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Poster

350. Patterning, Cell Death, and Proliferation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 350.01

Topic: A.01. Neurogenesis and Gliogenesis

Support: UMass Boston College of Science and Mathematics graduate research fellowship to M.B.
NIH R01 GM141843 to A.V.
NIH R01 HD085870 to A.V.

Title: Control of brain development by the DYRK1A kinase Minibrain

Authors: *M. BROWN, E. SCIASCIA, W. ADAM, A. VERAKSA;
Biol., Univ. of Massachusetts, Boston, Boston, MA

Abstract: Determining the mechanisms behind developmental diseases of the nervous system can provide insight into advancing treatments. The kinase DYRK1A plays a role in Down syndrome, microcephaly, and cancer, however the exact mechanism through which it functions is unknown. By studying the *Drosophila* homolog, Minibrain (Mnb), we can learn more about DYRK1A function. Neuronal precursor cells give rise to differentiated cells and the adult brain structure. During development, the proper number of neuronal precursors must be formed from neuroepithelial cells, and this transition is a carefully regulated process. Molecular marker analysis of mnb^{d419} null mutants has revealed alterations in the neuroepithelium and neuroblast regions of developing larval brains. We have also shown that Mnb is required for proper cell proliferation in larval brains using DNA content analysis from dissociated brain cells through fluorescence activated cell sorting (FACS). To determine how Mnb regulates brain development, we performed affinity purification-mass spectrometry (AP-MS) on embryos expressing an endogenously tagged form of Mnb. Several proteins identified in the Mnb interactome play a role in endocytosis. We hypothesize that Mnb may play a role in the endocytic regulation of signaling pathways, and thus regulate the critical neuroepithelium to neuroblast transition as well as cell proliferation in the larval brain.

Disclosures: M. Brown: None. **E. Sciascia:** None. **W. Adam:** None. **A. Veraksa:** None.

Poster

350. Patterning, Cell Death, and Proliferation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 350.02

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant RF1MH12460501

Title: Brain-wide cellular resolution mapping of GABAergic cells and microglia unveils spatially distinct developmental patterns in the early postnatal mouse brain

Authors: *J. K. LIWANG, J. MINTEER, F. KRONMAN, Y. KIM;
Penn State Col. of Med., Hershey, PA

Abstract: Microglia are the resident innate immune cells of the central nervous system which support cellular migration, initiate synaptic pruning, and sculpt developing neural circuits during early postnatal development. Particularly, microglia facilitate the programmed cell death of developing cortical neurons, including γ-aminobutyric acid-containing (GABAergic) interneurons. However, it is currently unknown whether microglial guidance of developing GABAergic cells occurs in region-specific manner. Furthermore, studies observing the developmental trajectories of microglia in the postnatal mouse brain are currently unknown due to the lack of cellular-resolution datasets. To resolve this knowledge gap, we used whole-brain quantitative cell type mapping methods to assess the temporal and spatial changes of microglial cell densities throughout postnatal mouse brain development. We have compared the developmental patterns of microglia to GABAergic cell types including somatostatin-, parvalbumin-, and vasoactive intestinal polypeptide-expressing neurons at postnatal day (P) 4, 6, 8, 10, 12, 14, 21, and 28 in mice. We find different spatiotemporal changes in microglial and GABAergic cell types in various cortical and subcortical brain regions. Microglia residing in the developing cerebellum show differential expression across cerebellar layers during maturation. Additionally, we identify white-matter-tract microglia with distinct, amoeboid morphologies migrating along developing commissural fibers, including the corpus callosum. In all, our study characterizes spatially distinct microglia during early postnatal development and presents a valuable resource for studying the interplay between microglia and GABAergic neurons in neurodevelopmental disorders and neuropsychiatric diseases.

Disclosures: J.K. Liwang: None. **J. Minteer:** None. **F. Kronman:** None. **Y. Kim:** None.

