as well as brain injury. Here, we report the development of an image analysis pipeline comprised of open source tools that allows us to perform fully automated analyses of whole rodent brain images generated by our STPT instruments (TissueCyte 1000s). The pipeline incorporates supervised machine learning (random forest model)-based feature extraction (e.g. autofluorescent blood vessels, damaged/scarred tissue, and fluorescent cell bodies and dendrites) with whole brain registration to the Allen Institute's Common Coordinate Framework, and is fully integrated with the UT Southwestern BioHPC high performance computing cluster to allow user-friendly batch processing of STPT datasets via a web interface. We have used these methods to localize and quantify exogenously introduced, fluorescently labeled immune cells throughout the mouse brain in order to assess their potential relevance to stroke recovery. Preliminary results across several additional experimental paradigms, including Fos-TRAP labeling of behaviorally relevant neuronal populations and viral-mediated neuronal tracing demonstrate the utility of this approach for identifying circuit-level dysfunction in a wide variety of complex brain disorders. We ultimately hope to apply our facility's large-scale, high-throughput model for brain-wide connectivity analysis to identify therapies that improve neural circuit connectivity in models of neuropsychiatric and neurodegenerative disorders.

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Poster

171. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Circuit Tracing

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 171.18/III32

Topic: I.03. Anatomical Methods

Support: NIH R01-EY014882 to H-KL

Title: Visualizing cross-modal connectivity via iDISCO+

Authors: *R. SAHA[1], H.-K. LEE[2]
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Abstract: Cross-modal plasticity is a unique adaptive feature of the brain, which involves cortical reorganization triggered by loss of one sensory modality leading to enhance performance in the remaining senses. We have observed after visual deprivation enhanced functionality of primary auditory cortex (A1) neurons, which manifest as increased sensitivity to sound stimuli and better auditory information encoding (Petrus et al., 2014). This study also demonstrated that vision loss produces a wide spread synaptic and circuit level adaptation even in the adult A1. The functional changes in A1 occurred quite rapidly (i.e. with a week of visual deprivation) suggesting that the cross-modal interaction between A1 and V1 is likely occurring through pre-existing anatomical connections. The anatomical circuitry which can mediate interaction between V1 and A1 is unclear, but it is likely through long-range connections between the two brain areas. To understand the anatomical basis for cross-modal interaction, we set out to investigate common targets of V1 and A1 projections. To achieve this, we are utilizing a new brain clearing method iDISCO+ for optical imaging. iDISCO+ is a brain clearing method based on removing lipid bilayers while preserving cellular proteins, hence rendering the tissue optically transparent. To determine the common projection targets of V1 and A1, we will inject AAV-GFP and AAV-mCherry to each area. We have adopted the iDISCO+ method for clearing the intact mouse brain, successfully performed immmunolabeling of fluorescent protein markers, and visualized cellular morphology using light sheet microscopy. We are currently optimizing double labeling to identify the common projection areas of V1 and A1. Results from this study will provide much needed information on potential long-range connectivity that may mediate cross modal plasticity.

Disclosures: R. Saha: None. **H. Lee:** None.

Poster

171. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Circuit Tracing

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 171.19/III33

Topic: I.03. Anatomical Methods

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: Transcriptomic correlates of long-range cortical projections revealed by barcode sequencing

Authors: *X. CHEN, Y.-C. SUN, A. M. ZADOR
Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: The three major classes of cortical projection neurons in the adult brain differ in their projection patterns, laminar locations, and transcriptomic profiles. Although neuronal projection patterns correlate with gene expression at these class-level divisions, it is unknown whether further correlations exist within a major class. To address this question, we developed a high-throughput approach that combines projection patterns and transcriptomic profiles in individual neurons. Our approach is based on two related techniques, MAPseq and BARseq, which allow high-throughput mapping of neuronal projections using barcode sequencing. In MAPseq, each neuron expresses a unique RNA sequence (barcode) that fills both the soma and its axons. Sequencing and matching axonal barcodes in distal brain areas with somatic barcodes thus reveals the projection patterns of individual neurons. BARseq additionally preserves the locations of the neurons by sequencing barcodes *in situ*. Here we present two complementary strategies that allow projectome and transcriptome mapping in the same neurons. In the first approach, we use single-cell RNAseq to read out both barcodes and endogenous transcript in the same neurons, thus combining projection mapping with an unbiased view of the transcriptome in the same neurons. In the second approach, we use targeted *in situ* sequencing to read out both barcodes and a panel of endogenous transcripts to correlate projections with specific gene expression, while preserving the locations of the neurons. Using these two approaches, we investigate the transcriptomic correlates of cortical projections in the mouse visual cortex.

Disclosures: X. Chen: None. **Y. Sun:** None. **A.M. Zador:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); equity owner/founder of MapNeuro.

Poster

171. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Circuit Tracing

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 171.20/III34

Topic: I.03. Anatomical Methods

Support: The Lundbeck Foundation

Title: Heterogeneity of dopaminergic neurons defined by their projections

Authors: *S. H. JØRGENSEN, S. NØRR, M. A. CHRISTIANSEN, U. GETHER, K. RICKHAG, A. TOFT SØRENSEN
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Abstract: Brain circuits consist of complex heterogeneous populations of neurons. In order to classify and determine their individual contribution to behavior, various viral techniques can disentangle subtypes of neurons and their projections, and ultimately link them to distinct behaviors. We and others have previously identified different subgroups of neurons within the dopaminergic midbrain nuclei based on their expression levels of different dopaminergic marker proteins, namely tyrosine hydroxylase (TH), dopamine transporter (DAT) and vesicular monoamine transporter 2 (VMAT2), suggesting a rather heterogeneous nature within the dopaminergic pool of neurons (Lammel et al., Neuron, 2015; Apuschkin et al., Eur J. Neurosci, 2016). To further determine to what extent this heterogeneity is defined by their projections, we utilize the ability of the Canine Adenovirus type 2 (CAV2) vector, rAAV2-retro vectors and RetroBeads to infect/enter neuronal processes and terminals and be retrogradely transported to the soma where their genetic cargo can be expressed. These retrogradely transported vectors carrying fluorophores are injected in different dopaminergic target regions of WT mice, the labeled neurons in the dopaminergic midbrain nuclei are stained for proteins of interest, and finally quantified and compared. As the study utilizes two distinct classes of viral vectors, namely CAV2 and rAAV2-retro, and also RetroBeads, the study also serves as a comparative framework to classify their retrograde efficiencies. While we achieve efficient retrograde transport of both vectors from both dorsal striatum and nucleus accumbens (NAc), the efficiency is much less pronounced from areas within prefrontal cortex. This obviously supports a sparser dopaminergic innervation within prefrontal areas, but we speculate that it may also reflect some limitations of the retrogradely transported viral vectors.

Disclosures: S.H. Jørgensen: None. **S. Nørr:** None. **M.A. Christiansen:** None. **U. Gether:** None. **K. Rickhag:** None. **A. Toft Sørensen:** None.

Poster

171. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Circuit Tracing

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 171.21/III35

Topic: I.03. Anatomical Methods

Support: EAGER Grant 1547967
NIH Grant U01 NS094288

Title: A viral receptor complementation strategy overcomes CAV-2 tropism for efficient retrograde targeting of neurons

Authors: *S.-J. LI, A. VAUGHAN, J. F. STURGILL, A. KEPECS
Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Objective: Deciphering how neural circuits are anatomically organized is crucial in understanding how the brain processes information. Retrogradely transported neurotropic viruses enable targeting neurons based on their long-range projections and have thus become indispensable tools for linking neural connectivity with function. A major limitation of viral techniques is that they rely on cell-type-specific molecules for uptake and transport. As a result, viruses fail to infect variable subsets of neurons depending on the complement of surface receptors expressed. This variable tropism presents a serious problem for viral technologies to reveal the ground truth in connectivity.
Results: Here we report a strategy to overcome this problem by enhancing the expression of receptors for the retrograde virus of interest, in this case canine adenovirus type 2 (CAV-2). We designed AAV vectors, which cause cells in the source region to express the coxsackievirus and adenovirus receptor (CAR) throughout candidate projection-neurons' axonal arbors. Enhancement of CAR expression greatly increased the ability of neurons to take up CAV-2, enhanced CAV-2 retrograde labeling rate and transgene expression level, which we demonstrated in several long-range projections in both rats and mice, including some resistant to other retrograde labeling techniques. We also combined the advantage of CAR complementation with multiple CRE-dependent optogenetic tools such as ChR2 and GCaMP, and demonstrated their use for *in vivo* functional studies during behavior.
Conclusion: We present a novel strategy to abrogate endogenous viral tropism and thereby facilitate efficient retrograde targeting for functional analysis of neural circuits. Our reagents improve on existing retrograde tracing techniques and provide useful toolbox for linking connectivity to function in neural circuits.

Disclosures: S. Li: None. **A. Vaughan:** None. **J.F. Sturgill:** None. **A. Kepecs:** None.

Poster

171. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Circuit Tracing

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 171.22/III36

Topic: I.03. Anatomical Methods

Title: MAPseq - democratizing high-throughput neuroanatomy using sequencing

Authors: *A. VAUGHAN[1], H. ZHAN[1], A. M. ZADOR[2]
[1]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [2]Zador Lab., Cold Spring Harbor Lab., Cold Spg Hbr, NY

Abstract: MAPseq is a novel technique for neuroanatomical tracing using RNA barcodes. This approach has the advantage of massive throughput compared to existing techniques, but is challenging for many researchers to implement in their lab. The overarching goal of MapNeuro, Inc is to facilitate this process, and to help disseminate MAPseq to the neuroscience community - enabling academic and non-academic researchers to integrate single-cell neuroanatomy into their research. To do so, we provide consulting and research services that help researchers perform the complex molecular biology and bioinformatics analyses necessary to incorporate MAPseq experiments into the standard workflow of systems neuroscience. Here, we outline many of the novel experimental designs made possible by MAPseq with specific attention to adapting this technique to a variety of research programs. By facilitating molecular connectomics, we aim to usher in a new understanding of neuronal circuits for all researchers.

Disclosures: A. Vaughan: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MapNeuro, Inc. **H. Zhan:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MapNeuro Inc. **A.M. Zador:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MapNeuro, Inc..

Poster

171. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Circuit Tracing

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 171.23/III37

Topic: I.03. Anatomical Methods

Support: NIMH U01MH114824

Title: Semantic segmentation framework for Neuronal cell detection and tracing

Authors: *K. UMADEVI VENKATARAJU[1], J. GORNET[3], A. NARASIMHAN[2], U. SÜMBÜL[4], H. SEUNG[5], P. OSTEN[2]
[1]Osten Lab., [2]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [3]Columbia Univ., New York, NY; [4]Allen Inst. for Brain Sci., Seattle, WA; [5]Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ

Abstract: Whole brain imaging of small mammals enables us to unravel the mysteries of structure and function of the central nervous system. Light microscopy methods such as serial 2-p tomography and light sheet imaging can provide the resolution, coverage and multiplexing to analyze the distribution of different cell types and reconstruct their somato-dendritic morphologies. Fluorescent markers can localize to subcellular structures such as the nucleus, soma, axons or just the spines. Here, we present a user annotation and machine learning based

segmentation framework to automatically segment these signals in such microscopy images. In regular semantic segmentation, tools such as ITK-SNAP and VAST assign a label to all the voxels of an image, which is a laborious process. Our framework leverages the local intensity variations within an image to obtain improved volumetric segmentations starting from basic topological segmentations. In particular, it enables the user to click at the signal positive cells and bootstrap the generation of volumetric labels with high pixel accuracy, which can be used for automated semantic segmentation. We demonstrate our approach on images acquired using TissueCyte Serial 2-photon tomography, LaVision Light sheet microscope, Zeiss Z.1 and custom built oblique light sheet tomography. This framework works both on slices and 3D volumes generated in these systems.

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Poster

171. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Circuit Tracing

Location: SDCC Halls B-H

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Program #/Poster #: 171.24/III38

Topic: I.03. Anatomical Methods

Support: NIMH U01MH114824
NSF REU Grant 1559816

Title: Generating brain atlases across diverse brain sample types

Authors: *J. GORNET[1], K. UMADEVI VENKATARAJU[2], U. SÜMBÜL[4], P. OSTEN[3]
[1]Neurosci., [2]Osten Lab., [3]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [4]Allen Inst. For Brain Sci., Seattle, WA

Abstract: Extracting structural and molecular information from the brain has been an important challenge in neuroscience. Whole-brain imaging through serial two-photon tomography or light-sheet fluorescence microscopy provides an extensive field-of-view with single-cell resolution. However, the increased dataset size requires a method to compare observations among brain regions across many different imaging modalities and mouse phenotypes. Brain atlases—such as the Allen Mouse Brain Atlas—provide a canonical coordinate framework that annotates different regions of the brain and allows researchers to narrow their focus to compare observations. Here we show a method for generating brain atlases from different imaging modalities and mouse phenotypes. To generate a brain atlas, the sample brains are imaged and registered to the serial two-photon Allen Mouse Brain Atlas. These registered sample brains are processed to create an initial sample brain atlas. To eliminate artifacts caused by incompletely registered sample brains, we propose an iterative algorithm: at each step, two-channel registration is performed using the

Allen Mouse Brain Atlas and the sample brain atlas of the previous step as fixed images. This two-channel registration and processing step is repeated until the mean-squared error between the consecutive sample brain atlases converges. In this presentation, we apply this method to light-sheet fluorescence microscopy mouse whole-brain images to generate a light-sheet fluorescence microscopy mouse brain atlas.

Disclosures: J. Gornet: None. **K. Umadevi Venkataraju:** None. **U. Sümbül:** None. **P. Osten:** None.

Poster

172. Physiological Methods: Optogenetics I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 172.01/III39

Topic: I.04. Physiological Methods

Support: DARPA CONTRACT W911NF-17-C-0059

Title: High channel count wireless neural recording and stimulation for *in vivo* electrophysiology for nhps

Authors: *J. C. MORIZIO, V. GO
Triangle Biosystems, Inc, Durham, NC

Abstract: Background: Over the last decade wireless technology advancements for neural recording and stimulation have evolved whereby they can now be designed high channel count *in-vivo* electrophysiology applications on freely moving non-human primates(NHPs). System level concepts for telemetric recording up to 384 simultaneous channels and bipolar constant current electrical and optogenetic stimulation up to 16 channels will be described. Key design challenges and tradeoffs of these wireless technologies and neural interfaces will be explained.
Materials and Methods: I will present integrated systems solutions for headstage technologies to acquire local field potentials and single units or spikes signals from NHP brains. Sub-system components and accessories will also be described that include electrodes or neural interfaces, monkey cap options, low noise integrated CMOS electronics, RF circuitry DAC analysis software used for neural recording. The same will follow for constant current electrical stimulation and optogenetic stimulation and combo stimulation/recording headstage implantable technologies.
Results: A high channel count wireless neural recording headstage system was presented showing real time single unit and local field data time data. The amount of records channels can be varied from 32 channels to 384 channels. Implantable Electrical and Optogenetic stimulation will also be demonstrated with 2 or 16 channel electrodes and 2 channel optrodes respectively. A variety of stimulation patterns will be presented.

Conclusions: Head mounted wireless recording up to 384 channels and stimulation headstage technologies up to 16 channels have been developed to be a viable and reliable alternative for NHPs. Recording channels can vary from 32 to 384 simultaneous electrode sites. Wireless stimulation headstages were demonstrated for 2 to 16 channels for bipolar constant current pulses and 2 channels of optogenetic blue, red and yellow wavelength. Technology roadmaps and behavior systems were also presented to show the technology migration of the complete systems.
Key Words: NHPs, High Channel Count Wireless Headstages, 384 channels neural recording, 16ch neural stimulation, single unit, local field potentials, electrical stimulation, optogenetic stimulation.

Disclosures: J.C. Morizio: A. Employment/Salary (full or part-time):; Full Time. **V. Go:** A. Employment/Salary (full or part-time):; Full time.

Poster

172. Physiological Methods: Optogenetics I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 172.02/III40

Topic: I.04. Physiological Methods

Support: AFOSR FA9550-17-0387
NIH Grant R01EB023232

Title: Simultaneous two-photon manipulation and imaging of neural activity based on spectral-temporal modulation of supercontinuum light

Authors: Y.-Z. LIU, C. RENTERIA, T. KOHLFAERBER, S. YOU, H. TU, P. SENGUPTA, *S. A. BOPPART
Beckman Inst. for Advanced Sci. and Technol., Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract: The rapid development of novel opsins and fluorescent indicators has introduced a large palette of biochemical probes for optogenetic stimulation and cellular imaging, which makes all-optical neural circuit excitation and neural activity recording possible. Compared to visible-light illumination, two-photon (2P) excitation and imaging not only avoids crosstalk between optogenetic probes and calcium indicators, but also provides deeper imaging penetration and higher spatial-temporal resolution for single-cell-level precise manipulation and imaging. Two-photon interactions frequently necessitate the use of high-power femtosecond (fs) sources with narrow bandwidth outputs. Multiple bulky and expensive fs lasers are usually required for simultaneous 2P optogenetic manipulation and recording. Here we propose a single source technique for simultaneous 2P stimulation and imaging based on the generation of fiber-based

broadband coherent supercontinuum (SC). A custom-made photonic crystal fiber is pumped by a fiber laser (1030 nm, 400 fs, 20 MHz) to generate a wide range of wavelengths, 880 - 1160 nm, which covers most of the 2P excitation wavelengths of the molecules used in optogenetics, e.g. ChR2 and RCaMP, and C1V1-2A-mCherry and GCaMP6s in our study. Because of the lower laser repetition rate and larger bandwidth, higher peak power for 2P excitation is achieved, compared to the more common 80 MHz narrow band fs lasers. A programmable pulse shaper, which consists with a grating to disperse and recombine different wavelengths (double-pass) and a 1D spatial light modulator to modulate the phases of targeted wavelengths, is utilized to tailor the temporal shapes of fs SC pulses. We have previously reported the use of chirped fs pulses to modulate light-evoked ionic current from ChR2 in brain tissue, and consequently the firing pattern of neurons, by manipulating the phase of the spectral component of the fs pulse. We believe this was the result of the quantum coherent control of the retinal-based protein (ChR2) system. In this study, together with whole-cell patch clamp, we programmably modulate the phase of the SC for simultaneous 2P calcium imaging and optogenetic stimulation. For the imaging band of the SC, pulse-width compression is applied to improve the SNR of video-rate calcium imaging. For the stimulation band of the SC, different types of phase modulation are applied to further explore the coherent control effect of the retinal-based optogenetic probes. This all-optical 2P imaging and stimulation technique by spectrally-temporally modulating SC pulses provides a new way to interrogate and investigate neural network activity in living tissues.

Disclosures: Y. Liu: None. **C. Renteria:** None. **T. Kohlfaerber:** None. **S. You:** None. **H. Tu:** None. **P. Sengupta:** None. **S.A. Boppart:** None.

Poster

172. Physiological Methods: Optogenetics I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 172.03/III41

Topic: I.04. Physiological Methods

Support: NSF Grant ECCS-1752241
NSF Grant ECCS-1734940
ONR Grant N00014161253

Title: A compact closed-loop artifact-free optogenetics system based on transparent graphene microelectrodes

Authors: Y. SHI, X. LIU, Y. LU, E. ISERI, *D. KUZUM
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Abstract: Electrophysiology is a decades-old technique widely used for monitoring activity of individual neurons and local field potentials. Optogenetics has revolutionized neuroscience

studies by offering selective and fast control of targeted neurons and neuron populations. The combination of these two techniques is crucial for causal investigation of neural circuits and understanding their functional connectivity. However, electrical artifacts generated by light stimulation interfere with neural recordings and hinder the development of compact closed-loop systems for precise control of neural activity. Here, we demonstrate that transparent graphene micro-electrodes fabricated on a clear polyethylene terephthalate film eliminate the light-induced artifact problem and allow development of a compact battery-powered closed-loop optogenetics system. We extensively investigate light-induced artifacts for graphene electrodes in comparison to metal control electrodes. We then design optical stimulation module using micro-LED chips coupled to optical fibers to deliver light to intended depth for optogenetic stimulation. For artifact-free integration of graphene micro-electrode recordings with optogenetic stimulation, we design and develop a compact closed-loop system and validate it for different frequencies of interest for neural recordings. This compact closed-loop optogenetics system can be used for various applications involving optogenetic stimulation and electrophysiological recordings.

Disclosures: Y. Shi: None. **X. Liu:** None. **Y. Lu:** None. **E. Iseri:** None. **D. Kuzum:** None.

Poster

172. Physiological Methods: Optogenetics I

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NIH Grant MH111359
NIH Grant NS057198
NIG Grant S10RR029050

Title: Deep 2-photon imaging and artifact-free optogenetics through transparent graphene microelectrode arrays

Authors: *X. LIU[1], M. THUNEMANN[1], Y. LU[1], K. KıLıÇ[1], M. DESJARDINS[1], M. VANDENBERGHE[1], S. SADEGH[1], P. SAISAN[1], Q. CHENG[1], K. WELDY[1], H. LYU[1], S. DJUROVIC[2], O. ANDREASSEN[3], A. DALE[1], A. DEVOR[1], D. KUZUM[1]
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Abstract: Recent advances in optical technologies such as multi-photon microscopy and optogenetics have revolutionized our ability to record and manipulate neuronal activity. Combining optical techniques with electrical recordings is of critical importance to connect the large body of neuroscience knowledge obtained from animal models to human studies mainly relying on electrophysiological recordings of brain-scale activity. However, integration of optical modalities with electrical recordings is challenging due to generation of light-induced artifacts. Here, we report a transparent graphene microelectrode technology that eliminates light-induced artifacts to enable crosstalk-free integration of 2-photon microscopy, optogenetic stimulation and cortical recordings in the same in vivo experiment. We achieve fabrication of crack- and residue-free graphene electrode surfaces yielding high optical transmittance for 2 photon imaging down to ~1 mm below the cortical surface. Transparent graphene microelectrode technology offers a practical pathway to investigate neuronal activity over multiple spatial scales extending from single neurons to large neuronal populations.

Disclosures: X. Liu: None. **M. Thunemann:** None. **Y. Lu:** None. **K. Kılıç:** None. **M. Desjardins:** None. **M. Vandenberghe:** None. **S. Sadegh:** None. **P. Saisan:** None. **Q. Cheng:** None. **K. Weldy:** None. **H. Lyu:** None. **S. Djurovic:** None. **O. Andreassen:** None. **A. Dale:** None. **A. Devor:** None. **D. Kuzum:** None.

Poster

172. Physiological Methods: Optogenetics I

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Program #/Poster #: 172.05/III43

Topic: I.04. Physiological Methods

Support: JST, CREST
KAKENHI

Title: Development of micro LED-based optical stimulation device combined with microdialysis for detecting the release of neurotransmitters

Authors: *Y. OHTA, K. NAGANUMA, M. KAWAHARA, A. KIMURA, M. HARUTA, T. NODA, K. SASAGAWA, T. TOKUDA, J. OHTA
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Abstract: Optogenetics has been remarkably developed and contributes to the variety fields of neuroscience. We have developed a new device that can simultaneously perform optical stimulation by micro LEDs and microdialysis. It is important to actually detect the release of neurotransmitters, especially when neural pathways and networks are unknown. For the following two reasons we use LEDs for optical stimulation instead of an optical fiber, which is conventionally used in optogenetics. The first reason is that a micro LED has a wider light output

distribution than that of an optical fiber and can stimulate a wider range of cells. The second reason is that micro LED is compact and lightweight so that arbitrary light stimulation pattern can be formed by arranging them. We have developed three types of devices utilizing this advantage of a micro LED. The first device comprises a ring shaped optical pattern and a hole for microdialysis probe insertion placed in the center of the ring. In the optical ring pattern, four micro LEDs are arranged to produce a uniform light output. The second device arranged four micro LEDs linearly along the dialysis probe. The first device was placed on the rodent's brain surface and the second was used to implant into the rodent's brain. The third device was developed to be placed on the brain surface of monkeys so as to stimulate a part of the cerebral cortex. It was equipped with 48 micro LEDs in the range of 6.5 mm × 9 mm and made nine holes for inserting the dialysis probe between the LEDs so as to be able to confirm the detailed part of neurotransmitter release. Actually, using the animals that expressed ChR2, it was confirmed by measurement that dopamine concentration changes in response to optical stimulation by micro LEDs. Optical stimulation by micro LED was performed in ventral tegmental area or prefrontal cortex, and at the same time the amount of dopamine released in nucleus accumbens or prefrontal cortex was measured by microdialysis. In the next step, we will construct a system that can study the neural activity in more detail by using it together with our developed brain-implanted imaging device.

Disclosures: Y. Ohta: None. **K. Naganuma:** None. **M. Kawahara:** None. **A. Kimura:** None. **M. Haruta:** None. **T. Noda:** None. **K. Sasagawa:** None. **T. Tokuda:** None. **J. Ohta:** None.

Poster

172. Physiological Methods: Optogenetics I

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The National Research Foundation of Singapore under its Competitive Research Programme (CRP Award no. NRF-CRP15-2015-03)

Title: Near-infrared deep brain stimulation via upconversion nanoparticle-mediated optogenetics

Authors: *S. CHEN[1], A. Z. WEITEMIER[4], X. ZENG[5], L. HE[2], X. WANG[3], Y. TAO[6], A. HUANG[3], Y. HASHIMOTODANI[7], M. KANO[8], H. IWASAKI[9], L. K. PARAJULI[9], S. OKABE[10], D. LOONG[11], A. H. ALL[12], I. TSUTSUI-KIMURA[13], K. F. TANAKA[14], X. LIU[5], T. J. MCHUGH[15]
[1]RIKEN Ctr. for Brain Sci., Wako-shi, Saitama, Japan; [2]Lab. for Circuit and Behaviour Physiol., RIKEN Ctr. for Brain Sci., Wakoshi, Japan; [3]RIKEN Ctr. for Brain Sci., Wako, Saitama, Japan; [4]RIKEN Brain Sci. Inst., Saitama, Japan; [5]Dept. of Chemistry, Natl. Univ. of Singapore, Singapore, Singapore; [6]Lab. for Circuit and Behavioral Physiol., RIKEN Brain Sci. Institute, BSI, Saitama, Japan; [7]Grad. Sch. of Brain Sci., Doshisha Univ., Kyoto, Japan; [8]Dept Neurophysiol, Grad Sch. Med, Univ. Tokyo, Tokyo, Japan; [9]Dept. of Cell. Neurobiology, Grad. Sch. of Med., The Univ. of Tokyo, Tokyo, Japan; [10]Univ. of Tokyo, Tokyo, Japan; [11]Singapore Inst. for Neurotechnology (SINAPSE), [12]Natl. Univ. of Singapore, Singapore, Singapore; [13]Harvard Univ., Arlington, MA; [14]Keio Univ. Sch. of Med. Dept. of Neuropsychiatry, Tokyo, Japan; [15]RIKEN BSI, Wako-shi, Japan

Abstract: Optogenetics, driven by the development of light-gated rhodopsins, has revolutionized the experimental interrogation of neural circuits and holds promise for next-generation treatment of neurological disorders. However, it is limited by the inability of visible light to penetrate deep inside brain tissue. Optical stimulation of deep brain neurons, for example, has hitherto required the insertion of invasive optical fibers because the activating blue-green wavelengths are strongly scattered and absorbed by endogenous chromophores. Red-shifted variants of rhodopsins have been developed, but their action spectra still fall out of the near-infrared (NIR) optical window (650-1350 nm) where light has its maximal depth of penetration in brain tissue. Here, we developed a novel approach for NIR optogenetics, where lanthanide-doped upconversion nanocrystals (UCNPs) were used to absorb tissue-penetrating 980 nm NIR and emit visible light for rhodopsin activation. Due to lanthanides' ladder-like electronic energy structure, the emission of UCNPs can be precisely tuned to a particular wavelength by control of energy transfer via selective lanthanide-ion doping. For instance, incorporation of Tm^{3+} into Yb^{3+} doped host lattices leads to blue emission (~470 nm) that matches the maximum absorption of channelrhodopsin-2 (ChR2) for neuronal activation, while the Yb^{3+}/Er^{3+} couple emits green light (~540 nm) compatible with activation of halorhodopsin (NpHR) or archaerhodopsin (Arch) for neuronal inhibition. We demonstrated that molecularly tailored UCNPs could serve as optogenetic actuators of transcranial NIR to functionally stimulate deep brain neurons in mice. Transcranial NIR UCNP-mediated optogenetics evoked dopamine release from genetically tagged neurons in the ventral tegmental area, induced brain oscillations via activation of inhibitory neurons in the medial septum, silenced seizure via inhibition of excitatory cells in the hippocampus, and triggered memory recall via excitation of a hippocampal engram. UCNP technology would open the door to less-invasive optical neuronal activity manipulation with the potential for remote therapy.

Disclosures: S. Chen: None. **A.Z. Weitemier:** None. **X. Zeng:** None. **L. He:** None. **X. Wang:** None. **Y. Tao:** None. **A. Huang:** None. **Y. Hashimotodani:** None. **M. Kano:** None. **H. Iwasaki:** None. **L.K. Parajuli:** None. **S. Okabe:** None. **D. Loong:** None. **A.H. All:** None. **I. Tsutsui-Kimura:** None. **K.F. Tanaka:** None. **X. Liu:** None. **T.J. McHugh:** None.

Poster

172. Physiological Methods: Optogenetics I

Location: SDCC Halls B-H

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Topic: I.04. Physiological Methods

Support: the NSFC Program 31700921
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the Shenzhen Governmental Research Grants JSGG20160428140402911

Title: Ultra-soft and highly stretchable hydrogel optical fibers for *in vivo* optogenetic modulations

Authors: *L. WANG, C. ZHONG, F. YE, J. TU, L. WANG, Y. LU
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Abstract: Keywords: optogenetics, neural modulation, stretchable
Optogenetics has been widely applied as a cell-specific technique with high temporal resolution for the modulation of neural circuitry *in vivo*, offering potential novel treatments for neuropsychiatric diseases. However, to date, the most widely used optogenetics waveguides remain silica optical fibers, which may lead to a mismatch in the mechanical properties between the implants and neural tissues. To resolve this issue, alginate-polyacrylamide hydrogel optical fibers can be fabricated in a simplified one-step process, and they show significantly improved characteristics for the *in vivo* optogenetic applications, including low light-propagation loss and Young's modulus, and high stretchability. After the expression of AAV-CaMKIIα-ChR2-mCherry, blue light pulses are delivered into hippocampus using a hydrogel-optrode array, and frequency-dependent neural responses can be observed. Moreover, optogenetic stimulation through the chronic implanted hydrogel optical fibers in the primary motor cortex can considerably modulate the animal's behavior. Hydrogel fibers significantly improve neuronal survival at the implant/tissue interface, compared with that observed using the silica optical fibers. Taken together, the results of this study demonstrate the feasibility and advantages of the hydrogel optical fiber use for chronic optogenetic modulation in free-moving animals. Hydrogel implant use may allow the development of novel therapeutic strategies for the treatment of neuropsychiatric disorders.

Disclosures: L. Wang: None. **C. Zhong:** None. **F. Ye:** None. **J. Tu:** None. **L. Wang:** None. **Y. Lu:** None.

Poster

172. Physiological Methods: Optogenetics I

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JST-PRESTO

Title: Optogenetic activation of cutaneous and proprioceptive afferent in the rat sciatic nerve

Authors: *S. KUBOTA[1,2], W. SIDIKEJIANG[1], M. KUDOH[1], K. INOUE[3], T. UMEDA[1], M. TAKADA[3], K. SEKI[1]
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Abstract: Selective manipulation of primary sensory neurons (dorsal root ganglion neurons; DRGs) by the optogenetics is a promising technique that will be beneficial for both basic research and therapeutic objectives. However, optogenetic implementation of the DRGs is still challenging, because of its difficulty in the transduction of the opsins with high selectivity and efficacy. So far, some attempts have been made to modulate the activity of DRGs with small-diameter for pain modulation, but little is established about the optogenetic control of the activity of cutaneous and proprioceptive afferents. In this study, we transduced Channelrhodopsin 2 (ChR2) into DRGs via nerve injection, and tested if their activity could be manipulated by the optogenetics. To select the serotype that could express the green fluorescent protein (GFP) in the DRGs with the best efficacy, we injected the adeno-associated virus (AAV) vectors (AAV1,2,6,8,9,10) into the sciatic nerve (titers; 8.2×10^{10} to 4.8×10^{11} gc/ml) of rats (n=12). After four weeks of survival time, each animal was sacrificed for the histological analysis. As a result, we selected the AAV9 since it transduced the DRGs with the best efficacy (81 % DRGs showed GFP positive), predominantly in the large-sized DRGs. Next, we transduced the ChR2 into the DRGs by AAV9 (AAV9-hSyn-ChR2(H134R)) and then performed terminal electrophysiological experiments under anesthesia (n=10). We illuminated the L4-L5 DRGs (exposed) with blue light (light-stimulation; LS) at various conditions (intensity, duration, intervals) and recorded the incoming volleys at the DR entry point. For a comparison, we also recorded the volley induced by an electrical stimulation (ES) of the sciatic nerve in the same animal. Amplitude of evoked

responses increased corresponding to the stimulus intensity in both conditions. However, the sensitivity to repetitive stimuli was different. For example, within the short intervals (~10 ms), the sensitivity of second response was decreased only in the LS, reflecting the desensitization of ChR2. Conduction velocity of volleys induced by LS was ranged from 20 to 50 m/s, which is comparable to that of Group I and/or II fibers. Overall, we established a way to manipulate the activity of cutaneous and proprioceptive afferents using the optogenetic technique. Since the intervention using optogenetics could suppress as well as facilitate the activity of DRGs with higher selectivity, this technique has a clear advantage over the manipulation using ES. This technique could also help to understand a role of the cutaneous and proprioceptive signals in the movement control and solve neurological disorders (e.g. spasticity).

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Poster

172. Physiological Methods: Optogenetics I

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Program #/Poster #: 172.09/III47

Topic: I.04. Physiological Methods

Support: SBF002\1033

Title: OptoStim: Opensource software for design and control of optogenetic experiments

Authors: *J. JOHNSTON[1], C. STEFENS[2], S. DUNKLEY[2]
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Abstract:

The ability to control the activity of single neurons, or discreet groups of neurons, enables the function and connectivity of neural circuits to be interrogated. Optical stimulation of optogenetic-actuator-expressing neurons is a powerful tool to realise this strategy. We have developed OptoStim, GUI driven software to facilitate targeted patterned illumination of neurons expressing optogenetic actuators. Using a digital-micromirror-device (DMD), OptoSim provides single photon patterned illumination to neurons identified with either 2-photon and/or epifluorescence microscopy. Alignment of the DMD and image planes is achieved with homography matrices automatically calculated from user defined points. Optical stimulation points can be selected from images read directly from micromanager supported cameras or from images imported from 2-photon microscopy. Using a low cost A/D board (Labjack), OptoStim can easily integrate with electrophysiology or 2-photon imaging experiments, where it can act as the "master" controlling the timing of all events or act as a "slave" waiting for signals from other equipment. In the protocol design, stimulus points can be incremented linearly or randomly with each loop of multiple trial experiments and all settings and experimental protocols can be saved and loaded via JSON files. OptoStim can also stream and display data from Arduino microcontrollers to display/monitor behavioural or physiological parameters which can be useful during in vivo experiments. OptoSim can also utilise inputs from microcontrollers to control closed-loop experiments.

Disclosures: J. Johnston: None. **C. Stefens:** None. **S. Dunkley:** None.

Poster

172. Physiological Methods: Optogenetics I

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Program #/Poster #: 172.10/III48

Topic: I.04. Physiological Methods

Support: Marie Curie Individual Fellowship to ASD

Title: Remote control of neural activity *in vivo* in zebrafish: Which opsin to chose and why?

Authors: *A. S. DUMITRESCU[1], C. DELEUZE[1], P. ANTINUCCI[2], F. KUBO[3], M. WU[1], H. BAIER[4], I. H. BIANCO[2], C. WYART[1]
[1]Inst. du Cerveau et de la Moelle Epinière, Paris, France; [2]Dept. of Neuroscience, Physiol. & Pharmacol., Univ. Col. London, London, United Kingdom; [3]Natl. Inst. of Genet., Mishima, Japan; [4]Max Planck Innstitute of Neurobio., Martinsried, Germany

Abstract: Background:
Thirteen years after the publication of the original Channelrhodopsin-2 proof of principle paper using cultured neurons, we now have available several excitatory and inhibitory opsins with marked improvements in kinetics, efficiency and different spectral properties. Despite these

advancements, the field if still facing several challenges. First, it is not a trivial matter to generate stable transgenic lines which express high levels of opsin fluorescence in specifically targeted cell populations. As a second point, there is currently a mismatch between the careful electrophysiological opsin characterisations performed in more accessible *in vitro* preparations, which are then followed by opsin-mediated activation or inactivation of neural activity *in vivo*, often deprived of re-calibrations in these new systems. Here we address both of these issues by taking advantage of the transparent zebrafish larva model to calibrate the performance of the best-expressing opsin transgenic lines available via electrophysiological recordings in motor neurons *in vivo*.

Results:

We used the *Tg(mnx1:gal4)* transgenic line to test the functional profile of the following excitatory opsin lines: UAS:ChR2-H134R-YFP, UAS:ReaChR-GFP, UAS:Chronos-tdTomato, UAS:CoChR-tdTomato, UAS:Chrimson-tdTomato, UAS:Cheriff-tdTomato, as well as the neural activity inhibitory effectors: UAS:eNphR3.0-YFP, UAS:eArch3.0-YFP, UAS:GtACR1-tdTomato, UAS:GtACR2-tdTomato. Our preliminary data show that the now "classical" excitatory ChR2-H134R-YFP opsin is able to elicit single spikes in primary and secondary MNs but fails to drive spiking at frequencies higher than 1Hz. In transgenic larvae, ReaChR-GFP on the hand, failed to elicit any spiking activity despite having excellent cellular targeting levels. In terms of inhibitory opsins, the first probe tested, UAS:eNphR3.0-YFP, seems to be an efficient tool to block neural activity. Experiments are ongoing to test the remaining opsin transgenic lines mentioned above.

Conclusions:

The present study is addressing an important gap in current methodology: the need for calibration of optogenetic tools expressed in a stable manner *in vivo*. Our ongoing opsin comparison experiments will shed light on the best available tools for eliciting spiking with high fidelity at physiological frequencies *in vivo*.

Disclosures: C. Deleuze: None. **P. Antinucci:** None. **F. Kubo:** None. **M. Wu:** None. **H. Baier:** None. **I.H. Bianco:** None. **C. Wyart:** None.