Poster

350. Patterning, Cell Death, and Proliferation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 350.03

Topic: A.01. Neurogenesis and Gliogenesis

Support: WSU OUR Grant
WSU Neuroscience Program

Title: Apoptosis in the developing zebrafish retina studied in an animal model of human autism

Authors: *A. M. STEED[1], E. SANDQUIST[2], J. B. HUTCHINS[3];
[1]Neurosci., [2]Zoology, [3]Hlth. Sci., Weber State Univ., Ogden, UT

Abstract: Development of the zebrafish (*Danio rerio*) retina is well-researched. Apoptosis of overproduced neurons is an important part of wiring the retinotectal pathway. In the past, apoptosis has been assayed with terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) which requires sectioning, a time-intensive process. Although this is the traditional method of tracking apoptosis, it is not the only way. Molecular Probes Lysotracker™ is a lipophilic dye which enters cells by simple diffusion. The dye is protonated by the acid pH of the lysosome, which traps the now-fluorescent dye. The lysosome drives apoptosis (as well as autophagy and other processes). We have used Lysotracker as an alternative method for studying the time course of apoptosis in normal and altered development. Sifuentes-Romero et al. (2020) have used this method in the cavefish lens and we have adapted it to our system. The probe is user-friendly and is used in living samples. There is no sectioning required which keeps the tissue in its original form for visualization by confocal microscopy. We have used this protocol to study apoptosis in developing zebrafish retina between about 2 and 6 days post-fertilization (dpf). We were able to confirm penetration of the Lysotracker dye by identifying known sites of apoptosis during development. We then applied the Lysotracker technique to investigate the role of apoptosis in autism. Our zebrafish model of autism involves treatment of zebrafish embryos with valproic acid (VPA) between 0 and 2 dpf. Prenatal exposure to VPA treatment is known to increase the incidence of human autism, and in zebrafish, produces a developmental phenotype with some resemblance to human autism (Zimmermann et al. 2015; Meshalkina et al. 2018; Dwivedi et al. 2019). Autism is thought to result from abnormally increased synaptic survival. We hypothesized that VPA would increase activity in developing neurons, leading to increased synaptic stabilization with decreased apoptosis. After Lysotracker exposure at various points in development, zebrafish were preserved in 4% formaldehyde. The fixed fish were mounted in low-melt agarose and labeling was visualized in an Olympus Fluoview 3000 confocal laser scanning microscope. There was more staining in retinas from VPA-treated fish at each age compared to the control fish, suggesting that cell death may be increased by VPA treatment. This contradicts our initial hypothesis. Lysotracker staining is a useful alternative for monitoring apoptosis and/or autophagy in developing zebrafish retinas and other developing zebrafish tissues and will help describe the role of cell death in normal and disordered development.

Disclosures: A.M. Steed: None. **E. Sandquist:** None. **J.B. Hutchins:** None.

Poster

350. Patterning, Cell Death, and Proliferation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 350.04

Topic: A.01. Neurogenesis and Gliogenesis

Support: EMBO Fellowship ALTF 180-2019
T32 GM007365-44

The Leona M. and Harry B. Helmsley Charitable Trust
NIDDK P30DK042086
The Alfred P. Sloan Foundation
The McKnight Foundation
NIH R01EB028171

Title: Regional cytoarchitecture of the adult and developing mouse enteric nervous system

Authors: R. HAMNETT[1], L. B. DERSHOWITZ[1], V. SAMPATHKUMAR[2], Z. WANG[1], V. DE ANDRADE[3], N. B. KASTHURI[2], S. DRUCKMANN[1], ***J. A. KALTSCHMIDT**[1];
[1]Stanford Univ., Stanford, CA; [2]Univ. of Chicago, Chicago, IL; [3]Argonne Natl. Lab., Lemont, IL

Abstract: The ENS autonomously controls secretory and motor functions of the gastrointestinal (GI) tract and ENS dysfunction contributes to the morbidity of a broad range of GI and neuropsychiatric illnesses. The ENS contains more neurons than the spinal cord and presents a translational model relevant to many human illnesses. However, ENS circuit development, connectivity and function is vastly understudied relative to that of the spinal cord or brain. We have adapted and developed state-of-the-art means to visualize ENS neurons and map their connectivity. By imaging large areas of the ENS, we have observed that the clusters of neurons that coordinate muscle activity are radially oriented and organized into stripes traversing the circumference of the intestine. Using computational approaches we have revealed marked differences in stripe width and stripe spacing between regions of the intestine. We have further found that specific enteric neuronal subtypes, defined by expression of neurotransmitters, neuropeptides, and calcium-binding proteins, are distributed in a highly region-dependent manner. Together, our work extends knowledge of ENS topography and provides a possible mechanistic basis for region-specific motility patterns in health and disease.

Disclosures: R. Hamnett: None. **L.B. Dershowitz:** None. **V. Sampathkumar:** None. **Z. Wang:** None. **V. De Andrade:** None. **N.B. Kasthuri:** None. **S. Druckmann:** None. **J.A. Kaltschmidt:** None.