Poster

172. Physiological Methods: Optogenetics I

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Topic: I.04. Physiological Methods

Support: ERC Grant 677683
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NIH Grant U01NS094190
imons Collaboration on the Global Brain 543037SPI

Title: Towards multipoint fiber photometry with tapered optical fibers

Authors: *F. PISANELLO[1], M. PISANELLO[2], F. PISANO[3], A. BALENA[4], E. MAGLIE[4], B. SPAGNOLO[4], L. SILEO[5], B. L. SABATINI[6], M. DE VITTORIO[2]
[2]Ctr. for Biomolecular Nanotechnologies, [1]Inst. Italiano Di Tecnologia, Arnesano, Italy; [3]Ctr. for Biomolecular Nanotechnologies, Italian Inst. of Technol., Arnesano, Italy; [4]Ctr. for Biomolecular Nanotechnologies, [5]Inst. Italiano di Tecnologia, Arnesano, Italy; [6]Neurobio., Harvard Med. Sch. Dept. of Neurobio., Boston, MA

Abstract: The recent advent of fiber photometry has increases the need of new methods to efficiently collect light from the neural tissue, better able to map functional connectivity using genetically encoded fluorescent indicators of neural activity. If technologies for optogenetic control of neural signaling have seen a strong focus of research activity in last 10 years, the development of implantable devices for combined optical control and monitoring of neural activity is still at its embryonal stage, and only a few works have proposed new neural interfaces specifically designed with this purpose. These include the use of microscopy-based endoscopes and integrated photodetectors [Biomed Opt Express 9, 1492 2018; PNAS 115, E1374 2018]. Here we demonstrate the potential of tapered optical fiber (TFs) to obtain multipoint optical monitoring of neural activity along the taper axis. Exploiting their mode division *demultiplexing* properties, TFs were already used to selectively trigger action potential in multiple brain areas [Nat Neuro 20, 1180 2017; Neuron 82, 1285 2014]. Using the system in reverse, e.g. exploiting the intrinsic *multiplexing* properties of the taper, allows instead for multipoint light collection with a single optical fiber. Light entering the taper close to the tip is coupled mostly to propagating modes with a low transversal component of the wavevector (k_t) within the waveguide, while light entering farther from the tip couples mostly to modes with high k_t. Therefore, measuring the collected k_t allows to identify the taper section at which light enters into the waveguide. This can be done by using a detection system based on a 4f optical path, imaging the far-field emission pattern of the fiber facet on a sCMOS camera. The result is a ring-shaped image, whose diameter allows to identify the k_t and therefore the taper section at which light enters the taper. By implementing image segmentation on the acquired images, fast detection of multiple fluorescence points along the fiber can be obtained, with spatial resolution of a few-hundreds of micrometers over an extent of about 2mm. The intrinsic multiwavelength operation of this approach [Sci Rep 8, 4467 2018], the low tissue reaction to the implant and the possibility to work with both uncoated and nanostructured TFs, let us believe that these devices can be used for simultaneous multipoint control and monitoring of neural activity.

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Poster

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Topic: I.04. Physiological Methods

Support: NIH
HHMI
several private foundations

Title: Optogenetic, calcium-sensing, voltage-sensing and chemogenetic mouse models available from The Jackson Laboratory

Authors: *J. BECKWITH, S. F. ROCKWOOD, C. LUTZ
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Abstract: A priority of the biomedical community is to advance the understanding of neural circuitry in both normal and disease states. To facilitate the response to this challenge, The Jackson Laboratory (JAX) Mouse Repository offers an impressive array of genetically-engineered tools enabling scientists to monitor the neural activity of intact mouse brain. Top most in this tool box are mouse lines employing optogenetic and transient-sensing (calcium-, voltage-) technologies. Opsins are light-activated proteins that alter membrane potential in neurons, so that stimulation with light allows rapid control of neuronal activity. Several mouse lines express improved/optimized opsins fused to fluorescent proteins. These include mice with channelrhodopsin expression directed by specific promoters. Additional control is available in mice with Cre- or Tet-dependent expression of channelrhodopsin or halorhodopsin.
Variants of GCaMP fluoresce in response to calcium binding and serve as an indicator of neuronal activation. These include *Thy1*-promoter driven GCaMP6 transgenic lines, Tet-dependent GCaMP6f or GCaMP6s transgenic lines and Cre-dependent GCaMP6f or GCaMP6s mouse lines. Both cytosolic- and membrane-targeted GCaMP6 mice are available.
Several strains utilize both Cre-lox and Tet-On/-Off functionality. Removal of a floxed-STOP allows Tet-dependent expression of channelrhodopsin (ReaChR/EYFP, $ChR2^{H134R}$/EYFP), GCaMP6s, GCaMP6f, RCaMP1.07, voltage-sensor (ASAP2s), bicistronic QuasAr voltage-indicator CheRiff channelrhodopsin (OptoPatch) or substrate-dependent reporter (ssAPEX2tm). This set includes mice created by the Allen Institute for Brain Science, the Genetically-Encoded Neuronal Indicator and Effector (GENIE) Project (Janelia/HHMI), Duke/MIT and several others.
Designer receptors exclusively activated by designer drugs (DREADDs) are mutant G-protein coupled receptors activated by the pharmacologically-inert molecule clozapine-*N*-oxide. Several chemogenetic strains have Cre- and/or FLP-inducible expression of DREADDs.
Repository offerings may be searched (**jax.org/mouse-search**). Researchers are encouraged to

donate their mouse lines via a very short online form (**jax.org/donate-a-mouse**). Visit The Jackson Laboratory Resources for Optogenetics, Cre-dependent Optogenetic Tools and Cre Strains for Neurobiology (**jax.org/optogenetics**). The JAX Mouse Repository receives support from NIH, HHMI and private foundations.

Disclosures: J. Beckwith: None. **S.F. Rockwood:** None. **C. Lutz:** None.

Poster

172. Physiological Methods: Optogenetics I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 172.13/III51

Topic: I.04. Physiological Methods

Support: DA036420
MHD007593
CA163069
MHD007586
GM059994

Title: An optogenetic approach to test the functional consequences of dopamine transporter multimer formation

Authors: *S. M. INGAM[1], T. RANA[2], N. BERRYMAN[4], J. S. GOODWIN[3]
[1]Morphology Core/ Cancer Biology/Biochemistry, [3]Biochem. and Cancer Biol., [2]Meharry Med. Col., Nashville, TN; [4]Life and Physical Sci., Fisk Univ., Nashville, TN

Abstract: The dopamine transporter (DAT) is essential for the reuptake of the released neurotransmitter dopamine (DA) in the brain. DAT is one of the main targets for psychostimulants leading to a disruption of DA homeostasis. Methamphetamine (METH), a widely abused psychostimulant, is a substrate for DAT. METH is a substrate for DAT and induces DA efflux into the synapse. DA efflux is known to contribute largely to the euphoric effect experienced when abusing METH. METH also induces an intracellular calcium response and the formation of DAT higher order (multimeric) complexes. However, the induced multimerization of DAT is not unique to METH as studies have shown 6-OHDA also induces multimerization in rats as well as in cell culture models. We find that there are differences in DAT multimer pattern and time-dependent formation in the presence of DA versus METH. The functional significance of DAT multimer formation is not currently known. To test the functional significance of DAT multimers we have generated an optogenetic fusion protein, Cry2-DAT-mCh that allows us to induce DAT multimers with blue light, and independent of DAT substrates such as METH and DA. Using pulsed blue-light in cells expressing this fusion construct, we were able to mimic DAT multimerization patterns. We used these specific pulses of blue light to

then test uptake and trafficking of these Cry2-DAT-mCh multimers. Our data reveal that Cry2-DAT-mCh has normal uptake activity and when pulsed with blue light increases uptake compared to control. Additionally, we find that blue light induced multimers of Cry2-DAT-mCh restore the uptake to control levels after pretreatment with nomifensine or METH.

Disclosures: S.M. Ingam: None. **T. Rana:** None. **N. Berryman:** None. **J.S. Goodwin:** None.

Poster

172. Physiological Methods: Optogenetics I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 172.14/III52

Topic: I.04. Physiological Methods

Title: A multi-channel injector for the introduction of viral vectors to the lateral surface of the rhesus monkey brain

Authors: *E.-M. JASKOT[1], M. A. G. ELDRIDGE[2], J. M. FREDERICKS[3], T. W. BENNETT[4], K. DASH[2], W. LERCHNER[2], B. RICHMOND[2]
[2]Lab. of Neuropsychology, [1]Natl. Inst. of Mental Hlth., Bethesda, MD; [3]Friedman Brain Inst., Icahn Sch. of Med. at Mount Sinai, New York, NY; [4]Section on Instrumentation, NIH, Bethesda, MD

Abstract: Genetically-based neural silencing technologies (e.g. optogenetics and chemogenetics) offer exciting opportunities for studying neural pathways. These techniques are often implemented in mice using germ-line modification or via the use of viral vectors; both these techniques are challenging to apply to the non-human primate (NHP). Germ-line modification is not currently available for studying monkeys. The use of viral vectors can be applied now in monkeys. One challenge in monkeys is getting virus into moderate volumes of tissue, e.g. whole architectonic brain regions. Currently we have had success delivering virus using either handheld or static, arm-mounted injections. Handheld injections usually give dense punctate expression with little or no expression between injection sites. With arm-mounted injections there is dense expression in the target tissue, and there is good coverage between injection sites. The arm-mounted method is time consuming to the point of limiting the number of injections that can be performed within a single surgery.
We produced two multi-channel injector arrays which allow for stable, parallel, slow infusions of large volumes of virus into regions of lateral cortex in monkey brains. Two 3D-printed array styles were produced: one with channels in a 2x2 ‘square’ arrangement (2 mm spacing between needles) and the other with a 1x4 ‘linear’ arrangement (1.5mm spacing between needles). The square array was fitted with 1.5mm 31 gauge needles and was designed to inject large lateral cortical regions. The linear array was fitted with 6mm 31 gauge needles and was designed to inject into the banks of sulci. Both arrays allow for four injections to be performed

simultaneously. The needles are connected via silicone tubing to glass syringes fitted into a 3D-printed syringe holder. The body of the injector array is connected to the syringe holder via a small metal dowel. The syringe holder is connected to an arm-mounted infusion pump which allows for stereotaxic control over the placement of the arrays. The pump permits infusion of four equal volumes at a slow rate (e.g. 0.5µL/min), thereby reducing the risk of backflow and increasing the homogeneity of the transduction of the target region.
Lentivirus expressing an hM_4Di-CFP fusion protein was co-infused with 0.5 mM manganese (an MRI contrast reagent) to permit immediate post-operative visualization of injection success. MR images show coverage of lateral cortex with spread between injection sites.

Disclosures: E. Jaskot: None. **M.A.G. Eldridge:** None. **J.M. Fredericks:** None. **T.W. Bennett:** None. **K. Dash:** None. **W. Lerchner:** None. **B. Richmond:** None.

Poster

172. Physiological Methods: Optogenetics I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 172.15/III53

Topic: I.04. Physiological Methods

Support: Howard Hughes Medical Institute
Simons Collaboration on the Global Brain
Helen Hay Whitney Foundation Fellowship

Title: Cellular-resolution recording and biasing of short-term memory in the anterior lateral motor cortex (ALM)

Authors: *K. DAIE[1], H. INAGAKI[1], K. SVOBODA[1], S. DRUCKMANN[2]
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Abstract: Short-term memory (STM) is believed to be an emergent property of neural circuits, arising from the interactions between populations of memory-less neurons. Recording the dynamics of neural populations is important to constrain circuit models of STM. But ultimately observation alone is insufficient to distinguish between circuit models. For instance, any pattern of persistent firing can be produced by multiple model networks that operate according to different principles. These principles in turn are reflected in the patterns of connections between neurons with specific activity patterns. Targeted cellular-resolution perturbations, where specific groups of neurons are chosen for perturbation based on their functional properties, can reveal circuit mechanisms by i) the population level response to a specific perturbation and ii) inference of the patterns of functional connections between neurons.
We trained mice to perform a two-alternative forced-choice task with a delay epoch. During the delay epoch, anterior-lateral motor cortex (ALM) neurons show persistent activity related to

future movement direction. This activity is a prospective STM, linking an instruction in the past to a future movement. We imaged activity in populations of ALM neurons. In some trials we used 2-photon excitation of soma-targeted Kv2.1-chrimsonR to photostimulate groups of neurons, chosen based on their selective activity during the delay epoch. We found that photostimulation of only 8 neurons produced significant changes in the activity of up to 40 of the 200 other active neurons within a 700x700 μm field of view.
Among the neurons that were not directly photostimulated, the largest changes were observed in neurons with similar task-related selectivity compared to the targeted group. In many cases, measured activity remained elevated for as long as five seconds following the offset of the photostimulation. Moreover, photostimulation produced small, but statistically significant, biases in behavior that were correlated with the selectivity of the targeted population. These results are consistent with a key assumption of many theoretical models of STM: strong positive feedback between neurons with similar selectivity.

Disclosures: K. Daie: None. **H. Inagaki:** None. **K. Svoboda:** None. **S. Druckmann:** None.

Poster

172. Physiological Methods: Optogenetics I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 172.16/III54

Topic: I.04. Physiological Methods

Support: Alberta Innovates-Health Solutions (AIHS) Postdoctoral Fellowship
Canadian Institutes of Health Research (CIHR) Postdoctoral Fellowship

Title: Engineering next-generation optogenetic pannexin-1 channels

Authors: *A. W. LOHMAN[1], R. E. CAMPBELL[3], R. J. THOMPSON[2]
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Abstract: Pannexin-1 (Panx1) forms large pore channels regulating synaptic plasticity, neuronal excitotoxicity and inflammatory signaling in the brain. Our understanding of Panx1 in neuro(patho)physiology has expanded since their discovery in 2000, but detailing the exact roles in these phenomena is limited by non-specific tools to isolate channel activity. Many of the pharmacological agents used to block these channels have relatively low affinity and are predominantly non-selective, inhibiting connexin based channels, maxi anion channels and other plasma m membrane channels. Additionally,, Panx1 knockout animals often show compensatory upregulation of the Panx3 isoform, hindering the ability to specifically assess the contribution of Panx1 in a multitude of signaling cascades. To overcome this experimental barrier, we recently developed a first-generation light-controlled Panx1 channel (Opto-Panx1) that is irreversibly

activated by cleaving the auto-regulatory C-terminal domain (4.65 min latency to activation). This tool has aided in defining the sufficiency and necessity of Panx1 in driving neuronal death during ischemic stroke. Due to the irreversible nature of Opto-Panx1, applications are limited to studying neuropathological paradigms. In order to fine-tune control of Panx1 activity, we engineered a new channel with improved temporal dynamics. The light-oxygen-voltage (LOV) domain from *Avena sativa* was cloned into the auto-regulatory C-terminal domain of Panx1 to create a library of chimeric channels. In the dark state, LOV2 has a structured J alpha-helix folded atop an anti-parallel beta-sheet "sandwiching" an FMN co-factor. Upon blue light (~450nm) illumination the J alpha-helix unfolds and can be harnessed to impart conformational changes to regulate protein activity. The J alpha-helix restructures into its folded confirmation in the dark state reversing any light-induced changes. Using Panx1 dye uptake assays, ATP release assays and whole-cell patch clamp electrophysiology, we identified a chimera (Panx1-LOV) with improved temporal control (35.2s latency to activation) that closes in the dark state. This new optogenetic tool will significantly advance the study of Panx1 signaling across research disciplines and can be harnessed to dissect the specific functions of these channels in neurophysiology and pathology.

Disclosures: A.W. Lohman: None. **R.E. Campbell:** None. **R.J. Thompson:** None.

Poster

172. Physiological Methods: Optogenetics I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 172.17/III55

Topic: I.04. Physiological Methods

Support: CIHR
NSERC
CFI
Brain Canada

Title: Two-colour optogenetic control of cAMP and cGMP in target synapses and neurons of brain regions

Authors: *M. VALENCIA[1,2], K. OKAMOTO[1,2]
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Abstract: Intracellular messengers cAMP and cGMP are thought to invoke synaptic plasticity and contribute to learning/memory and disease. Pharmacological reagents and genetic manipulations have been used to study their functions, however, their signalling dynamics and interactions at synapses remain elusive because these approaches have limited cellular precision

and spatiotemporal specificity. Here we introduce a two-colour optogenetic approach by the combination of blue light-sensitive adenylyl cyclase (PAC) and green light-sensitive rhodopsin guanylyl cyclase (RhGC) for selectively activating cAMP and cGMP signals by light in living neurons. To characterize these enzymatic activities, we measured photoactivation with different colors of light and determined distinct excitation wavelengths for cAMP and cGMP synthesis. To target photoactivation at the synapse level within brain tissue, we next characterized the two-photon excitation spectrum of the enzymes and optimized a combination of two-photon excitation wavelengths for selective photoactivation of PAC and RhGC *in vitro*. By co-expressing these optogenetic enzymes in CA1 pyramidal neurons in living hippocampal brain slices, we selectively manipulated levels of cAMP and cGMP at target dendritic spines using two-photon photoactivation, and demonstrated their bidirectional function in the regulation of structural synaptic plasticity in dendritic spines. Furthermore, we co-expressed the enzymes in target neurons of the mouse brain and validated their photoactivation. Thus, our established two-colour optogenetic approach provides powerful tools to directly study spatiotemporal cAMP/cGMP functions in an unprecedented way.

Disclosures: M. Valencia: None. **K. Okamoto:** None.

Poster

172. Physiological Methods: Optogenetics I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 172.18/III56

Topic: I.04. Physiological Methods

Support: NIH Grant K99 MH110597
NIH Grant F32 NS083369

Title: Opsin-independent suppression of striatal neuron firing by high-intensity illumination

Authors: *S. F. OWEN[1], M. H. LIU[2], A. C. KREITZER[1]
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Abstract: Optogenetics has emerged as a powerful tool to investigate specific cell types and circuits, and relies upon the assumption that light entering the brain only affects the activity of neurons that are expressing opsins. Here we examine recent controversy surrounding the function of striatal parvalbumin-positive (PV) interneurons to highlight how optogenetic stimulation can have non-specific effects on neuronal firing rate. PV cells are canonically thought to suppress medium spiny neurons (MSNs), but recent optogenetic studies have produced conflicting results. We find that at <3mW of illumination, optogenetic inhibition of PV cells disinhibits MSNs within 10-20 ms. However, increasing light power to >10mW suppresses

both MSNs and PV cells, even in the absence of opsin expression, on a time scale of 500-700ms. Importantly, the kinetics of these processes differ by an order of magnitude, and the opsin-independent effects are consistent with heating of brain tissue. We demonstrate that this opsin-independent suppression of spiking in MSNs can be replicated in the acute slice preparation, and is accompanied by activation of a cesium-sensitive potassium conductance. Taken together, these results suggest that researchers need to exercise extreme caution in the application of high-intensity illumination for optogenetic experiments.

Disclosures: S.F. Owen: None. **M.H. Liu:** None. **A.C. Kreitzer:** None.

Poster

172. Physiological Methods: Optogenetics I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 172.19/III57

Topic: I.04. Physiological Methods

Support: ERC grant #677683
ERC Grant #692943
NIH Grant U01NS094190
Simons Collaboration on the Global Brain Grant #543037SPI

Title: Light delivery in extended brain structures by high NA tapered fibers

Authors: *E. MAGLIE[1,2], M. PISANELLO[1], F. PISANO[1], G. MANDELBAUM[3], L. SILEO[1], E. BELLISTRI[4], B. SPAGNOLO[1], B. L. SABATINI[3], M. DE VITTORIO[1,2], F. PISANELLO[1]
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Abstract: Spatiotemporal optogenetic control of neuronal activity at depth requires adjustable light delivery using implantable technologies. In this field, neuroscientists are making efforts to develop devices for reaching deeper and extended brain regions [1]. Among existing technologies, Tapered Optical Fibers (TFs) represent a promising low invasive alternative [2-4].We present TFs with increasing Numerical Apertures (NA) for wide-volume or site-selective light delivery across different brain structures [4]. TFs can stimulate brain areas extending up to 3 mm by increasing the NA from 0.22 to 0.66 and by modulating the taper angle (Fig.1a). We characterized the optical properties of TFs in stained brain slices at both 473 and 561 nm by changing the input angle θ of light into the fiber [3, 4]. To do this, a setup based on a galvanometric mirror was implemented (Fig.1b) [4], allowing to change the sub-portion of the taper which is emitting light in a linear fashion as a function of θ. Fig. 1c demonstrates

simultaneous injection of two wavelength inputs (473 nm and 561 nm), showing the ability of the method to stimulate and inhibit neural activity in different brain regions at the same time with a single implanted device. Moreover, on the base of these results, we set up ray tracing simulations which validated the experimental data [4].

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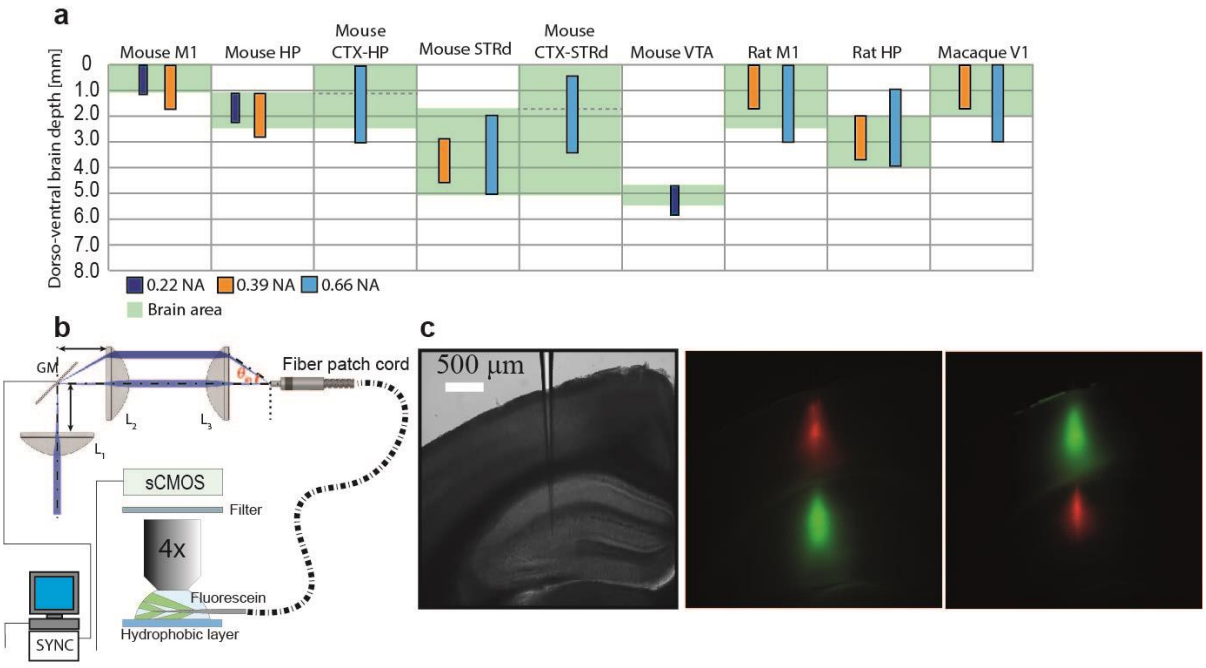

Figure 1

Disclosures: E. Maglie: None. **M. Pisanello:** None. **F. Pisano:** None. **G. Mandelbaum:** None. **L. Sileo:** None. **E. Bellistri:** None. **B. Spagnolo:** None. **B.L. Sabatini:** None. **M. De Vittorio:** None. **F. Pisanello:** None.

Poster

172. Physiological Methods: Optogenetics I

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 172.20/III58

Topic: I.04. Physiological Methods

Support: FAPEMIG APQ-00476-14

Title: Optogenetic stimulation of prefrontal glutamatergic transmission drives hyperkinesia and ameliorates motor dysfunctions following depletion of dopamine

Authors: *L. V. MAGNO, H. TENZA-FERRER, M. COLLODETTI, A. P. C. RODRIGUES, M. F. G. AGUIAR, R. S. SILVA, D. V. F. ROSA, M. A. ROMANO-SILVA
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Abstract: Although cortical circuit dysregulation is correlated with PD, it has been challenging to elucidate the relevant target cell types of cortical stimulation to modulate basal ganglia activity. In this study we infected the secondary motor (M2) neurons with an AAV vector carrying the channelrhodopsin-2 (ChR2) gene driven by the CaMKII promoter to provide evidence if cortical stimulation could be a valuable therapeutic strategy in PD. We observed that the selected prefrontal area harbored glutamatergic pyramidal neurons that projected almost entirely ipsilateral to subcortical structures including the dorsal part of the striatum, pallidum, thalamus, substantia nigra, superior colliculus and pons. Interestingly, the PFC axons showed synaptic contacts with TH-positive neurons in the lateral SNc. Because the PFC glutamate axons projected to brain areas critically involved in motor control, we implanted an optical fiber above the cortical axons in the dorsomedial striatum and addressed whether optogenetic stimulation of the PFC excitatory would affect the locomotor behavior. Compared to mice infected with control virus expressing eYFP only, photostimulation of ChR2:mice reliably evoked locomotor activity such as increased contralateral rotations, ambulation and total distance traveled during the light-on epochs. Relative to the not stimulated hemisphere, tissue levels of dopamine and its metabolites DOPAC and HVA were increased in stimulated striatum and midbrain. Pharmacological studies showed that the light-induced locomotor activity could be attenuated by selective inhibition of DA receptors. Brain infusions of glutamate antagonists NBQX and AP5 (or saline in control mice) via an internal infusion cannula did not change locomotor activity however abolished light-induced hyperlocomotion. In a series of experiments using unilateral or bilateral depletion of dopamine nigrostriatal neurons, we have shown that optical stimulation also improved the behavior impairments consistent with parkinsonian motor dysfunction in mice. Taken together, these findings provide evidence that activation of PFC neurons can ameliorate motor deficits in a dopamine-depleted mouse model of Parkinson’s disease. Future studies will be required to characterize the role of this neurocircuit activity as a novel future therapeutic target for in Parkinson’s disease.

Disclosures: L.V. Magno: None. **H. Tenza-ferrer:** None. **M. Collodetti:** None. **A.P.C. Rodrigues:** None. **M.F.G. Aguiar:** None. **R.S. Silva:** None. **D.V.F. Rosa:** None. **M.A. Romano-silva:** None.

Poster

173. Physiological Methods: Electrophysiology: Neural Networks

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 173.01/III59

Topic: I.04. Physiological Methods

Support: Start-up to AJN

Title: Pharmacological investigation of transcranial magnetic stimulation evoked short and long-latency afferent inhibition

Authors: *C. V. TURCO[1], J. EL-SAYES[1], M. LOCKE[1], S. BAKER[2], R. CHEN[4], A. J. NELSON[3]
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Abstract: Peripheral nerve stimulation paired with transcranial magnetic stimulation (TMS) is a non-invasive technique to assess the influence of the sensory afferent volley on corticospinal excitability. By varying the interstimulus interval, different phases of afferent inhibition can be assessed, termed short-latency afferent inhibition (SAI) and long-latency afferent inhibition (LAI). Previous studies have demonstrated that SAI is reduced by lorazepam and scopolamine, suggesting that SAI is modulated by GABAergic and cholinergic activity. At present, the neural mechanisms that underpin the genesis of LAI remains unknown. The aim of the present study was to test whether LAI is mediated GABA-receptor activity. In a double-blinded, placebo-controlled study, healthy right-handed individuals (n=14, age 22.7 ± 1.9 years) participated in three sessions, receiving 2.5mg of Lorazepam ($GABA_A$ agonist), 20mg Baclofen ($GABA_B$ agonist) or placebo. Median nerve stimulation and TMS were coupled to assess the change in SAI and LAI following administration of each pharmacological agent. SAI was assessed at interstimulus intervals relative to the latency of the N20 (N20 +4ms, N20 + 6ms). LAI was assessed at 200ms, 400ms, and 600ms. Our findings demonstrate no significant change in SAI or LAI following ingestion of Baclofen. In contrast, Lorazepam significantly reduced SAI (one-tailed paired t-test, $p = 0.025$), supporting previous work, and also LAI (two-tailed paired t-test, $p = 0.027$). This is the first study to demonstrate LAI is mediated by $GABA_A$-receptor activity and confirm the previously reported findings that SAI is also $GABA_A$-receptor mediated. Follow-up studies will further examine the influence of $GABA_B$ receptor activity on afferent inhibition.

Disclosures: C.V. Turco: None. **J. El-Sayes:** None. **M. Locke:** None. **S. Baker:** None. **R. Chen:** None. **A.J. Nelson:** None.

Poster

173. Physiological Methods: Electrophysiology: Neural Networks

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 173.02/III60

Topic: I.04. Physiological Methods

Title: Interlamellar dentate gyrus neurons in hippocampus

Authors: *G. CHOI[1], H. KANG[4], S. PAK[2], S. YANG[5], S. YANG[3]
[1]City Univ. of Hong Kong, Kowloon, Hong Kong; [2]City Univ. of Hong Kong, Hong Kong, Hong Kong; [3]City Univ. of Hong Kong, Kowlong, Hong Kong; [5]Nano-bioengineering, [4]Incheon Natl. Univ., Incheon, Korea, Republic of

Abstract: Information into hippocampus begins in dentate gyrus (DG) and then flows into the CA3-CA1 circuit. The lamellar network from DG to CA1 has been a central subject for memory and cognition of the brain. While the lamellar organization of DG-CA1 neurons has been well-investigated, the neural circuit of interlamellar DG-DG neurons remained to be determined. Here, we show DG-DG connection between granule cells using two-photon calcium imaging, glutamate uncaging, and optogenetics. We found that DG granule cells project to neighboring DG. Furthermore, there was the dense number of boutons along thin axons in the DG-DG projection, suggesting heavy synaptic formation along the interlamellar DG plane. The results stimulate further investigation of the contribution of DG neurons to memory and cognition. It would provide an invaluable asset for deeper understanding of hippocampal circuit and functions.

Disclosures: G. Choi: None. **H. Kang:** None. **S. Pak:** None. **S. Yang:** None. **S. Yang:** None.

Poster

173. Physiological Methods: Electrophysiology: Neural Networks

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 173.03/III61

Topic: I.04. Physiological Methods

Title: Detection of electroencephalographic changes in rats during auditory processing and reward anticipation using a two-tone auditory discrimination task

Authors: *B. LAURSEN, J. F. BASTLUND
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Abstract: The two-tone auditory discrimination task (TTADT) is widely used for detection of event-related changes in the electroencephalographic (EEG) signal during auditory discrimination and decision making in clinical studies. In recent years, the TTADT has also been applied succesfully in different preclinical studies where evoked potentials comparable to human event-related potentials have been reported in rats. However, very little is known about the electrophysiological responses in humans during reward anticipation and almost nothing is known about it in rats. Here, we investigate the potential to characterize EEG changes during reward anticipation using the TTADT. Male Sprague Dawley rats (n = 35) were conditioned to press a lever following 7.5 kHz target tone presentations and to reject 6 kHz non-target tone presentations. Target and non-target tones were presented at 30:70 7.5/6 kHz cue ratio. Only lever presses to target tones were rewarded by a food pellet. Intracranial recordings were performed in the auditory cortex, nucleus accumbens shell, and infralimbic cortex as these areas have been shown to be activated during the TTADT. Additionally, behavioral indices were evaluated. Event-related potential responses were quantified and spectral analyses of oscillatory activity within and between subcortical areas were investigated. Clear auditory evoked potentials were obtained from all recording sites and differential oscillatory EEG activity was prominent in the investigated areas. The evoked responses and time-frequency analyses revealed a concise portrayal of the spatiotemporal evolution of EEG changes from target tone presentation to correct lever press response to reward delivery 2 seconds later. Our findings indicate that the TTADT may be used for investigation of neural responses related to reward anticipation in rats. Future studies should address the pharmacological sensitivity and whether neural responses in humans performing the TTADT translate to the present findings.

Disclosures: B. Laursen: None. **J.F. Bastlund:** None.

Poster

173. Physiological Methods: Electrophysiology: Neural Networks

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 173.04/III62

Topic: I.04. Physiological Methods

Support: NIH R15HD075207
NDF IIS1608147

Title: Changes in ventral horn networks when cocultured with astrocytes or striated muscle

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Abstract: Co-cultures are a traditional method for studying the cellular properties of cell to cell interactions among different cell types. How network properties in these multicellular synthetic systems vary from monocultures are of particular interest. Understanding these changes in cell behavior can provide new insights into *in vivo* systems and how to develop models that better reflect physiological conditions - something of paramount importance to the progress of synthetic biology.
Culture models of spinal motor neurons have been customarily studied as a monoculture, and the overwhelming consensus is that in culture they are different in nature from their *in vivo* counterparts. As a control we used E7 embryonic chick spinal ventral horns cultured on a hydrogel matrix substrate, we compared them with those co-cultured with spinal astrocytes or striated muscle also from embryonic chicks.
We studied the electrophysiological properties of these networks using the 64 channel multielectrode array (MEA) system from Alpha Med Scientific. As compared with controls, there was a decrease in total detected spikes with myocyte cocultures while there was an increase seen amongst astrocyte cocultures. This is in stark contrast to the population of active neurons which increased in myocytes but decreased in astrocytes. The average amplitude of sorted spikes in ventral horn cultures and astrocyte cocultures showed an increase in amplitude over time, while myocyte cocultures showed a decrease in amplitude over time. Significant differences were also found between coculture types in the number of bursts, burst duration, and interspike interval.
Traditional culturing techniques involving a monolayer of uniform cell type might not be the best way to mimic *in vivo* systems. A synthetic ecosystem of various cell subtypes is beneficial to replicating cell behavior *in vitro*, thus is a necessary refinement to the commonly used technique of cell culture. With a more physiological model system, hypotheses about interacting systems can be better addressed and the outcomes will have greater relevancy.

Disclosures: A. Tharaneetharan: None. **M.A. Harrington:** None.

Poster

173. Physiological Methods: Electrophysiology: Neural Networks

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 173.05/III63

Topic: I.04. Physiological Methods

Support: NIH grant MH110217
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Title: Electric field dynamics in the brain during phase-varying transcranial alternating current stimulation

Authors: *I. ALEKSEICHUK[1], A. Y. FALCHIER[2], G. LINN[2], T. XU[3], M. P. MILHAM[2], C. E. SCHROEDER[2,4], A. OPITZ[1]
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Abstract: Transcranial alternating current stimulation (tACS) is a method of non-invasive, frequency-specific manipulation with the brain oscillations. One recent application of multi-electrode tACS is the hyper- or de-/synchronization of distant brain regions. To do so, researchers vary the phase of applied stimulation currents between the stimulation electrodes at different scalp sites. However, the effect of this manipulation on the intracranial electric fields has not yet been directly demonstrated. Here, we performed intracranial recordings in two non-human primates during multi-electrode tACS and systematically evaluated the effect of varying input phases on the electric fields. Monkeys were implanted with three recording arrays (8-12 sites each) along the anterior-posterior axis. Three stimulation electrodes were placed on the scalp over the medial prefrontal (anterior target), left occipital (posterior target), and left temporal ("return" electrode) locations. We applied TACS with phase differences of 0° to 360° in 15° steps between the prefrontal and occipital electrodes. The electric field was calculated as the numeric gradient along the recording arrays. We found that phase differences between the stimulation electrodes significantly affect the electric field magnitude in the brain in a systematic, albeit non-linear fashion (non-parametric ANOVA, sbj 1: $\chi_{(224,700)} = 396.2$, $p = 4.5\times10^{-69}$; sbj 2: $\chi_{(224,525)} = 164.3$, $p = 6.95\times10^{-23}$). The weakest field was found for the 0° / 360° stimulation condition (RMS $E_{sbj\ 1} = 0.37$ V/m; $E_{sbj\ 2} = 0.57$ V/m per 1 mA), and the strongest for the 180° condition (RMS $E_{sbj\ 1} = 0.74$ V/m; $E_{sbj\ 2} = 1.3$ V/m per 1 mA). Changes between the stimulation conditions following a sinusoidal curve ($R^2_{adj} = 0.99$). The 0° stimulation condition generates an "anti-phase" electric field with opposite phase angles at its anterior and posterior ends. The 180° stimulation condition leads to an "in-phase" electric field with a zero-degree phase difference along the arrays. Moreover, the intermediate conditions, such as 90° or 270°, create a mixed picture where the electric field changes its spatial configuration in a traveling wave like manner. Previous studies using "in-phase" or "out-of-phase" tACS are not in concordance with revealed biophysics of generated electric fields. Nevertheless, our measurements demonstrate the ability to create stimulation conditions with varying phase relationships. Further, we identify novel stimulation parameters leading to the traveling-wave like stimulation fields. Accompanying computational efforts will enable future multi-electrode tACS protocols based on validated biophysical principles.

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Poster

173. Physiological Methods: Electrophysiology: Neural Networks

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Program #/Poster #: 173.06/III64

Topic: I.04. Physiological Methods

Support: NIH Grant DP2 EY025446

Title: Navigating in neural and behavioral manifolds with closed-loop multi-site electrical microstimulation system

Authors: *S. TAFAZOLI[1], C. MACDOWELL[1], K. LETAI[1], D. CHE[1], T. BUSCHMAN[2]
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Abstract: Electrical microstimulation is often used as a tool for mapping sensory-motor functions and studying the neuronal basis of cognition. We have recently shown that multi-site microstimulation can produce a multi-dimensional neural response close to natural sensory stimulation. However, it is not clear if the relationship between dynamics of responses produced by microstimulation and sensory stimulation would allow control over brain dynamics. To address this, we built a novel large-scale, closed-loop stimulation system which can learn how to generate spatiotemporal patterns of activity across populations of neurons. This closed-loop system uses both stimulation and recording to learn to control the activity of populations of neurons. Stimulation and recording were performed with 64-channel silicon probes placed in primary visual cortex (V1) of awake head-fixed mice. Using 32 channels for recording and 32 channels for stimulation allowed us to simultaneously record from small populations of single neurons while patterning stimulation at nearby sites. To achieve a desired population firing pattern, the appropriate pattern of electrical microstimulation was then learned using a novel adaptive algorithm that minimizes the difference between the measured evoked neural response and the desired output. In this way, our closed-loop system could achieve sophisticated control over neural population firing patterns. In a subsequent work, we are using this system to bias perception of animal in a perceptual discrimination task. These findings suggest that closed-loop electrical stimulation system can achieve sophisticated control over neural population activity and provides a building block for probing complex neural dynamics as well as new generation of neural prosthetics.

Disclosures: S. Tafazoli: None. **C. MacDowell:** None. **K. Letai:** None. **D. Che:** None. **T. Buschman:** None.

Poster

173. Physiological Methods: Electrophysiology: Neural Networks

Location: SDCC Halls B-H

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Program #/Poster #: 173.07/III65

Topic: I.04. Physiological Methods

Support: NSFC Program (31700921)
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Shenzhen Governmental Research Grants (JSGG20160428140402911)

Title: Fabrication and modification of implantable optrode arrays for *in vivo* optogenetic applications

Authors: *C. ZHONG, L. WANG, Y. CAO, S. PAN, K. HUANG, L. WANG, Y. LU
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Abstract: Keywords: optogenetics, neural interface, neural electrodes
Recent advances in optogenetics have established a precisely timed and cell-specific methodology for understanding the functions of brain circuits and the mechanisms underlying neuropsychiatric disorders. However, the fabrication of optrodes, a key functional element in optogenetics, remains a great challenge. Here, we report reliable and efficient fabrication strategies for chronically implantable optrode arrays. To improve the performance of the fabricated optrode arrays, surfaces of the recording sites were modified using optimized electrochemical processes. We have also demonstrated the feasibility of using the fabricated optrode arrays to detect seizures in multiple brain regions and inhibit ictal propagation in vivo. Furthermore, the results of the histology study imply that the electrodeposition of composite conducting polymers notably alleviated the inflammatory response and improved neuronal survival at the implant/neural-tissue interface. In summary, we provide reliable and efficient strategies for the fabrication and modification of customized optrode arrays that can fulfill the requirements of in vivo optogenetic applications.
This work was partially sponsored by the NSFC Program (31700921) and the Shenzhen Governmental Research Grants (JSGG20160429184327274, JSGG20160428140402911).

Disclosures: C. Zhong: 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.; 31700921, JSGG20160429184327274, JSGG20160428140402911. **L. Wang:** None. **Y. Cao:** None. **S. Pan:** None. **K. Huang:** None. **L. Wang:** None. **Y. Lu:** None.