Poster

350. Patterning, Cell Death, and Proliferation

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 350.05

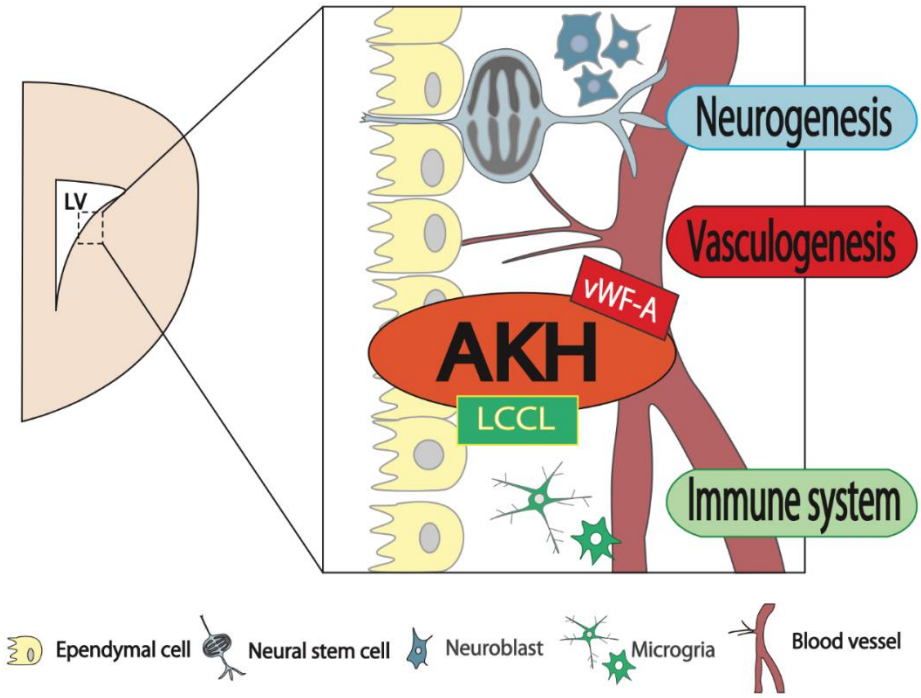
Topic: A.01. Neurogenesis and Gliogenesis

Support: JSS Grant (2022-4026)
IMEG Kumamoto University
SPRING Grant

Title: Akhirin, a Secreted Protein, Regulate Innate Immune Responses During Brain Development and Maintains Homeostasis in the Neural Stem Cell Niche.

Authors: *M. KUDO, N. MATSUO, T. IRIE, K. NAKASHIMA, K. OHTA;
Kyushu Univ., Kyushu Univ., Fukuoka, Japan

Abstract: Previously, we identified Akhirin (AKH) as a novel secretory molecule expressed in the chick embryo's lens epithelium. AKH contains one LCCL domain (involved in the innate immune response from bacterial infections in the inner ear) and two vWF domains (one of the famous blood coagulation factor), exhibits heterophilic cell adhesion property (Ahsan et al., 2005). AKH is expressed explicitly in the neural stem cell (NSC) niche region (microenvironment where NSCs are present) of the central nervous system (eye, spinal cord, and brain) (Ahsan et al., 2005, Athary et al., 2015, Anam et al., 2020). Our findings suggest that AKH is one of NSCs niche regulator and plays a crucial role in their development, however the detail molecular function of AKH in brain development is still unclear. Here, using an anti-AKH polyclonal antibody, we show that AKH is expressed in the neuronal ependymal cells layer, choroid plexus ependymal cells layer, and the cerebral spinal fluid at embryonic brain. Compared to wild-type mice, AKH knock out mice ($AKH^{-/-}$) have aberrant LV expansion and suppressed NSC proliferation, as well as behavioral abnormalities. Furthermore, we revealed the abnormal blood vessels formation and the increase of activated microglia in the $AKH^{-/-}$ brain. Recently, microglial association has been reported for maintenance of homeostasis in the NSC niche. To elucidate the reason behind microglial activation induced by AKH deficiency, we observed that AKH is involved in the innate immune response of the developing mouse brain. In the developing brain, the blood-brain barrier (BBB) is immature, and the maturation of the BBB is complex and remains unclear because it involves factors such as vascularization and glial maturation. We propose that AKH regulates innate immunity in the brain during development, when the brain's immune system is still underdeveloped, and is involved in maintaining homeostasis of NSC niche regulation to maintain normal brain development.