Poster

173. Physiological Methods: Electrophysiology: Neural Networks

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 173.08/III66

Topic: I.04. Physiological Methods

Support: LDRD 17-SI-002

Title: Recapitulating complex neuronal-glia networks in an *in vitro* system

Authors: *H. A. ENRIGHT, D. LAM, A. SALES, J. CADENA, J. OSBURN, S. PETERS, D. SOSCIA, K. KULP, E. WHEELER, N. O. FISCHER
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Abstract: Organ-on-a-chip systems are designed to recapitulate the microenvironment of human tissue and organ systems, and hold promise in evaluating new drugs, characterizing toxicants, and to aid in elucidation of disease mechanisms. Multi-electrode arrays (MEAs) are used to non-invasively interrogate neuronal health and function and are traditionally used to evaluate networks of primary neurons in vitro. While these cultures can provide key insights into neuronal response and function, they fail to recapitulate the cellular complexity in vivo as they lack supporting glia including astrocytes, microglia, and oligodendrocytes. In this work, we have developed a complex in vitro system which incorporates supporting glial cell types using primary rat cortical neurons and glia on a MEA device. Immunostaining was used to identify each cell type and its phenotypic state, evaluate cell morphology and network maturity. Electrophysiology was used to compare between complex and simple neuronal cultures over several weeks in vitro. Significant differences in electrophysiology were observed as neuronal complexity increased, including an earlier firing response and greater synchrony. Alignment of myelin basic protein with mature axons was observed in the complex culture system. These results suggest that complex neuronal/glial cultures may provide additional insight and relevance when evaluating the effects of new drugs and toxicants on primary neurons in vitro. This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344 through LDRD award 17-SI-002. (LLNL-ABS-750623)

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Poster

173. Physiological Methods: Electrophysiology: Neural Networks

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DARPA Contract HR0011-14-C-0102

Title: Enabling multimodal interrogation of the primate brain via a modular neural interface

Authors: *J. KLEINBART[1], A. L. ORSBORN[1], J. CHOI[1], C. WANG[2], S. QIAO[1], M. F. KHAZALI[1], B. FERRENTINO[1], J. VIVENTI[3], B. PESARAN[1]
[1]Ctr. for Neural Sci., New York Univ., New York, NY; [2]BME, Duke Univ., Durham, NC; [3]Electrical and Computer Engin., Duke Univ. Dept. of Electrical and Computer Engin., Durham, NC

Abstract: The macaque brain consists of billions of neurons that can be studied across multiple spatial scales using electrical and optical techniques for recording and stimulation. Applying different techniques to study the same population of neurons offers powerful capabilities for high-resolution circuit mapping and manipulation. Typical approaches to studying neural populations in the macaque brain involve neural interfaces that are designed for use with a single recording or manipulation modality. Repeated measurements from the same population with a different modality thus becomes impractical, as it requires repeated surgical interventions. To permit repeated measurements and manipulations of the same volume of cortical tissue in the macaque monkey using different experimental modalities, we developed a chronic multimodal chamber-based system. The surgically-implanted chronic portion of the interface, the 'base,' is low-profile to facilitate non-surgical hardware changes depending on the desired experimental modality. Magnetic resonance imaging (MRI) data is reconstructed into a three-dimensional model of the skull in order to custom-form the base to the skull. This maintains a low profile for the base and allows for the best possible cement seal. Subjects for whom MRI data is not readily available can be implanted with a generic low-profile base. The inner diameter of the base is 25mm and is designed to accommodate an artificial dura to allow for dura removal for better access to cortical tissues. At present, the implant supports untethered electrophysiological recording with a subdural microelectrocorticography (μECoG) array over an area of 254.47mm^2, untethered electrophysiological recording and stimulation with penetrating electrodes over an area of 251.71mm^2, awake wide-field imaging over an area of 153.94mm^2, and awake

optogenetic manipulation within an area of 254.47mm^2. Neural data using all of these modalities has been successfully acquired from two non-human primate subjects, with additional µECoG and penetrating electrode data sets acquired from one other subject. Each subject has been implanted with the chronic portion of the interface from several months to over a year with minimal necessary surgical intervention. This work demonstrates the feasibility of large-scale multimodal, multiscale investigations of the macaque brain and its utility for high-resolution circuit mapping and manipulation.

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Poster

173. Physiological Methods: Electrophysiology: Neural Networks

Location: SDCC Halls B-H

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Program #/Poster #: 173.10/III68

Topic: I.04. Physiological Methods

Title: High-throughput and high-speed data acquisition of compounds responses on calcium oscillation of human ipsc-derived cortical neurons with astrocyte

Authors: *J. LU[1], K. XU[2]
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Abstract: There is a surge of interest in high-throughput fluorescence measurement of calcium oscillations in neuronal cultures, both normal and disease models. However, progress has been limited by the long imaging acquisition time for an entire 96- or 384-well plate, and also by the heterogeneity of neurons derived from human iPSCs. With the advent of new camera technologies as well as availability of highly enriched, functionally mature human cortical neurons derived from iPSCs, these limitations can now be overcome. We utilized a newly developed fast data acquisition protocol (200 Hz for 96-well plate) on the FDSS/µCELL calcium imager to capture fluorescence measurements of calcium oscillations from human cortical neurons. These cortical glutamatergic neurons were seeded in recently developed rapid maturation medium in 96-well plates, loaded with the calcium indicator dye, and calcium dynamics were recorded. We evaluated cultures at different stages of development (6-21 days in vitro) and at different neuronal densities by monitoring calcium activity over 10-minute periods with high data acquisition speed. Several different culture media and measurement buffers were also evaluated to determine the optimal condition for producing neuronal oscillation. Finally, we applied a set of excitatory and inhibitory agonists and antagonists to study the contribution of voltage-gated ion channels and AMPA, NMDA and GABA receptors to neuronal oscillation.

Disclosures: J. Lu: None. **K. Xu:** None.

Poster

173. Physiological Methods: Electrophysiology: Neural Networks

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 173.11/JJJ1

Topic: I.04. Physiological Methods

Title: Brain signal acquisition with miniaturized electronic systems for the investiga-tion of local neural networks

Authors: *A. BAHR
Inst. of Electrical and Information Engin., Univ. of Kiel, Kiel, Germany

Abstract: In neuroscience research the development of the brain and the treatment of diseases like certain forms of epilepsy are analyzed with genetic mouse disease models. For the special case of the recording from neonatal mice (2-3 cm, 3-5 g) an implantable system has been developed, that enables chronic recordings. To achieve this, an application specific inte-grated circuit has been developed in an advanced 130 nm CMOS technology. Moreover, an implant and a recording system for live view of neural data have been presented. The functionality of the integrated circuit and the suitability of the implant system have been confirmed with in-vivo experiments with adult and 12 days old mice.
[Andreas Bahr, Aufnahme von Hirnsignalen mit extrem miniaturisierten elektronischen Systemen zur Untersuchung von lokalen neuronalen Vernetzungen, Buch, erschienen im Logos Verlag Berlin GmbH, Serie "Wissenschaftliche Beiträge zur Medizinelektronik, Bd. 6", 2017, Herausgeber Wolfgang Krautschneider]

Disclosures: A. Bahr: None.

Poster

173. Physiological Methods: Electrophysiology: Neural Networks

Location: SDCC Halls B-H

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Program #/Poster #: 173.12/JJJ2

Topic: I.04. Physiological Methods

Title: Magnetic platform for modulating and monitoring neuronal networks

Authors: *O. SHEFI[1], M. MARCUS[2], N. VARDI[3], H. SCHORI[2], A. SHARONI[4]
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Abstract: Stable and specific neuron-electrode correspondence is necessary for monitoring individual neuron activity in a neuronal network for an extended time. However, cultured neurons on flat substrates tend to migrate over time, thus the common used Multi Electrode Arrays (MEAs) lack the one-to-one neuron-to-electrode specificity. Attempts to overcome this limitation include chemical and physical approaches. In our study we designed a magnet-based platform for modulating neuronal networks formation and monitoring electrical activity. We developed a unique electrode array with dual function: (i) localization and immobilization of cells at electrode sites, (ii) efficient recording of neuronal electrical activity. Our device combines magnetic and conductive properties in order to direct cells towards electrodes sites and record individual neuron activities for an extended time. We use magnetic nanoparticles as mediators to transform cells into magnetic sensitive units. By incorporating MNPs within cells, the magnetic complex has the potential to be remotely-guided to specific sites in response to external magnetic field gradients. We studied nanoparticle uptake by cells and evaluated their magnetic properties. We fabricated the multi-functional device embedded with micro-sized electrodes. By applying controlled external magnetic forces we located cells at electrode sites and achieved stable and specific neuron-electrode correspondence, enabling to monitor individual neurons electrical activity for long term. Our unique electrode array device enables magnetic manipulations of neuronal motility together with monitoring neuronal growth pattern and activity. Using this interface opens new possibilities for studying neuronal network organization and maturation.

Disclosures: O. Shefi: None. **M. Marcus:** None. **N. Vardi:** None. **H. Schori:** None. **A. Sharoni:** None.

Poster

173. Physiological Methods: Electrophysiology: Neural Networks

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 173.13/JJJ3

Topic: I.04. Physiological Methods

Title: Seizure Detection based on “imaged-EEG” signals through statistical learning

Authors: *C. GOMEZ[1], P. A. ARBELÁEZ[2], M. VALDERRAMA[3]
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Abstract: The diagnosis of many brain diseases relies on the tools that have been designed to measure biological signals. For instance, monitoring brain activity through the electroencephalogram (EEG) is one of the standard methods used to diagnose epilepsy. Patients with epilepsy are prone to experience epileptic seizures, which have physical manifestations and specific patterns on the EEG activity. Due to the spontaneous nature of these episodes, long-lasting recordings must be taken, making seizure detection a time consuming and subjective task. We developed an automatic method for seizure detection using EEG signals by changing from signals domain to image domain producing an "imaged-EEG" representation of the signals. We proposed to apply high-level methods used in Computer Vision object recognition task to classify brain activity into two classes: ictal and interictal states.
To accomplish this task, we analyzed scalp EEG recordings from 24 pediatric patients belonging to the public CHB-MIT Scalp EEG Database. The dataset consists in several hours of recording in the standard 10-20 EEG system. In total, we used more than 600 hours containing 141 annotated seizures. To change from signals to images domain, we took the raw amplitude of the EEG signals as the pixel attribute and divided the recordings into four-second windows to generate ictal and interictal instances. We used Fully Convolutional Neural Networks (FCN) to make predictions of variable-length recordings. The data from both classes was distributed into two cross-patient models: 3 fold Cross-Validation (3FCV) and Leave-one-out (LOO), in which we train one model per patient. We explored different FCN architectures and image generation in the 3FCV model and applied the best combination in the LOO model. We evaluated the performance of the algorithms using the standard metrics of detection problems: precision, recall, F-measure and Average Precision (AP). Our method in the LOO model achieves an average precision of 44.2%, recall of 46.3%, F-measure of 40.2% and AP of 34.1% when evaluating in all the recordings (n=642). Even though, hard patients decreased the overall performance, we obtained satisfactory results for patients with a detection precision over 90% and recall over 70% (n=196 recordings). Additionally, we performed a qualitative comparison between the predicted seizures and the annotated ones, and found overlapping regions between them when high precision and recall were reported. Using our experimental setup, we validated the models using "imaged-EEG" signals as inputs to distinguish between ictal and interictal activities.

Disclosures: C. Gomez: None. **P.A. Arbeláez:** None. **M. Valderrama:** None.

Poster

173. Physiological Methods: Electrophysiology: Neural Networks

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 173.14/JJJ4

Topic: I.04. Physiological Methods

Title: Virtual reality embodied therapy technique for increasing emotional processing

Authors: *C. KROGMEIER
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Abstract: Cognitive behavioral therapy via Virtual Reality has been proven effective for a variety of patients across multiple trainings such as increased social cognition in those with high-functioning autism and increased empathy/fear-detection of victims in domestic violence offenders. Individuals with schizophrenia suffer from a plethora of symptoms that are often resistant to the antipsychotic treatments that alleviate positive symptoms such as hallucinations. In recent years body psychotherapy, using body movement in order to treat disorders of thought and emotion, has been proven effective in reducing negative symptoms of schizophrenia such as anhedonia and inabilities in processing emotion. Previous studies have shown schizophrenic subjects experienced a 20% mean reduction in negative symptoms through sessions of body psychotherapy (Martin, Koch, Hirjak & Fuchs, 2016). While antipsychotics can be effective in alleviating positive symptoms of schizophrenia, they do little to tackle negative symptoms, eliminate side effects or treat the patient long-term. Although a schizophrenic individual is described by the DSM-5 as exhibiting a decreased emotional range, little research into virtual reality body psychotherapy development for the exploration of increased emotional processing exists currently. While a variety of pathologies such as autism, depression, narcissistic personality disorder as well as schizophrenia involve emotional processing deficits, and because virtual reality cognitive behavioral therapy has been proven effective for alleviating symptoms of various disorders, it is feasible to design a virtual reality body psychotherapy paradigm; focusing on the immersive properties of virtual reality in order to foster an embodied perceptual reorganization for the purpose of increasing emotional processing abilities in schizophrenic and other individuals long-term, with fewer to none of the side effects of antipsychotics among other drugs used for treatments. In this technique development, a virtual reality body psychotherapy system will be programmed and designed in Unreal Engine for the purposes of embodying the patient in a world that uses his or her movements in order to increase emotional processing abilities. Experimental design with this technique will involve allowing for EEG recordings, specifically in the temporoparietal junction, in order to evaluate changes in TPJ activation during VR body psychotherapy in schizophrenic patients, as well as to allow for the exploration of brain waves during this therapy, and to investigate potential treatment directions that result from improved brain wave understanding.

Disclosures: C. Krogmeier: None.

Poster

174. Physiological Methods: Electrophysiology: Stimulating Neurons

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 174.01/JJJ5

Topic: I.04. Physiological Methods

Support: JSPS KAKENHI 17J02997

Title: Inter-day repeatability of spinal reflexes evoked by transcutaneous spinal cord stimulation

Authors: *A. SAITO[1,2], Y. MASUGI[3], K. NAKAGAWA[1,2], H. OBATA[4], K. NAKAZAWA[1]
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Abstract: Transcutaneous spinal cord stimulation (tSCS) is a relatively new technique that can evoke spinal reflexes in multiple lower-limb muscles simultaneously, yet its repeatability remains unknown. The purpose of this study was to examine inter-day repeatability of recruitment properties of the spinal reflexes evoked by tSCS, and to compare their properties to the Hoffmann-reflex (H-reflex), which is considered to be "gold standard" for evaluating monosynaptic spinal reflex. As a reference of the spinal reflex, the recruitment curve of H-reflex in the soleus was measured in two consecutive days (n = 9). In addition, recruitment curve of spinal reflexes of 8 muscles in the lower-limbs (i.e., foot, shank, and thigh muscles) were evoked by tSCS in two consecutive days (n =20). To confirm that responses were caused by activation of the sensory fiber, a double-pulse stimulation with 50 ms inter-pulse interval was delivered. Peak-to-peak amplitude of the first response was calculated for each muscle when no response was observed in the second response. Threshold intensity of the responses evoked by H-reflex and tSCS was defined as the minimum stimulation intensity that produced the responses, and maximal slope of the responses was determined by fitting six-order polynomial function to the recruitment curve. Inter-day repeatability of the recruitment parameters was quantified using intra-class correlation coefficients (ICC). Regarding the soleus H-reflex, ICCs of threshold intensity and maximal slope were 0.936 and 0.751, respectively. Using tSCS, ICCs of threshold intensity and maximal slope of the soleus were 0.702 and 0.964, respectively. ICCs of threshold intensity evoked by tSCS for each muscle ranged between 0.487 and 0.874, and ICCs of maximal slope ranged between 0.474 and 0.964. These results suggest moderate to high repeatability of the parameters of the recruitment curve of spinal reflexes in lower-limbs, as well as H-reflex. Therefore, spinal reflexes evoked by tSCS could be useful in longitudinal neurophysiological studies.

Disclosures: A. Saito: None. **Y. Masugi:** None. **K. Nakagawa:** None. **H. Obata:** None. **K. Nakazawa:** None.

Poster

174. Physiological Methods: Electrophysiology: Stimulating Neurons

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 174.02/JJJ6

Topic: I.04. Physiological Methods

Title: Regulation of viral vector gene expression in the hippocampus by medial septal nucleus deep brain stimulation

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Abstract: Introduction: Deep brain stimulation (DBS) is a well-established treatment for several neurological diseases; however, the mechanisms by which DBS exerts its effects remain controversial. Electrical stimulation has clear effects on nearby cells but can also affect distant neuronal populations within the same circuitry. This proof-of-principle study aims to examine whether DBS can regulate gene expression from a virally delivered vector by first transducing neurons with an adeno-associated virus containing the gene for a fluorescent reporter protein downstream of a DBS-responsive promoter and then stimulating with DBS from a remote target. Methods: Adult Sprague-Dawley rats underwent a unilateral right hippocampal injection of adeno-associated virus serotype 5 containing a DNA construct for green fluorescent protein (GFP) under control of the chicken beta actin promoter and TdTomato (TdT) under control of the c-fos promoter (AAV5-c-fos). Two weeks later, a stimulating depth electrode was implanted into the medial septal nucleus (MSN). In the first experiment, 1 week after implantation, rodents received no DBS, 7.7 Hz (theta frequency) DBS, or 130 Hz (gamma frequency) DBS for 1 hour, and tissue analysis was performed 1.5 hours after the conclusion of stimulation. In the second experiment, rodents received DBS treatment 1 week after implantation followed by another treatment 1 week after the initial treatment with no DBS, 7.7 Hz DBS, or 130 Hz DBS. Fluorescent protein expression levels were analyzed with confocal microscopy to evaluate for spatio-temporal control of the reporter's expression in the hippocampus. Results: In the first experiment, we found that, two weeks after AAV5-c-fos injection, either 1 hour of continuous 7.7 Hz, or 130 Hz MSN stimulation resulted in an increase in TdT reporter expression levels relative to no stimulation. Results from the second experiment demonstrated that TdT levels were no longer increased 1 week later with no additional stimulation but TdT expression could be induced again with repeat 7.7 Hz or 130 Hz MSN stimulation. Conclusions: Here, we demonstrate that viral vector-mediated gene expression can be induced using both low- and high-frequency DBS to a distal target along a circuit. Taken together these data suggest that DBS can regulate gene expression both spatially and temporally. Successful control of gene expression by DBS will warrant further investigation into stimulation responsive promoters for use in clinical applications.

Disclosures: D.J. Lee: None. **C. McKinnon:** None. **M. De Snoo:** None. **A. Fomenko:** None. **E. Lee:** None. **V. Agapova:** None. **S. Ngana:** None. **C. Hamani:** None. **A. Lozano:** None. **L.V. Kalia:** None. **S.K. Kalia:** None.

Poster

174. Physiological Methods: Electrophysiology: Stimulating Neurons

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 174.03/JJJ7

Topic: I.04. Physiological Methods

Support: C. J. Gauthier Program for Exploratory Studies

Title: Heat transport from stimulated activity in neurons studied through transient intracellular temperature measurements

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Abstract: For the central nervous system (CNS), a 2 ºC temperature change from physiological baseline can impede cognitive functions such as memory encoding, attention, and information processing [1]. The apparent sensitivity of CNS to temperature can be traced to the reactions at the cellular level. For a single neuron, a 1 ºC temperature change can change its firing rate by 0.33 Hz and membrane potential by 0.53 mV. The fundamental coupling of heat transport in neuronal activities remains widely disputed [2]. To this end, we study simultaneous measurements of temperature and electrical activity of neurons at the intracellular level using a custom-designed, micro-fabricated thermocouple probe that is accurate to ± 54 mK at 300 ± 10 K. We use proton uncouplers to induce thermogenesis at the mitochondria in neurons from the sea slug *Aplysia californica.* Previous studies of mitochondrial thermogenesis report a steady 2-5 K increase that exceeds analytically predicted available energy by a factor of 10^2-10^3[3]. In our study, we perform systematic control experiments to identify the baseline temperature signals from heat of mixing, extracellular bulk heating, and other convection effects. By isolating extraneous signals, we measure transient intracellular temperature responses in the range of 2-6 K over ~20 s that are close to the predicted available energy in *Aplysia* neurons. Using the measurements of mitochondrial thermogenesis as a benchmark for our intracellular thermometry technique, we then study the temperature response arising from evoked electrical activity in the neurons. We electrically stimulate the neurons to generate spikes at or exceeding the frequencies at which they endogenously or synaptically generate. By capturing the temperature response over one to few action potentials, we identify transient heat generation and absorption cycles. Previous micro-calorimetric studies that isolated neurons to study thermal response from action potentials reported reversible heat release [2]. However, through our *in vitro* measurements that provide a natural milieu to the neurons, we observe predominant heat release over few action potentials. Our measurements of transient temperature changes during neuronal firing activity

pave the way to understanding heat transport in neurons. Through concurrent measurements of intrinsic and synaptic conductances with thermal activity, more insights into coupled heat and mass transport mechanisms can be elucidated.
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[2] Nag, K. ed., 2008. Structure and Dynamics of Membranous Interfaces. Hoboken, NJ: Wiley.
[3] Baffou, Guillaume, et al. Nature methods 2014.

Disclosures: M. C. Rajagopal: None. **J.W. Brown:** None. **K.V. Valavala:** None. **D. Gelda:** None. **D. Llano:** None. **R. Gillette:** None. **S. Sinha:** None.

Poster

174. Physiological Methods: Electrophysiology: Stimulating Neurons

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 174.04/JJJ8

Topic: I.04. Physiological Methods

Title: Characterization of goat's retinal ganglion cells firing patterns evoked by pulses of different wavelengths and widths through the evaluation of inherent features for stimulus-response mapping between light and electrical stimulation parameters

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Abstract: Although outer retinal diseases cause a progressive damage of photoreceptors, artificial stimulation can be exploited as an attempt to restore vision by exciting surviving retinal cells. The development of effective prosthesis requires the characterization of retinal ganglion cells (RGC) response to the external stimulus. Here, we investigate a method of RGC excitation through light pulses, for generating precise temporal patterns of spikes reproducing those normally evoked in healthy vision. The responses to optical stimulus could further be used for mapping those due to electrical stimulation, on which the existing devices are being tested in clinical trials.
The retina isolated from eyeballs of a healthy goat, collected within few minutes after decapitation, cut into approximately 2 mm x 2 mm square samples and covered with AMES media, was placed on a multi Cr/Au micro electrodes array (200 μm diameter), fabricated on polydimethylsiloxane (PDMS) substrate. In a dark room inside the lab, light stimulation was performed with red, green, blue and white pulses, characterized by 1, 5, 10, 15 and 20 ms width and repeated 10 times, programmed through a microcontroller. Spikes were recorded from 15 channels with auto-thresholding by Grapevine Scout processor (Ripple LLC, USA).
Spike sorting, based on principal component analysis, distinguished 5 waveforms suggesting 5

types of neurons. Shapiro-Wilk test rejected normality of data-sets. To characterize neuronal response to a precise stimulus, 3 inherent features were evaluated: mean firing rate (FR), mean interspike interval (ISI), with the related coefficient of variation (CV), and first spike latency. Among the waveforms, one type was always firing regardless of pulse width and wavelength, with a FR ranging between 66.5 and 72.5 Hz. Since these values did not significantly differ between colours and width, as confirmed by Kruskal-Wallis test, it was assumed as the spontaneous neuronal activity. FR of 2 other waveforms were statistically different for all the wavelengths. For blue light pulses, FR was 26.6 and 34.8 Hz, followed by white, green and red. FR of red, 5.4 and 13.5 Hz, can be considered negligible. This assumption is consistent with the dichromatic vision of goats, that have only S-cones (maximum absorbance at 443.3 nm) and L-cones (552.5 nm). Significant variations of ISI and latency for 2 waveforms were observed by changing the pulse width. The ISI and the related CV were lower for spontaneous activity than for specific neuronal response. The average latency ranged from 0.011 to 0.034 s. These features could then be used for mapping of the responses between light and electrical stimulation parameters.

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Poster

174. Physiological Methods: Electrophysiology: Stimulating Neurons

Location: SDCC Halls B-H

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Program #/Poster #: 174.05/JJJ9

Topic: I.04. Physiological Methods

Support: ERC Advanced Grant 2015 - 694829 “neuroXscales”

Title: Single-neuron sub-cellular-resolution electrical stimulation with high-density microelectrode arrays

Authors: *S. RONCHI, M. FISCELLA, C. MARCHETTI, J. MUELLER, V. VISWAM, U. FREY, A. HIERLEMANN
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Abstract: Non-invasive electrical stimulation is a consolidated technique to study and control neural activity in the brain and peripheral nervous system. It is used, e.g., for controlling Parkinson's disease or to induce sensation in paralyzed patients (Armenta Salas et al., 2018), as well as for attempts to restore vision (Fan et al., 2018; Grosberg et al., 2017) and hearing (Wilson & Dorman, 2008). A common requirement in electrical stimulation is the precise and controlled stimulation of individual targeted neurons. For achieving this purpose, it is necessary that electrodes can stimulate and record extracellular signals at sub-cellular resolution.

Furthermore, it is important to design efficient stimulation pulses to be delivered to the neurons. In the present work we used an CMOS-based high-density microelectrode array (HD-MEA) (Ballini et al., 2014), featuring 26'400 bidirectional electrodes with a pitch of 17.5 µm, which was designed for in-vitro applications. This high-resolution was used to test electrical stimulation parameters in vitro, which then could potentially be adapted to elicit single-cell action potentials *in vivo*. In this work we used different stimulation parameters, such as waveforms, amplitudes and durations (Grosberg et al., 2017; Wagenaar, Pine, & Potter, 2004), using 5x9 µm² electrodes to target sub-cellular structures in single neurons. E-18 Wistar rat cortical neurons were stimulated at days-in-vitro 10, 15, 20 and 25 using randomized voltage and current stimulation modalities. Axon initial segments of individual neurons (Radivojevic et al., 2016) were targeted for stimulation, enabled by the HD-MEA device. We found that voltage biphasic anodic-cathodic waveforms were less efficient than biphasic cathodic-anodic waveforms in eliciting action potentials in a single neuron. Moreover, it was possible to detect action potentials directly at the cell soma, which ensured a reliable confirmation of successful neuron stimulation. Finally, HD-MEA technology enabled to elicit action potentials in single-neurons embedded in high-density cell cultures. The obtained results can be used to optimize *in vivo* single-cell targeting for stimulation and read-out.

Disclosures: S. Ronchi: None. **M. Fiscella:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MaxWell Biosystems AG. **C. Marchetti:** None. **J. Mueller:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MaxWell Biosystems AG. **V. Viswam:** None. **U. Frey:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MaxWell Biosystems AG. **A. Hierlemann:** None.

Poster

174. Physiological Methods: Electrophysiology: Stimulating Neurons

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 174.06/JJJ10

Topic: I.04. Physiological Methods

Support: NIH Grant R01GM104948
UK Dementia Research Institute
UK National Institute for Health Research
Wellcome Trust

Title: A method for automated, long-term neural stimulation with temporally-interfering electric fields in freely-moving mice

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Abstract: Deep brain stimulation (DBS) has been used as a therapy for various neurological and psychiatric conditions, such as depression, obsessive-compulsive disorder, schizophrenia, epilepsy, and chronic pain, among others. DBS is an effective, but invasive, technique that requires surgical implantation of the electrodes. A non-invasive alternatives to DBS is transcranial magnetic stimulation (tMS), but has the disadvantage of requiring large size equipment to be performed. A more feasible alternative is transcranial electrical stimulation (tES), which can either be driven by direct or alternating currents. Recently, a new method has been proposed that uses temporally-interfering (TI) alternating currents to stimulate a well-localized target area without affecting the surrounding tissue, which has not been possible with standard techniques, using either direct or alternating currents. However, both TI and tES have required the return electrodes to be placed outside of the skull to achieve an effect in deeper brain structures. Until now, TI has been tested in anesthetized mice by placing the electrodes on the skin of the cheeks or chest, while tES have been tested in awake rats by implanting metal plates below the skin of the chest. Here, we describe how to use lightweight, non-metallic conductive materials as non-implanted return electrodes to extend the use of TI to freely-moving mice. We avoid the need to implant metallic return electrodes, which can produce charge build-up that results in tissue damage during long-term stimulation protocols. Furthermore, we combined this setup with chronic neural recordings and automated delivery of stimulation schedules, which allows for long-term testing of different stimulation parameters without human intervention. We also incorporated electromyography and movement detection to assess possible effects of TI on muscle tone and overall behavioral activity. Our method will prove useful in the assessment of TI safety and efficacy in non-anesthetized rodents, which is fundamental for the translational applications of the technique.

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Poster

174. Physiological Methods: Electrophysiology: Stimulating Neurons

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 174.07/JJJ11

Topic: I.04. Physiological Methods

Support: Taiwan Ministry of Science and Technology grant

Title: Defensive effect of deep brain stimulation on hippocampus apoptosis in the rat

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Abstract: Objective: To evaluate the neuro-protective properties of hippocampus in a rodent model, pedunculopontine tegmental nucleus (PPTg) deep brain stimulation (DBS) was used as a potential tool. **Methods:** PPTg DBS (50 Hz, 2.5 V, and 500 μs) was applied on rats for 1 h. Caspase-3 activity, myelin basic protein (MBP), Bcl-2, and BAX levels, lipid peroxidation (LPO), interleukin (IL)-6 levels, and brain-derived neurotrophic factor (BDNF) levels were evaluated at 1h, 3h, and 6h. **Results:** PPTg DBS significantly decreased caspase-3 activity at 6h and MBP at 1h, 3h, and 6h. PPTg DBS increased Bcl-2 expressions and inhibited BAX expressions at 6h. Furthermore, the ratio of Bcl-2/BAX was also increased. IL-6 and LPO levels were not affected by PPTg DBS. PPTg DBS significantly increased the nitric oxide levels and the BDNF expressions at 6h. **Conclusion:** PPTg DBS reveals the protective effects on hippocampus apoptosis in rodent model. BDNF may get partly involved in the regulation of Bcl-2 and BAX expression under PPTg DBS. **Significance:** The effects of PPTg DBS on hippocampus have never been investigated in rodent models, DBS were mainly used in the management of Alzheimer disease (AD) and Parkinson's disease (PD). Nevertheless, this novel study will pave a way for better understanding for further research in the field of neuro-motor disorders.

Disclosures: C. Peng: None. **S. Chen:** None.

Poster

174. Physiological Methods: Electrophysiology: Stimulating Neurons

Location: SDCC Halls B-H

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Program #/Poster #: 174.08/JJJ12

Topic: I.04. Physiological Methods

Support: Swiss National Science Foundation grant 320030_146789

Title: No effect of transcranial direct current stimulation (tDCS) on auditory-evoked potentials

Authors: *K. KUNZELMANN, L. MEIER, Y. MORISHIMA, T. DIERKS
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Abstract: Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation technique to modulate cortical excitability. Various investigations have shown tDCS to modify behavior as well as symptom severity in psychiatric disorders. Despite growing popularity, underlying neural mechanisms causing behavioral effects are still hardly understood. Additionally, results in recent investigations of tDCS effects on auditory processing, i.e. on auditory-evoked potentials (AEP), were inconsistent. In these recent investigations, sample sizes were small and reported results restricted to effects after stimulation. Thus, we aimed to investigate the effects of tDCS on auditory processing in more detail by including a larger population compared to earlier publications and by comparing effects of stimulation before, during, and after tDCS application. We included 24 healthy subjects in a crossover design to receive anodal or sham tDCS in two sessions with one week apart. During an AEP paradigm, we applied 20 min of tDCS on the primary auditory cortex. Results in earlier studies suggest an increase of P50 amplitude after anodal compared to sham tDCS as well as a shortening of N100 latency. In the current study, measures of amplitude and latency for P50, N100, and P200 were compared between three time points (pre, during, after stimulation) and for two types of stimulation (anodal, sham). Our results showed no difference in AEP for anodal compared to sham stimulation and no difference in AEP after stimulation compared to baseline. Hence, we were not able to replicate earlier results even when investigating the expected effects of tDCS in a larger cohort and with a longer duration of stimulation. Our results suggest that tDCS fails to substantially modify basic processing in the auditory cortex. Further evidence for the actual effectiveness of tDCS on auditory processing and its underlying mechanisms is warranted.

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Poster

174. Physiological Methods: Electrophysiology: Stimulating Neurons

Location: SDCC Halls B-H

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Program #/Poster #: 174.09/JJJ13

Topic: I.04. Physiological Methods

Support: KU Leuven Research Funding: STG/14/024 and EGM-D2929-C24/17/091
EIT Health Innovation by Ideas, NEURO-WEAR Project

Boateng Asamoah is SB PhD fellow at FWO

Title: Resolving the tACS paradox: If the electric field in the brain is too weak to cause neural entrainment, how can the observed behavioural effects be explained?

Authors: *B. ASAMOAH, A. KHATOUN, M. MC LAUGHLIN
Neurosci., KU Leuven, Leuven, Belgium

Abstract: Rationale
Transcranial alternating current stimulation (tACS) is a neuromodulation method where electrodes are placed on the scalp. A sinusoidal current passes through the scalp, skull and CSF before a weak electric field reaches the brain. It is implicitly assumed that this weak field modulates the membrane potential and affects spike timing. This is thought to be the underlying mechanism driving the behavioural effects in many human studies. Importantly, recent studies have challenged the assumed underlying mechanism by showing that the electric fields generated in the human brain may be too weak to modulate neurons and drive behavioural effects. Thus there is a paradox: on one hand tACS has been shown to cause behavioural effects in humans but on the other hand the electric field reaching the brain is not strong enough to cause neural entrainment.
Objective
We propose a hypothesis that may resolve this paradox: the relatively strong current in the scalp stimulates peripheral nerves which give input to the brain and cause neural entrainment. We tested this hypothesis in a rat model by separating the two possible underlying mechanisms – namely transcranial and transcutaneous.
Methods
In 7 anaesthetised male Wistar rats we isolated the transcranial mechanism by opening the scalp and directly stimulating on the skull while extracellularly recording single neuron activity in the motor cortex. We then isolated the transcutaneous mechanism by stimulating the skin of a limb (away from the head) while recording from the same neurons. In both cases, we applied the same stimulation protocol: 1 minute of NO-stimulation followed by 1 minute of sinewave stimulation followed by another 1 minute of NO-stimulation.
Results
We confirm that transcranial stimulation can entrain neurons in the rat motor cortex. Importantly, we also find that transcutaneous stimulation entrains the same neurons in a very similar way to transcranial stimulation. Preliminary analysis indicated that the transcranial and transcutaneous stimulation mechanisms also have similar temporal dynamics.
Conclusions
We found that both transcranial and transcutaneous stimulation entrain neurons in the rat motor cortex. Our results may help resolving the tACS paradox: Stimulation of peripheral nerves in the scalp which then give input to the brain could be driving some of the effects observed in humans. Further experiments in humans are needed to separate these competing mechanisms.

Disclosures: B. Asamoah: None. **A. Khatoun:** None. **M. Mc Laughlin:** None.

Poster

174. Physiological Methods: Electrophysiology: Stimulating Neurons

Location: SDCC Halls B-H

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Program #/Poster #: 174.10/JJJ14

Topic: I.04. Physiological Methods

Support: NIH Grant R21MH107671
NSF Grant 1631329

Title: A user-friendly, universal, robust algorithm for real-time detection and phase prediction of oscillatory episodes in the LFP for closed-loop phase-locked stimulation

Authors: *C. M. RODRIGUEZ RIVERO, SR[1], J. DITTERICH[2]
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Abstract: Synchronized oscillatory activity has been proposed to play important roles in modulating functional connectivity between populations of neurons and in memory storage/retrieval processes. Testing these hypotheses requires techniques that allow manipulation of the timing relationship between neural activity in different brain areas. Despite the existence of several technique papers on closed-loop phase-locked stimulation, hardly any experimental papers using these techniques have been published. We believe that this is due to the proposed algorithms not being particularly user-friendly and due to the non-stationarities in LFP signals of behaving animals, which pose considerable challenges for the detection and prediction of oscillatory activity. Here, we use a collection of real LFP signals that have been recorded from a variety of different species and different brain areas as well as a collection of artificial LFP signals with known properties to assess the detection and prediction performance of several algorithms under a variety of conditions. Based on the assessment of the weaknesses and strengths of these algorithms when dealing with oscillatory episodes over a wide range of frequencies, signal-to-noise ratios, episode durations, and non-stationarities in the oscillation frequency, we propose a strategy for a universal, user-friendly algorithm that can perform well over a wide range of realistic conditions. The user specifies the frequency range of interest within which oscillatory episodes are to be tracked, but frequency-specific detection thresholds are determined automatically and filter parameters are adjusted automatically based on the short-time LFP power spectrum. Estimates of instantaneous frequency and instantaneous phase are used for phase extrapolation.

Disclosures: C.M. Rodriguez Rivero: None. **J. Ditterich:** None.

Poster

174. Physiological Methods: Electrophysiology: Stimulating Neurons

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 174.11/JJJ15

Topic: I.04. Physiological Methods

Support: SC INBRE Grant

Title: Effects of high definition transcranial direct current stimulation on inhibitory control

Authors: *M. T. RODRIGUEZ[1], A. E. ROACH[2]
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Abstract: High definition transcranial direct current stimulation (HD-tDCS) is a targeted method of non-invasive brain stimulation that has been shown to enhance synaptic plasticity. Approximately 20 minutes of 1 to 2 mA of stimulation can improve certain cognitive processes, including executive functions such as response inhibition. We aimed to replicate the findings of Hogeveen et al., (2016) who applied anodal HD-tDCS to the right inferior frontal cortex to enhance response inhibition during a stop signal task (SST). In a within subjects design, participants were counterbalanced to receive 20 minutes of 1 mA stimulation on two separate occasions; once while simultaneously completing a control task, and once while simultaneously completing the SST. The electrode montage consisted of an anodal electrode over FC6, with surrounding return electrodes at F2, F10, CP2, and TP8. Both sessions consisted of participants completing the SST prior to the HD-tDCS (pretest) and after the HD-tDCS (posttest), with the only difference between the two sessions being whether they practiced the SST during stimulation or practiced a control task that did not require stopping a manual response during stimulation. Hogeveen et al. found a significant improvement in the posttest stop signal reaction time (SSRT), the time required by each participant to inhibit their planned response, when participants received stimulation while completing the SST, but did not show the same improvement in posttest SSRT when stimulation was during the control task. The SSRT is calculated by subtracting the average stop signal delay from the average reaction time on correct "Go" trials. The difference in SSRT between pretest and posttest is called the gain score. We subjected gain scores for each of the two sessions to a paired samples t-test which yielded significant results: $t(19) = -2.467$, $p = .023$, indicating a significant improvement in response inhibition when HD-tDCS is combined with SST training (Cohen's $d = -.552$). Our findings replicate the results of Hogeveen et al., providing additional evidence that 20 minutes of 1 mA stimulation, when combined with practicing the SST response inhibition paradigm, significantly improves subsequent performance on the SST when posttest immediately follows the stimulation session. These results bolster the findings of Hogeveen et al., and provide further evidence of the efficacy of HD-tDCS in promoting neural plasticity.

Disclosures: M.T. Rodriguez: None. **A.E. Roach:** None.

Poster

174. Physiological Methods: Electrophysiology: Stimulating Neurons

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Topic: I.04. Physiological Methods

Support: Research supported by European Commission, ICT-FET FP7/2007-2013, FET Young Explorers, grant agreement N° 284772 BRAIN BOW (www.brainbowproject.eu).