Disclosures: **M. Kudo:** None. **N. Matsuo:** None. **T. Irie:** None. **K. Nakashima:** None. **K. Ohta:** None.

Poster

350. Patterning, Cell Death, and Proliferation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 350.06

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant RO3HD10176701
NSF Graduate Research Fellowship

Title: NMDA receptor-mediated signaling is required for the regulation of neural progenitor cell proliferation in the forebrain of zebrafish larvae

Authors: ***A. J. NAPOLI**[1], J. D. ZOODSMA[1], B. BIJU[1], O. MUCOLLARI[1], S. SCHUBEL[1], C. APREA[1], A. SAYED[1], L. P. WOLLMUTH[2], H. I. SIROTKIN[2];
[1]State Univ. of New York At Stony Brook, Stony Brook, NY; [2]Neurobio. & Behavior, Stony Brook Univ., Stony Brook, NY

Abstract: Normal brain development depends upon precise spatiotemporal regulation of neural stem and progenitor cells (NSPC) to ensure that the appropriate number of neurons are generated and balanced neuronal wiring can emerge. Aberrations in this process contribute to neurodevelopmental disease (NDD) pathogenesis. N-Methyl-D-Aspartate receptors (NMDAR) are glutamate-gated cation channels known to play essential roles in neurodevelopment, yet their role in the regulation of neurogenesis is not fully understood. Some studies report suppression of NMDAR-mediated signaling promotes neurogenesis, while other studies support the opposite view. Discord in the literature is, in part, due to the perinatal lethality of NMDAR knockout in rodents, which precludes analysis of postembryonic neurodevelopment. We have developed a mutant zebrafish line that lacks all NMDAR-mediated signaling (*grin1* double mutant) yet survives to 10 days post fertilization (dpf), far beyond the comparable age of rodent models. Thus, our model provides an opportunity to examine the role of NMDARs in all stages of neurodevelopment. To assess the cytoarchitecture of the *grin1* double mutant brain at the end of embryonic neurogenesis, we performed detailed quantification of 12 forebrain cell populations in 3 dpf zebrafish. We found that relative to wild type fish, *grin1* double mutants show increased cell densities in the anterior regions of the forebrain. At 5dpf, increased cell densities were observed in all forebrain cell populations. We performed GFAP and PSA-NCAM Immunohistochemistry (IHC) and determined that these cells were neurons, not glial cells. Morphometric analysis demonstrated that increased neuronal density occurs without any gross anatomical changes to the brain. Activated caspase-3 IHC indicated that the supernumerary neurons did not result from diminished programmed cell death. We then assayed the NSPCs using IHC to determine the origin of dysregulation. We find that *grin1* double mutants have a higher percentage of mitotic neuroblasts, as indicated by PCNA expression, and are

predominantly clustered abventricurlarly. These data, taken together, imply a delay in their differentiation into mature neurons, which inappropriately prolongs their mitotic state and amplifies the neuronal population. These data suggest that NMDAR signaling is required for suppression of neurogenesis in the neuroblast transit amplifying cell populations, without which proliferation progressively increases unchecked. Furthermore, as NMDAR mutations are correlated with NDDs, as are supernumerary neurons, this work also suggests an unexplored mechanism of NMDAR-mediated NDD etiology.

Disclosures: **A.J. Napoli:** None. **J.D. Zoodsma:** None. **B. Biju:** None. **O. Mucollari:** None. **S. Schubel:** None. **C. Aprea:** None. **A. Sayed:** None. **L.P. Wollmuth:** None. **H.I. Sirotkin:** None.

Poster

350. Patterning, Cell Death, and Proliferation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 350.07

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant EY07060
NIH Grant EY07003
NIH Grant OD28612
Foundation Fighting Blindness

Title: In zebrafish, Il10 governs the proliferation of Müller glia-derived progenitors during the selective regeneration of photoreceptors

Authors: *M. NAGASHIMA[1], P. F. HITCHCOCK[2];
[2]Dept Ophthalmol, [1]Univ. of Michigan, Ann Arbor, MI