Title: A closed-loop architecture to allow hybrid bi-directional interaction between neuronal assemblies *in vitro*

Authors: *S. BUCCELLI[1,2], I. COLOMBI[1,2], Y. BORNAT[3], V. PASQUALE[1], L. MARTINES[2], F. DIFATO[1], A. AVERNA[1], J. TESSADORI[1], M. TEDESCO[2], P. MASSOBRIO[2], T. LEVI[3], M. CHIAPPALONE[1]
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Abstract: The motor and cognitive deficits following a brain injury represent a major challenge for the entire society. Recent improvements in the field of bioelectronic medicine are creating new opportunities to deliver an electric therapy with possibly less side effects than standard pharmacological approaches. To test scientific and technological questions related to the design and implementation of new stimulation strategies in vitro models of brain lesions can be adopted. Here we realized a prototype of a neuroprosthetic device able to interact with dissociated cortical neurons from rats plated over a 60-channel Micro-Electrode Array (MEA). Neurons were plated according to a bimodular layout by means of custom-made PDMS masks, which allowed to create two interconnected sub-populations of cells. During the in vitro development, the biological connections between the two neuronal assemblies gave rise to several, almost synchronous, network events called network bursts (NB). During experiments, we performed a laser ablation to resect the biological connections between the two neuronal sub-populations, resembling an experimental model of focal lesion. The main effect of the lesion was related to the correlation between the spiking activity of the two modules. Before lesion, the correlation was high and stable, indicating a functional communication, but following the injury it collapsed to zero, thus proving both an anatomical and a functional disconnection. In order to solve this problem, we envisaged a reconnection strategy through our neuroprosthetic device. Such a device consisted of a Field Programmable Gate Array (FPGA) board, able to perform a real-time NB detection and trigger an activity dependent stimulation from one module to the other and vice versa (Bi-directional Bridging, BB). Through the BB strategy, we were able to partially recover the cross-correlation features without imposing any preferred direction. A different

strategy, called Hybrid Bidirectional Bridging (HBB), was applied when we aimed at replacing one entire module by means of a Spiking Neural Network (SNN). We used NB events as the main feature to control the stimulation and we obtained a partial recovery of the cross-correlation without affecting the firing of the survived module. In conclusion, the developed neuroprosthetic prototype can be seen as a starting point towards a new stimulation paradigm aimed at restoring neuronal functionality following a brain injury.

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Poster

174. Physiological Methods: Electrophysiology: Stimulating Neurons

Location: SDCC Halls B-H

Time: Sunday, November 4, 2018, 8:00 AM - 12:00 PM

Program #/Poster #: 174.13/JJJ17

Topic: I.04. Physiological Methods

Support: National Institute of Health Grant R01MH111871

Title: Large-scale modulation of spontaneous neural activity in sensorimotor cortex using somatosensory electrical stimulation

Authors: *A. K. HISHINUMA[1,2], T. GULATI[1], M. BURISH[1], K. GANGULY[1,2]
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Abstract: Background: Somatosensory Electrical Stimulation (SES) of the forelimb area has been shown to induce reorganization of the sensorimotor cortex, which may improve motor ability and functional outcomes in injuries such as stroke. However, studies to understand mechanisms of action were primarily done in humans using noninvasive, lower resolution recordings. In order to better understand the underlying neural mechanisms behind SES, it is important to understand how it directly modulates cortical activity. Here, using a rodent model, we investigated how SES modulates neural activity in the contralateral primary motor cortex (M1). Methods: We performed acute extracellular recordings in 7 intact adult Long Evans rats under ketamine-xylazine anesthesia while they received transcutaneous electrical nerve stimulation of the ulnar and radial nerve for one hour. We recorded single unit spiking and local field potentials (LFP) in the contralateral M1 of the stimulated arm, and compared changes in neural firing rate, spike-field coherence (SFC), and power spectral density before and after stimulation. Results: Following SES, firing rate of units increased. For many of these neurons, these changes were persistent, and lasted several hours. Interestingly, SFC increased specifically in the low frequency range (0.5-4Hz). An increase in power spectral density in the low frequency

range was also observed with SES. Conclusions: Afferent stimulation of the radial and ulnar nerves increased firing rate, as well as SFC and power specifically in the low frequency range. This frequency range has been previously associated with greater functional recovery after stroke. Our results thus suggest that SES may be able to modulate low-frequency cortical oscillatory activity. In future studies we aim to examine if this also holds in the injured brain. Research reported in this publication was supported by the National Institute Of Health under Award Number R01MH111871.

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Poster

174. Physiological Methods: Electrophysiology: Stimulating Neurons

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Topic: I.04. Physiological Methods

Support: NIH R01 NS085167
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DARPA N66001-17-2-4011

Title: Flat electrodes for peripheral nerve stimulation and non-invasive dose-response curves of vagus nerve stimulation

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Abstract: Vagus nerve stimulation (VNS) is emerging as a means to treat a variety of disorders. The majority of available systems for stimulation use either a helical or a circumferential stimulation contact pattern, which produces a largely uniform voltage field around the circumference of the nerve. A flat electrode that only contacts a fraction of the circumference of the nerve may provide advantages including simpler implantation, ease of production, and more resistance to damage. However, it is possible that a flat contact configuration will yield inefficient fiber recruitment due to a less uniform voltage field across the nerve. Additionally, no system is in place to measure nerve activation and calibrate stimulation parameters. Here we tested the hypothesis that flat electrode contacts will recruit fibers less effectively than industry standard designs, and we evaluated the use of oxygen saturation and heart rate to create dose-response curves of VNS. Computational modeling suggested that changing from circumferential contacts to flat contacts reduced fiber recruitment, but other design parameters such as the inner

diameter of the cuff appeared to be much more impactful. Modeling results were confirmed with *in vivo* experiments on multiple nerves in multiple species. Initial results demonstrated similar fiber recruitment in the rat vagus and sciatic nerves with quasi-flat and circumferential electrodes. Follow up experiments comparing true flat electrodes to circumferential electrodes on the rabbit sciatic nerve confirmed that fiber recruitment was equivalent between the two designs. These findings suggest that flat electrode contacts are a viable design that may provide advantages over the current circumferential designs. Dose-response curves of VNS with drops in oxygen saturation were robust and could be used to calibrate stimulation, but drops in heart rate were inconsistent.

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Poster

174. Physiological Methods: Electrophysiology: Stimulating Neurons

Location: SDCC Halls B-H

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Program #/Poster #: 174.15/JJJ19

Topic: I.04. Physiological Methods

Support: NSF INSPIRE (CBET-1343193)

Title: Design and testing of a multi-channel asynchronous neurostimulator with stimulation artifact rejection for cortical prosthesis

Authors: *S. ELYAHOODAYAN[1], D. SONG[2]
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Abstract: Cortical Prosthesis is defined as a closed-loop system that bypasses a damaged / diseased brain region by reinstating its input-output properties to restore cognitive functions. Different from deep brain stimulation (DBS), which typically utilizes synchronous, fixed-interval trains of electrical stimulation pulses from a small number of channels to modulate brain functions, a cortical prosthesis requires delivery of large-scale spatio-temporal patterns of asynchronous electrical pulses to mimic neural codes underlying cognitive processes. In addition, cortical prosthesis can greatly benefit from the capability of recording feedback signals from the same region being stimulated for real time adjustment of stimulation parameters. We have developed a highly configurable multi-channel stimulator that can be triggered to generate arbitrary spatio-temporal patterns of stimulation pulses continuously and in real time. This design also enables validation and optimization of the stimulation by allowing recording neural responses from the same electrodes. Taking advantage of the sparse nature of neural spiking activity, which seldom requires simultaneous pulse generation from multiple electrodes, we utilize a highly efficient multiplexed system, where the output of a single current pulse generator

is fed into a multiplexer and then expanded to 32 independent channels with negligible sacrifice in the temporal precision. The multiplexer is controlled by 5 select lines, and driven by a firmware-implemented multi-input, multi-output (MIMO) nonlinear dynamical model, which mimics the input-output spike transformational properties of a brain region. To prevent saturation of recording amplifiers by stimulation artifacts, the stimulators are synchronized with a set of serially controlled CMOS switches to completely block the artifacts from the recording amplifiers during stimulation. This system has been tested in phosphate buffered saline using a PtIr-coated Parylene electrode array. Results show that the stimulator can generate arbitrary spatio-temporal patterns of current pulses and completely remove the stimulation artifacts. Future work will aim to demonstrate the functionality of the microstimulator in the hippocampus of behaving rats.
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Disclosures: S. Elyahoodayan: None. **D. Song:** None.

Poster

174. Physiological Methods: Electrophysiology: Stimulating Neurons

Location: SDCC Halls B-H

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Topic: I.04. Physiological Methods

Support: JST KAKENHI 17K17684
The Precise Measurement Technology Promotion Foundation

Title: Modifying behavior of a rat during a Brain-Machine Interface experiment by deep brain stimulation

Authors: *N. SUDO, O. FUKAYAMA, K. MABUCHI, T. ISOYAMA
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Abstract: Rats are often used in neural recording experiments for developing Brain-Machine Interface (BMI) system because of their manageability and commonality with humans in their basic brain structure. However, determining rat's intention is difficult so that there is a possibility that different neural signals were recorded even though the rat takes same behavior on the appearance. In addition to this, trainings that are usually required to make the rat to perform a specific behavior under a particular situation take relatively long time and have a possibility to change original neural structures during the trainings. Therefore, we intended to regulate rat's intention and control their behavior during an experiment by deep brain stimulation. For surgical preparation, 5 rats were implanted 4-bundle tungsten electrode to the lateral hypothalamus (LH) region. Any 2 of 4 wires of the electrode were used for stimulation. During experiments, a pair of LED was attached behind the connector of the electrode to detect rat's position in an

experimental filed optically. Following 10 minutes sham trial, a circular “attracting zone” was designated where stimuli were applied to the rat when it was in the zone and faced its center (“target”). In the beginning of the trial, the target was placed the center of the field and had a radius barely covered all area of the field. As the rat got the stimuli, the radius gradually shrank. After the radius shrank to the minimum size (0.14 m), the position of the target was changed randomly without noticing to the rat. As a result, 2 of 5 rats were attracted to the target by either a pair of 2 wires. They also searched and followed the attracting zone when the position of the target was changed. The other rats did not show such behaviors, instead showed no response to the stimuli or disliked and avoided the stimuli. These results suggest that the electrical stimulation to the LH region can modify rats’ behavior. LH is known as one of reward system related region, so that it is supposed that the rats recognized the stimuli as a reward came to want to get more reward and attempted to enter the attracting zone. This process is similar to learning in operant conditioning, while our method could induce the specific behavior instantly. About the rest that failed to reach the target, it is considered that the stimulated brain area was inappropriate for the stimuli to work as a reward, where was not LH region or LH neurons that are not related to reward recognition. Our method can regulate intention of a rat subject during a BMI experiment and control their behavior, that may be useful for neural recordings to estimate relationship between a specific behavior and neural signals.

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Poster

174. Physiological Methods: Electrophysiology: Stimulating Neurons

Location: SDCC Halls B-H

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Program #/Poster #: 174.17/JJJ21

Topic: I.04. Physiological Methods

Support: DARPA Electrx Grant HR0011-16-2-0023

Title: Wireless, injectable neural interfaces (WINI) for peripheral nerve neuromodulation *in vivo*

Authors: *A. SRIDHARAN[1], S. CHIRANIA[1], B. TOWE[1], J. MUTHUSWAMY[2]
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Abstract: Conventional electrical neuromodulation approaches have stability and reliability challenges when chronically interfacing with peripheral nerves due to lead drift and tissue inflammation reactions. Novel, implantable microstimulators (~200 µm to 1 mm feature size) offer significant advantages of high-throughput stimulation and localized selectivity for obtaining high degrees of freedom in peripheral nerve control. Previous work demonstrated that high frequency, AC signals are capable of wirelessly stimulating nerve fibers via placement of microscale rectifiers on the nerve *in vivo* in a chronic, rodent sciatic nerve model. In addition,

implanting microscale stimulators inside the nerve leads to a more selective neuromodulation. The main objective of this work is to characterize the ability of the wireless, injectable neural interfaces (WINI) to stimulate a rodent sciatic nerve using an external AC excitation. Off-the-shelf rectifiers were modified to study the effects of orientation (cathodic vs. anodic), changes in dipole length (100 µm to 1 mm) and external stimulation frequency dependence (2-1000 kHz) on electromyogram (EMG) threshold, latency, and amplitude in a rodent sciatic nerve model *in vivo*. Commercially available nerve cuffs with 8 annular electrodes annular spaced 250 um apart were placed externally on the nerve to wirelessly deliver AC currents to the modified rectifiers. Overall, it was found that the currents required to reach EMG threshold ranged from 100-700 µA. Above 20 kHz, control experiments with no rectifiers showed higher EMG threshold currents compared to experiments that included rectifiers. EMG thresholds increased linearly with frequency until 50 kHz, beyond which the increase in EMG threshold was nonlinear. Increases in pulse duration (250 µs to 1 ms) and increasing dipole length resulted in lower EMG thresholds. Orientation of the WINI with respect to cuff leads also changed EMG thresholds in cathodic configurations. EMG recruitment curves also varied with changes in latencies (3-5 ms) with changes in orientation and muscle selection. AC current stimulation with implanted WINIs showed increasing EMG response amplitudes without reaching saturation, while control AC current stimulation without implanted WINIs showed no EMG responses. Preliminary TEM based histology of sciatic nerve sections implanted with WINIs & cuff versus nerve sections with cuff only show similar distribution of myelinated and unmyelinated fibers with minimal damage in tissue sections near the implanted microstimulators. More comprehensive studies are being currently being conducted to assess microscale damage.

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Poster

174. Physiological Methods: Electrophysiology: Stimulating Neurons

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Program #/Poster #: 174.18/JJJ22

Topic: I.04. Physiological Methods

Support: KHIDI Grant HI16C0132
GIST Research Institute (GRI)

Title: The effect of transcranial direct current stimulation (tdcs) in a rodent postoperative delirium

Authors: *K. KIM, J. HAM, J. OH, D. CHO, B. LEE
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Abstract: Background: Delirium is a common manifestation of postoperative patients, especially in ICU patients. It is associated with a high mortality and extended hospital day, therefore, a new treatment is needed for rapid recover from delirium. Meanwhile, tDCS which is a kind of noninvasive brain stimulation has been investigated to apply to various brain disorders such as stroke, dementia, and depression. It can induce a change of cortical excitability by applying direct electric current. It is well known that anodal tDCS can increase cortical excitability while cathodal tDCS can decrease that. Furthermore, it has been reported that those change of cortical excitability can affect neuroplasticity. In this study, we hypothesized that anodal tDCS can prevent or recover the symptoms of postoperative delirium.
Method: 8 adult male Sprague Dawley rats (17.5±3.7 weeks, 400.21±52.52g) were divided into two groups: the anodal tDCS and sham groups. For recording local field potential, bilateral electrodes were implanted into the frontal cortex (AP: +5.50mm, ML: ±3.00mm) and the parietal cortex (AP: -4.36mm, ML: ±4.00mm). The tDCS electrode was placed at the right frontal cortex (AP: +2.00mm, ML: +1.50mm). All rodents induced delirium by a surgery were treated with tDCS for 20min after surgery. For the anodal tDCS group, a constant-current of 200μA was applied, while a constant-current which is close to zero was applied for the sham group. To assess the recovery of delirium, we performed buried food test at before surgery, 9hr and 48hr after surgery and EEG (electroencephalography) recording at 9hr and 48hr after surgery.
Results: In comparison with before surgery on buried food test, the anodal tDCS group did not show increased time to find food at 9hr and 48hr after surgery. In the sham group, however, the time to find food was increased at 9hr and 48hr after surgery. The normalized time by the baseline was also reduced in the tDCS group compared to the sham group at 48hr after surgery. In delta frequency from the right frontal cortex on EEG analysis, the average of relative normalized power was reduced, at 9hr after surgery in the tDCS group.
Conclusion: In summary, the tDCS group, which was treated with tDCS after surgery, shows a better behavioral test result at 48hr after surgery and a reduced delta power on EEG analysis at 9hr after surgery. The tDCS in postoperative delirium may induce activation of the frontal cortex on early stage and help improve the behavioral symptom afterward. Our results suggest that the possibility of a rapid recovery in the postoperative delirium with tDCS.

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Poster

174. Physiological Methods: Electrophysiology: Stimulating Neurons

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Program #/Poster #: 174.19/JJJ23

Topic: I.04. Physiological Methods

Title: Combining non-invasive brain technologies to detect and stimulate brain activity

Authors: *A. J. GARBIN, A. M. HOOYMAN, J. J. KUTCH, B. E. FISHER
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Abstract: Research objective: To develop a protocol for the combined use of Transcranial Magnetic Stimulation and Electroencephalography (TMS-EEG) that facilitates measurement of cortical properties of non-motor areas during an upright postural task.
Rationale: Combined neuroimaging techniques afford brain measurements that each technique alone cannot provide. TMS-EEG enables measurement of both motor and non-motor cortical properties thus providing valuable information with a single TMS pulse, such as connectivity and excitation/inhibition. Despite the value of TMS-EEG, there has been limited widespread use as the TMS pulse induces significant artifacts into the EEG signal. Therefore, we sought to develop a TMS-EEG protocol that attenuates these artifacts and is suitable for standing postural tasks.
Methods: Utilization of concurrent TMS-EEG requires a TMS-compatible EEG system. With this system, there are still several artifacts introduced to the EEG recordings by the electromagnetic TMS pulse. To attenuate these artifacts during data collection, we have added a thin layer of foam to the TMS coil to remove 60hz noise, utilized earplugs to mitigate auditory-potentials, and stimulated with slight separation between the TMS coil and EEG cap to prevent electrode movement. Following collection, we filtered the data and performed an independent component analysis to determine physiologic and electric artifacts that can subsequently be removed. With these techniques, we performed two trials of TMS-EEG consisting of approximately 40 pulses each and assessed the amplitude and inter-trial variability of the signal pre- and post-artifact removal.
Results: Artifact removal resulted in significant reduction of signal amplitude and decreased inter-trial variability.
Conclusions: Our methodology allows for collection and interpretation of TMS-EEG signals that measure motor and non-motor cortical properties. This will facilitate future research examining the association between activity and connectivity of somatosensory and primary motor cortices with the lower extremity stiffening strategy in older adults with a concern of falling.

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Poster

174. Physiological Methods: Electrophysiology: Stimulating Neurons

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Program #/Poster #: 174.20/JJJ24

Topic: I.04. Physiological Methods

Support: GRF15102417

Title: Behavioral and functional assessment of low frequency low intensity ultrasound stimulation on *Caenorhabditis elegans*

Authors: *Q. XIAN[1], Z. QIU[2], J. GUO[2], L. SUN[3]
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Abstract: Ultrasound stimulation is a promising modality for probing brain function and treating brain diseases. Low intensity ultrasound has made great advances in brain stimulation and neuro-modulation. Ultrasonic waves can be non-invasively steered and focused into mm-scale regions across the human skull, facilitated to produce controlled modulation of neuronal activity. However, how ultrasound stimulation modulate neural activity is unclear and some of the mechanisms remain elucidate. Here we developed a top-down strategy for behavioral and functional testing of ultrasound effects *in vivo*. A transgenic line of C elegans with GCaMP6s expression in every neuron was utilized as the model to simultaneously monitor of behavioral and neural activity upon low frequency low intensity (LILF) ultrasound stimulation (US). In addition, all the neurons are visualized during ultrasound stimulation by which we can assess the effects of ultrasound stimulation on different neuron types and the interaction with the chemo and thermos cues. Our results show that LILF US is capable of initiating behavioral responses and activating neurons of C. elegans' whole body under the stimulation. The calcium spikes in the whole body can be visualized longitudinally with single neuron resolution in free moving C. elegans. There are about 84.38% worms responded to the ultrasound stimulation exhibiting a shock behavior, and generating more reversal and omega turning behavior after ultrasound stimulation. In vivo calcium imaging also showed the neurons of calcium fluorescence dynamic in the head respond to ultrasound stimulation. Compared with high frequency ultrasound and microbubble assisted ultrasound effects on C elegans, the present study with 0.5 MHz and 1 MHz is more clinical relevant because its capability of penetrate through the skull. Surprisingly, the predominant responses were found in the tail with about 65.517% worms of tail respond to ultrasound stimulation, some kinds of tail's calcium neurons light up. We confirmed that low frequency ultrasound which can modulate neurons in C elegans and it can also integrate with natural cues to modulate neuron activity. This study confirmed that ultrasound can activate neurons directly and the platform we developed can be used to further investigate the mechanism of ultrasound brain stimulation *in vivo*.