Abstract: Inflammation is the tissue-based response to invasive pathogens and cellular injury. Following an injury to the central nervous system, activated microglia release inflammatory cytokines, a diverse group of soluble factors that function to activate, amplify, and resolve inflammation. Il10 is an anti-inflammatory cytokine that plays a critical role in resolving neuroinflammation and promoting wound healing. In the zebrafish retina, which has the capacity to regenerate neurons and photoreceptors, acute inflammation is required to reprogram Müller glia, which function as an intrinsic stem cell, and regulate proliferation of the Müller glia-derived progenitors (MGDPs), the immediate antecedents of regenerated neurons. In this study we used wild-type (WT) zebrafish, *il10*$^{-/-}$ mutants, Q-PCR, and tissue-based proliferation assays to determine expression and function of Il10 during the selective death and regeneration of photoreceptors. Following photoreceptor death, in WT animals *il10* expression peaks at 2 days post lesion (dpl) and returns to baseline by 5 dpl. Q-PCR assays of *tnfa*, *tnfb*, and *il1b*, and their downstream target, *cjun*, show that these pro-inflammatory cytokines are significantly elevated in *il10*$^{-/-}$ mutants, indicating that in the zebrafish retina, IL10 is required to resolve the inflammatory response. At 5 and 7 dpl in WT animals, MGDPs have exited the cell cycle and are

differentiating into regenerated photoreceptors. In contrast, in *il10*$^{-/-}$ mutants, the MGDPs continue to proliferate. At 14 dpl in WT animals, regenerated photoreceptors have mature morphologies, whereas in the il10$^{-/-}$ mutants, photoreceptor morphology is markedly abnormal. Further, at this time point activated microglia persist in the subretinal space of il10$^{-/-}$ mutants. These results indicate that in the zebrafish Il10 governs the proliferation of Müller glia-derived progenitors by determining the duration of the pro-inflammatory response. Further, the persistent inflammation in *il10*$^{-/-}$ mutants compromises the maturation of regenerated photoreceptors.

Disclosures: M. Nagashima: None. **P.F. Hitchcock:** None.

Poster

350. Patterning, Cell Death, and Proliferation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 350.08

Topic: A.01. Neurogenesis and Gliogenesis

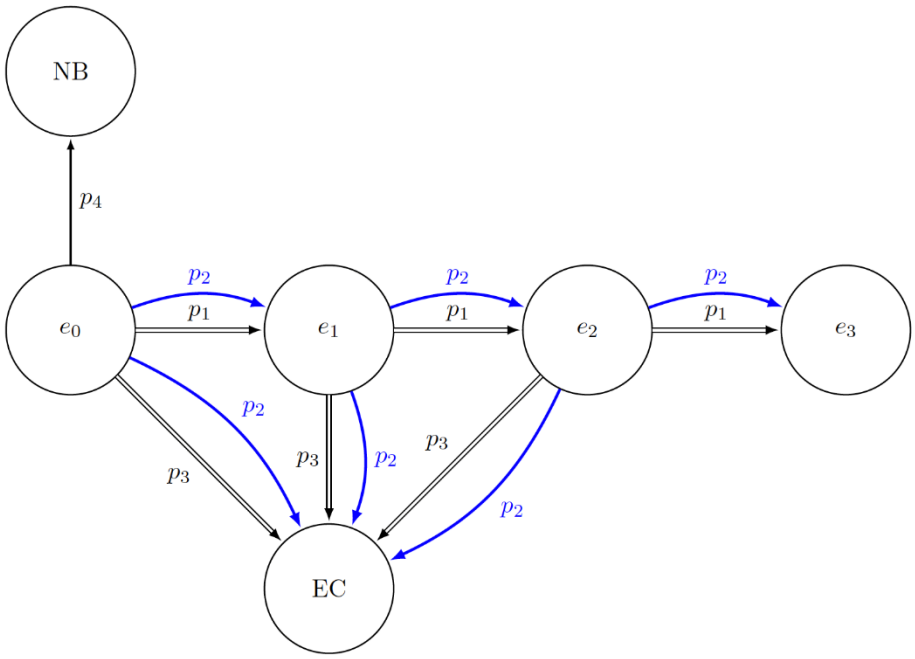
Support: NS110586
UCONN IBACS

Title: Predictive Modeling of Stem Cell Fate Decisions During Early Brain Development

Authors: *B. PARK[1], H. NGUYEN[2], M. MAJID[1], P. BRIODY[1], A. PAUL[1], M. MACEY[1], B. HAIM[2], J. CONOVER[1];
[1]Physiol. and Neurobio., [2]Dept. of Statistics, Univ. of Connecticut, Mansfield, CT