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Poster

174. Physiological Methods: Electrophysiology: Stimulating Neurons

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Topic: I.04. Physiological Methods

Support: NIH Grant 1RF1MH114233-01

Title: Examining local direct neural activation during transcranial focused ultrasound neuromodulation

Authors: *X. NIU[1], K. YU[2], B. HE[1]
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Abstract: Introduction
Transcranial focused ultrasound (tFUS) is a noninvasive neuromodulation method with high spatial resolution (mm) and deep penetration. However, researchers need to investigate careful controls to ensure the recorded observations are free from confounding activation in the auditory cortex, reported with EEG source imaging, calcium imaging and multiunit recordings. The auditory effects are presumed to be due to the mechanical coupling between the tFUS pulsation induced vibrations with the cochlea. Here, we present evidence of direct brain activation by tFUS, not dictated by the auditory pathway (n = 6).
Methods
Anesthetized adult rats received baseline auditory brain stem response (ABR) tests to determine baseline hearing thresholds. 4 rats were then chemically deafened with furosemide and gentamicin and re-tested with the same ABR test 72 hours after injection. After deafening, a 32-channel recording electrode microarray was inserted into the primary somatosensory region (S1) to record the neural activities elicited by tFUS. The 500 kHz ultrasound stimulation targeting at S1 was administered at a pulse repetition frequency of 1.5 kHz at 2.5-second intervals, with intensities below 720 mW/cm^2. As a sham comparison on the deafened model, tFUS was directed to skull's frontal part 10-15 mm away from S1 in order to rule out confounding activation of auditory pathways due to mechanical forces propagating along the skull.
Results
All rats injected with furosemide and gentamicin exhibited significant ABR response after injections, while control rats did not experience significant changes. We observed a local rise in local field potential within 300 ms of tFUS stimulation at S1 in both control subjects and subjects with hearing reduction (240 trials). This suggests that side-effect activations in the auditory pathway from tFUS-induced hearing percepts do not dictate activation of S1 cortex, such activation is confined at a targeted local brain region. Peri-stimulus time histograms during tFUS stimulation shows increased neuronal firing within 100ms of tFUS stimulation well above the baseline firing in S1 of deafened rats (confidence level 99%, 480 trials).
Conclusion
In this preliminary study, we performed control studies to examine the direct activation of the rat S1 cortex *in vivo*. Through chemically induced deafening, we can successfully eliminate the confounding activation pathway propagating from the cochlear activation, thus proving a direct brain activation by tFUS.
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Poster

174. Physiological Methods: Electrophysiology: Stimulating Neurons

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Program #/Poster #: 174.22/JJJ26

Topic: I.04. Physiological Methods

Support: MCubed, University of Michigan

Title: Assessing optimal transcranial magnetic stimulation parameters for probing inhibitory function in the visual cortex

Authors: *D. KHAMMASH[1], M. SIMMONITE[2], T. A. POLK[4], S. F. TAYLOR[5], S. K. MEEHAN[3]
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Abstract: Short-interval cortical inhibition (SICI), an inhibitory phenomenon elicited by paired-pulse transcranial magnetic stimulation (TMS), has been extensively used to probe intracortical networks in the motor cortex. We previously used phosphenes, short-lived TMS induced visual percepts, to demonstrate that SICI-like inhibition is present in visual cortex, albeit at much lower conditioning stimulus intensities than those employed in motor cortex studies. The current study investigates the time course of SICI in the visual cortex. Here, we assessed changes in phosphene area across a range of conditioning-test stimulus interstimulus intervals (ISI). Participants completed a single 2-hour session, during which they were seated in the dark facing a 117cm screen spanning their visual field. Phosphene threshold (PT) was defined as the minimum stimulator intensity at which phosphenes were induced on 5 out of 10 stimulations. After each stimulation, participants traced the area of the computer screen covered by the resulting phosphene. Test stimuli (TS, 120% of PT) were preceded by a conditioning stimulus (CS, 45% of PT). Using a CS intensity of 45% of PT elicited optimal inhibition in visual cortex in a previous study. The interstimulus interval between the TS and CS was tested at ISIs of 2, 3, 5, 10, 15, 50, 100, and 200ms. Preliminary results (N=19) demonstrate equivalent inhibition of the phosphene response at all tested ISIs with the exception of 15ms. In motor cortex, a 2-3ms ISI is ideal for elicitation of SICI. This study therefore further demonstrates that parameters that are optimal for motor cortex are not necessarily optimal for visual cortex and provides a new approach to studying cortical inhibition in the visual cortex.

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Poster

174. Physiological Methods: Electrophysiology: Stimulating Neurons

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Topic: I.04. Physiological Methods

Support: DARPA-GG012363

Title: Focused ultrasound reversibly suppresses action potential propagation

Authors: *Y. BABA[1], B. U. HOFFMAN[1], S. A. LEE[2], E. E. KONOFAGOU[3], E. A. LUMPKIN[1]
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Abstract: Ultrasound enables non-invasive stimulation of inaccessible areas, such as deep brain tissue, and has shown promise as both a therapeutic tool and a technique to study basic neuronal mechanisms. We have previously shown that brief pulsed focused ultrasound stimulation (FUS) directly evokes action potentials in sensory neurons in mouse skin-nerve preparation recordings. Interestingly, parameter space exploration revealed that longer, low-intensity sonication induced a decrease in excitatory neuronal activity. To identify FUS parameters that suppress action potential propagation in neurons, we employed two complementary approaches: invertebrate preparation (cockroach), which provides a tractable identified neurons within intact circuits; and an *ex vivo* mouse skin-nerve preparation, which is a well-characterized system to analyze mammalian mechanoreceptors. In the cockroach system, action potentials in ascending interneurons were elicited by air-puff stimulation to mechanosensory organs. Action potentials were recorded in the posterior nerve of the meta-thoracic ganglion (MTG) and the anterior nerve of the terminal abdominal ganglion (TAG). FUS was applied at the midpoint between the two recording locations (frequency: 3.57MHz; pressure: <7.2 MPa; pulse duration: 1 ms, duty cycle: 1-5%; stimulus duration: 0.5-1.0 s). After FUS stimulation, we observed a reversible decrease in air-puff evoked action potentials in the MTG, but not the TAG, which were proximal and distal to FUS stimulation, respectively. These results indicate that low-intensity sonication induced action potential propagation failure at the site of FUS stimulation. This failure lasted for up to 2 h after sonication but recovered in all recordings (n=10/10). In mouse preparations, touch stimulation was applied to receptive fields, while simultaneously delivering FUS stimuli (parameters as above; ≤9.3 MPa) to a proximal region of the saphenous nerve. We observed four types of responses to FUS sonication: no effect (n=9/30), a reversible decrease in mechanically evoked spikes (n=10/30), a non-reversible gradual decrease in mechanically evoked spikes (n=5/30), and an immediate decrease in mechanically evoked spikes (n=6/30). The increased variability of FUS induced responses in the mouse compared to cockroach preparations could be

due to the presence of myelinated axons, or the absence of cell bodies in the mouse preparation. Together, these studies illuminate an exciting parameter space in which ultrasound reversibly silences neural activity, providing a springboard for the development of ultrasound as a clinical therapeutic in sensory dysfunction.

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Poster

174. Physiological Methods: Electrophysiology: Stimulating Neurons

Location: SDCC Halls B-H

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Program #/Poster #: 174.24/JJJ28

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Title: Remote magnetomechanical stimulation of sensory neurons mediated by magnetic nanodiscs

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Abstract: A new technique has been developed for magnetic nanoparticle-based neural stimulation. Unlike magnetothermal neural stimulation, which is based on the transfection of neurons to sensitize them to heat, this magnetomechanical approach does not rely on transgenes, making it potentially safer for clinical applications. In contrast to previously reported magnetomechanical stimulation techniques, in which neurons are typically hundreds of microns or less from a magnetic field source, the field required for this technique (1–50 mT, 1–20 Hz) is produced at the scale of an entire rodent model using a simple solenoid and a 200 W audio amplifier. This advantage in stimulated volume is enabled by magnetic nanodiscs with volumes hundreds of times larger than conventional magnetic nanoparticles, but which have favorable colloidal properties due to their disc shape. These magnetic nanodiscs possess a spin vortex state, which nearly eliminates stray field and results in less inter-particle attraction compared to isotropic magnetic particles of similar volume. The neural stimulation technique enabled by these magnetic nanodiscs has been demonstrated to robustly induce calcium influx in sensory neurons in rat dorsal root ganglion (DRG) explant cultures and enhance rat expression of an

immediate early gene c-fos in DRGs of adult rats. This technique may find applications in basic studies of neural circuits as well as pave way for future neuromodulation therapies.

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Poster

174. Physiological Methods: Electrophysiology: Stimulating Neurons

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Topic: I.04. Physiological Methods

Support: NSF IGERT 120104

Title: Magnetoelectric materials for miniature, wireless neural interfaces

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Abstract: Miniaturized and wireless neuromodulation devices would improve the treatment for neurological disorders in humans and enable new studies of neural circuits in freely behaving animals. While many of these miniaturized devices operate using electromagnetic inductive coupling between a transmitting coil and an implanted receiver coil, there are fundamental limits on the size reduction that is possible due to decreased energy transfer efficiency and high angle sensitivity for small receiver coils. Additionally these small coils require ~MHz frequency electromagnetic fields to carry the power, which is absorbed by biological tissue, placing strict limits on the amount of power that can safely transferred at these frequencies. New methods capable of efficient wireless power transfer to small devices are required to develop more effective miniature biomedical devices. Here we show magnetoelectric devices capable of transforming external magnetic fields to electric fields strong enough to stimulate neurons without any genetic manipulation. These devices rely on the coupling of piezoelectric and magnetostrictive materials driven at an acoustic resonance. In contrast to traditional inductive coupling, we show magnetoelectric materials are scalable and still capable of generating large voltages with a small device footprint. Our results demonstrate that magnetoelectric materials can be used to develop versatile lightweight wireless neural implants. We lay the foundation for further developing these materials to be used for many different applications in neuroscience.

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Poster

174. Physiological Methods: Electrophysiology: Stimulating Neurons

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Title: Non-invasive mesenchymal stem cell transplantation and changes of Intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 by low intensity focused ultrasound in the rat brain

Authors: *J. LEE[1,2], J. SHIN[1,2], C. KONG[2], Y. SEO[2], B.-W. SONG[3,4], B.-S. KIM[3,4], W. CHANG[2], J. CHANG[1,2]
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Abstract: Introduction: There have been reports of promising results regarding transplantation of mesenchymal stem cells for neurodegenerative diseases through the use of neuronal differentiation or control of microenvironment. However, traditional surgical transplant methods, such as parenchymal or intravenous injection, possess limitations like secondary injuries in the brain, infection, & low survival rate of stem cells in the target site. Meanwhile, several studies have shown that disrupting the blood-brain barrier using focused ultrasound induced various cytokines associated with stem cell homing & migration. Therefore, this study investigates a possibility for non-invasive focused ultrasound use in targeted stem cell transplantation into the brain **Materials & methods:** In this study, male Sparague-Dawley rats (200-220g) & bone marrow-derived MSC (5p) were used. Low intensity focused ultrasound(LoFUS) was applied with parameters of 0.25Mpa, 300s (Targeted hippocampal region: AP -3.5, ML ±2). Three hours after sonication, bone marrow-derived MSC (BM-MSC) (3*106/200ul) was injected into the tail vein. The other rats were injected with BM-MSC only intravenously. Then twenty-four hours after stem cell injection, all rats were sacrificed. **Results:** The position of blood-brain barrier

opening was shown by the rat injected with Evans blue & BM-MSC were detected in both groups of the hippocampus region. After comparing FUS+MSC & MSC-only rats, it was confirmed that LoFUS increases BM-MSC homing in the sonicated brain region. In addition, some cytokines, which are involved in the stem cell homing process like ICAM-1(Intercellular adhesion molecule 1) & VCAM-1(Vascular cell adhesion molecule 1), were increased in the sonicated brain tissue. Also, brain-derived neurotrophic factor(BNDF) were increased in the sonicated brain region **Conclusion:** Noninvasive LoFUS is a promising approach in targeting & maximizing stem cell delivery by stimulating cytokine expression(ICAM-1 & VCAM-1) & brain-derived neurotrophic factor(BNDF) in sonicated tissue before cell injection. The results of this study suggest mechanisms of stem cell homing process through use of LoFUS. However, further study regarding the function of stem cells transplanted in the brain & a more detailed mechanism of stem cell homing by LoFUS is needed.

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Poster

174. Physiological Methods: Electrophysiology: Stimulating Neurons

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Title: Improvements of cognitive function by focused ultrasound associated with adult hippocampal neurogenesis in immunotoxin 192-Saporin rat model of cholinergic degeneration

Authors: *C. KONG[1], J. SHIN[1,2], J. LEE[1,2], C. KOH[1], J. SIM[1,2], Y. NA[3], W. CHANG[1], J. CHANG[1,2]
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Abstract: Introduction: Alzheimer's disease is irreversible, progressive neurodegenerative disorder that destroys memory and cognitive function. Recently, focused ultrasound (FUS) has been demonstrated that FUS-mediated BBB opening induces an increase in hippocampal neurogenesis in adult rodents. In this study, we investigated the effects of FUS on memory and cognitive function after 192 IgG-saporin lesion. **Materials and Methods:** The present study utilized adult male Sprague-Dawley rats (200-250 g). Animals were divided into the three

groups: Sham group (PBS injection), Lesion group (saporin injection), FUS group (saporin + FUS treatment). Lesion groups were injected bilaterally into the lateral ventricle. Rats were sonicated using a single-element transducer with microbubble. The acoustic parameters used for each sonication are: pressure amplitude 0.3 MPa, pulse length 10 ms, burst repetition frequency 1 Hz, and a duration of 120 s. BrdU was intraperitoneally injected two times per day for 4 consecutive days starting 24 hours after sonication. Two weeks after IgG-saporin administration, spatial memory was tested with the Morris water maze training. **Results:** In the water maze test, the FUS groups were significantly increased in number of crossing and platform zone, compared to the lesion group. We confirmed that the number of $BrdU^+$, DCX^+, and $NeuN^+$ were significantly increased in the dentate gyrus following FUS sonication, compared to the lesion groups. Also, we found that the expression level of BDNF and TrkB increased in FUS group. **Conclusion:** Our results suggest that FUS treatments led to spatial memory improvement in cholinergic deficits rat model. Therefore, this provides evidence that the behavior changes may be mediated by increased acetylcholine activity and neuronal plasticity. Furthermore, FUS may represent a promising treatment for neurodegenerative disease, including Alzheimer's disease because neurogenesis is associated with memory and cognitive function.

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Poster

174. Physiological Methods: Electrophysiology: Stimulating Neurons

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Title: The role of vesicular zinc on adult hippocampal neurogenesis induced by focused ultrasound

Authors: *J. SHIN[1,2], B. CHOI[3], C. KONG[1], S. LEE[3], J. SIM[1], J. CHANG[1,2], W. CHANG[1], S. SUH[3]
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Abstract: Introduction: Transcranial focused ultrasound (FUS) has gained attention for its potential application as a method to locally open the blood-brain barrier (BBB) and facilitate

drug delivery into the brain parenchyma. It has been demonstrated that FUS-mediated BBB opening induces an increase in hippocampal neurogenesis in adult rodents. However, the mechanism underlying FUS-induced neurogenesis is unclear. Recent evidence suggests that zinc is a central actor in regulating stem cell proliferation and neurogenesis in the adult brain. Here we speculate that vesicular zinc may relate to the increased hippocampal neurogenesis after focused ultrasound sonication.
Materials and Methods: The present study utilized adult male Sprague-Dawley rats (2-3 months, 250-300 g). Rats were sonicated using a single-element transducer (frequency 0.5 MHz) with microbubble. The acoustic parameters used for each sonication are: pressure amplitude 0.3 MPa, pulse length 10 ms, burst repetition frequency 1 Hz, and a duration of 120 s. BrdU was intraperitoneally injected 2 times per day for 4 consecutive days starting 24 hours after FUS sonication. Histological and western blot analysis were performed at 24hours, 5 or 21 days after FUS sonication and western blotting was BDNF and TrkB expression were measured via western blotting. **Results:** We found that the number of $BrdU^+$ and DCX^+ cells were significantly increased in the FUS-treated dentate gyrus (DG) following 5 days of FUS sonication, compared to the contralateral untreated DG. Zinc transporter 3 (ZnT3), a transporter of zinc into synaptic vesicles, was also seen to increase in the DG at 5 days after FUS sonication. Furthermore, the total number of $NeuN^+/BrdU^+$ cells in FUS-treated DG was significantly increased at 21 days after FUS sonication, compared to the untreated DG. Tweenty-four hours after sonication, the expression of BDNF and TrkB were significantly increased in the FUS-treated hippocampus, compared to contralateral hippocampus.
Conclusion: The present study demonstrates that increased vesicular zinc by FUS induces adult hippocampal neurogenesis. Therefore, this study suggests that vesicular zinc may be involved in FUS-induced neurogenesis.

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Poster

174. Physiological Methods: Electrophysiology: Stimulating Neurons

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Title: Manipulation of rat movement with liquid crystal polymer based electrodes in 3d-maze

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Abstract: In this paper, we controlled locomotion behavior of rats using liquid crystal polymer (LCP) electrodes. Medial Forebrain Bundle (MFB) was stimulated by depth electrodes and direction cues were delivered in barrel field cortex (S1BF) by surface type electrodes. The efficacy of our LCP based stimulation system was verified by success rate of turning response which was measured the percentages of each side of turning with ipsilateral cues. To evaluate rat movement control, 3D-maze test was conducted. Our result showed that LCP electrodes based stimulation can control locomotion activities and directions of rats successfully. Previously, metal electrodes were applied for stimulation but still there are some disadvantages to use. To tackle this limitation, we used LCP electrode, which is flexible and biocompatible. Additionally, we also designed semi-invasive electrodes for epidural cortex stimulation. LCP depth electrode was implanted in the MFB and surface type of LCP electrode was located upon epidural cortex of S1BF in rats. After implantation, we conducted Intra Cranial Self - Stimulation (ICSS) in the MFB to develop operant condition. For turning behavior training, we delivered epidural stimulation on each S1BF area. We trained as left and right S1BF area stimulation for each ipsilateral turning direction with rewarding cues. We measured the success rate in T-maze test and confirm the controlling the movements in complex 3D-maze which have different kinds of fearful obstacles. The success rate of turning behaviors, measured left turning when left turning cue delivered and vice versa. The average success rate of each turning was 80% (Left turning: 79 ± 1.2% and Right turning: 81 ± 1.5%). Confirming the direction control of rats, T-maze test were followed. We controlled rats to random direction (left or right) using S1BF-cue and MFB reward-stimulation. The success rate was 100%. Moreover, in complex 3D-maze, rats were well-followed operator's instruction to many obstacles which rats may not go voluntarily. We developed an LCP electrode based rat behavior control model and proved the possibility of using LCP in freely moving animals. We believe that LCP can be a good alternate for brain stimulation with high efficient and less side effects. Furthermore, LCP electrodes can be applied not only for behavior controlling in animals but also deep brain stimulation and/or semi invasive cortex stimulation for therapeutic use. With many advantages of LCP electrodes, there are magnificent usages possibility opened in future.

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