Abstract: How is an intricate pattern of stem cells and post-mitotic ependymal cells generated along the **lateral wall** of the lateral ventricle, while an uninterrupted monolayer of ependyma forms along the **medial wall**? In late embryonic development, neural stem cells give rise to ependymal cells forming a monolayer barrier along the ventricle surface. This process of ependymogenesis is likely achieved by two different modes of cell division: symmetric and asymmetric division. During embryonic to postnatal development, a combination of cell division strategies generates two different cytoarchitectures at the ventricle surface: 1) a monolayer of ependymal cells interspersed with the apical process of stem cells, forming pinwheel-like structures, across the lateral wall and 2) an uninterrupted ependyma along the medial wall. The goal of our project is to computationally predict stem cell lineage commitment decisions that give rise to the distinct cellular patterning observed along the lateral versus medial wall of the lateral ventricle. Our model, based on the Markov chain Monte Carlo (MCMC) method, predicts the changing cytoarchitecture of the lateral and medial walls by subjecting stem cells to 4 possible events: 1. symmetric division (two stem cells, *p1*) 2. asymmetric division (one stem cell and one ependymal cell, EC, *p2*) 3. terminal symmetric division (two EC, *p3*) 4. terminal neurogenesis (two neuroblasts, NB, *p4*). Each stem cell is assigned an ‘energy level’ (*e*) - a variable that defines the total number of cell divisions a single stem cell can undergo - that diminishes with every division. For initial data input, cell counts by type (stem cell or EC) at the

ventricle surface (n≥3) and ventricle surface area (n≥3) from 6 embryonic to postnatal periods are used. To support our model, cell lineage-based frequency of asymmetric versus symmetric division at the lateral ventricle surface are calculated using lineage-tracing mouse models with timed EdU labeling. MCMC predictive modeling will serve as a powerful program to determine cytoarchitectural changes during early brain development.



Disclosures: **B. Park:** None. **H. Nguyen:** None. **M. Majid:** None. **P. Briody:** None. **A. Paul:** None. **M. Macey:** None. **B. Haim:** None. **J. Conover:** None.

Poster

350. Patterning, Cell Death, and Proliferation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 350.09

Topic: A.01. Neurogenesis and Gliogenesis

Title: Continuous turnover of astrocytes-derived niches support long-term neurogenesis in the lesioned striatal parenchyma

Authors: *M. FOGLI[1], G. NATO[1], P. GREULICH[2], J. PINTO[1], P. PERETTO[1], A. BUFFO[1], F. LUZZATI[1];
[1]Univ. of Turin, Turin, Italy; [2]Univ. of Southampton, Southampton, United Kingdom

Abstract: In the adult brain, subsets of astrocytes act as neural stem cells in two anatomically defined neurogenic niches: the sub-ventricular zone and the hippocampal dentate gyrus.

Surprisingly, in specific physiologic and pathologic conditions, neurogenic progenitors can activate also in the mature brain parenchyma, particularly in the striatum. In this region, at least after excitotoxic lesion and stroke, these progenitors turned out to be local astrocytes, however little is known about the organization of neuronal genesis outside the canonical niches specialized microenvironment. Here, through genetic lineage-tracing, BrdU birth-dating analyses and 3D reconstructions coupled with mathematical modelling and spatial analyses, we thus evaluated the spatio-temporal dynamics of parenchymal astrocytes activation and lineage progression after excitotoxic lesion. Our results indicate that starting from the third week after lesion, neurogenic astrocytes are activated in a sparse and asynchronous manner preferentially around the lesion border. Once activated each astrocyte locally generate a cluster of clonally related proliferating neuronal precursors that transiently expand for about 10 days. The exhaustion of some of these niches however is continuously counterbalanced by the establishment of new ones, so that the total number of niches remains constant at least up to 8 weeks post lesion. The analyses of cellular composition revealed that striatal niches are initially composed of activated astrocytes and transient amplifying progenitors that further divide and differentiate into proliferating neuroblasts. Both cell types stochastically undergo symmetric divisions that are uncoupled from cell differentiation. Conversely, the differentiation rate of transit amplifying progenitors and proliferating neuroblasts deterministically increases in an exponential manner. Finally, post-mitotic neuroblasts initially accumulate in the niche before dispersing in the striatum as individual cells. Furthermore, spatial and clonal analyses indicated that striatal niches are independent of each other both regarding their astrocytic origin and their expansion and maturation dynamics.In conclusion, long-term striatal neurogenesis emerges from the asynchronous activation of multiple scattered striatal astrocytes stablishing transient independent neurogenic niches. Overall, these data suggest that the neurogenic potential is widespread among striatal astrocytes, and that the striatal parenchyma is largely permissive for de-novo establishment of neurogenic niches.

Disclosures: **M. Fogli:** None. **G. Nato:** None. **P. Greulich:** None. **J. Pinto:** None. **P. Peretto:** None. **A. Buffo:** None. **F. Luzzati:** None.

Poster

351. Cell Lineage, Fate, and Migration

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 351.01

Topic: A.01. Neurogenesis and Gliogenesis

Support: ERC BRAIN-MATCH 819894
FP7 2007-2013 615253

Title: Single nucleus RNA sequencing atlas of human hindbrain development identifies cellular origins of pediatric brainstem tumors

Authors: *P. JOSHI[1,2], K. OKONECHNIKOV[1,2], M. SEPP[3], I. SARROPOULOS[3], K. LEISS[3], H. KAESSMANN[3], L. M. KUTSCHER[1,2], S. M. PFISTER[1,2,4];
[1]Deutsches Krebsforschungszentrum, Heidelberg, Germany; [2]Hopp-Children's Cancer Ctr. (KiTZ), Heidelberg, Germany; [3]ZMBH Univ. of Heidelberg, Heidelberg, Germany; [4]Heidelberg Univ. Hosp., Heidelberg, Germany

Abstract: The lower brainstem (pons and medulla oblongata) is a key central nervous system structure involved in communication between the brain and the spinal cord, integrating sensory information and coordinating motor responses. Dysregulation in hindbrain development may lead to tumor formation and frequently leads to behavioral disorders, stressing the importance of understanding the intricacies of human hindbrain development. Here we performed RNA-sequencing of over 300,000 single nuclei to construct a developing human hindbrain atlas ranging from embryonic to adult stages, profiling complete neural and glial populations of the hindbrain, from progenitors to mature cell types. We complemented the transcriptomic atlas with single nuclei ATAC atlas from same or stage matched samples. We describe the transcriptional and regulome identity of neuronal cells constituting hindbrain nerve centers including precerebellar, respiratory, auditory and vestibular nuclei, and their differentiation trajectory from the progenitor domains in the embryonic rhombencephalon. To identify the origins of pediatric brain tumors, we mapped ~2,900 bulk and ~50 published single-cell transcriptome tumor datasets to the atlas of brainstem development, focusing on medulloblastoma and pediatric low- and high-grade glioma, and determined cell populations and differentiation states associated with specific tumor groups. We confirmed migrating lower rhombic lip derivatives as the lineage of origin for WNT medulloblastoma. Furthermore, we ascertained the early ventral neural progenitor-derived oligodendrocyte lineage as the source of diffuse midline glioma, H3 K27-altered and postnatal oligodendrocyte progenitors as the origin of pilocytic astrocytoma. We also identified the ependymal lineage as the best match for ependymomas. We show that tumor development has a component that transcriptomically follows its cellular lineage of origin, and identify genes with preserved expression between the respective normal and malignant cell populations. We also identify tumor-specific genes, which represent promising therapeutic candidates. We plan to make our data and analysis pipeline publicly available via user-friendly graphical interface for unrestricted use of the generated results.

Disclosures: **P. Joshi:** A. Employment/Salary (full or part-time):; DKFZ. **K. Okonechnikov:** A. Employment/Salary (full or part-time):; DKFZ. **M. Sepp:** A. Employment/Salary (full or part-time):; ZMBH, University of Heidelberg. **I. Sarropoulos:** A. Employment/Salary (full or part-time):; ZMBH, University of Heidelberg. **K. Leiss:** A. Employment/Salary (full or part-time):; ZMBH, University of Heidelberg. **H. Kaessmann:** A. Employment/Salary (full or part-time):; ZMBH, University of Heidelberg. **L.M. Kutscher:** A. Employment/Salary (full or part-time):; DKFZ. **S.M. Pfister:** A. Employment/Salary (full or part-time):; DKFZ, University of Heidelberg.

Poster

351. Cell Lineage, Fate, and Migration

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 351.02

Topic: A.01. Neurogenesis and Gliogenesis

Support: NINDS Grant K08NS099502

Title: Developing hindbrain motor neurons show spatial and temporal transcriptomic diversity mapping to wiring decisions

Authors: *M. ROSE[1], A. P. TENNEY[2], T. P. RAY[2], D. CREIGHTON[2], A. GELBER[4], M. TISCHFIELD[5], E. MURRAY[6], T. COLLINS[2], A. A. NUGENT[2], P. ANG[2], S. C. IZEN[7], M. BAUER[2], W. HUANG[8], R. SATIJA[9], O. ROZENBLATT-ROSEN[6], E. MACOSKO[10], F. CHEN[6], A. REGEV[6], E. ENGLE[3];
[1]Univ. of California Irvine, Irvine, CA; [3]Neurol. Res. - Engle Lab., [2]Boston Children's Hosp., Boston, MA; [4]Bioengineering, UCSD, San Diego, CA; [5]Cell Biol. and Neurosci., Rutgers Univ., Warren, NJ; [6]The Broad Inst., Cambridge, MA; [7]Univ. of Massachusetts Boston, Boston, MA; [8]MIT, Cambridge, MA; [9]New York Genome Ctr., New York, NY; [10]Stanley Ctr. for Psychiatric Res., Broad Inst., Brookline, MA

Abstract: The brainstem ocular motor neurons (OMNs) mediate eye movements and are differentially affected in some disorders, compared with other motor neurons (MNs). In congenital cranial dysinnervation disorders (CCDDs) such as Duane Syndrome, OMN subpopulations show disrupted or aberrant innervation, while in Amyotrophic Lateral Sclerosis (ALS), OMNs continue to function while other MNs degenerate. Here we define unique gene expression patterns among developing MNs, and generate a toolbox of protocols and genetic markers to help study these disorders. We combine various mouse genetic reporter lines with intersectional temporal and spatial transcriptomics (bulk-, single cell-, and single nuclei RNA-seq, and Slide-seq) to isolate and compare eight distinct mouse MN populations from embryonic days E9.5-E18.5: the three ocular motor nuclei (CN3, CN4, CN6) and the other primary MN types (CN5, CN7, CN9/10, CN12 in brainstem, and spinal MNs). Gene expression was validated with database analysis, *in situ* hybridization, antibodies, and genetic axonal labeling. We correlate gene expression differences with cell age by both EdU labeling and tamoxifen-mediated temporal CreER induction, and visualize iDISCO- and EyeDISCO-cleared whole embryos by light sheet microscopy. Each MN population shows a unique genetic fingerprint, including novel markers of spatially- and temporally-distinct OMN subpopulations. Some OMN nerve branches correspond with cell birthdate and selectively contribute to specific aberrant branches in the *Mafb*-knockout mouse model of Duane Syndrome. Overall, this MN transcriptomic atlas uncovers distinct developmental gene expression patterns and markers of the various cranial motor neurons, and provides new tools to study their differential vulnerability in the CCDDs and other motor neuron disorders.

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Poster

351. Cell Lineage, Fate, and Migration

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 351.03

Topic: A.01. Neurogenesis and Gliogenesis

Support: MD/PhD Endowment Grant
5R01NS118580-02
5R01NS118580-01S1
1F31NS120608-01A1

Title: Mapping the Cellular Composition of Resected Cortical Tubers and Perituberal Tissues

Authors: *J. S. ARCENEAUX[1], R. KHURANA[2], A. A. BROCKMAN[2], M.-B. L. CHALKLEY[2], L. C. GEBEN[3], R. P. CARSON[3,4,5], K. C. ESS[2,4,5], R. A. IHRIE[2,6];
[1]Biochemistry, Cancer Biology, Neuroscience, and Pharmacol., Meharry Med. Col. / Vanderbilt Univ., Nashville, TN; [2]Cell and Developmental Biol., [3]Pharmacol., Vanderbilt Univ., Nashville, TN; [4]Neurol., [5]Pediatrics, [6]Neurolog. Surgery, Vanderbilt Univ. Med. Ctr., Nashville, TN

Abstract: Tuberous sclerosis complex (TSC) arises due to heterozygousmutations in TSC1 or TSC2 and affects approximately 1 in 6000 births.Neuropsychiatric symptoms of this disorder include autism spectrum disorder(ASD), developmental delay, intellectual disability, and epilepsy. Mutations inTSC2 are often associated with worse symptoms and severity. Epilepsy in TSC patientsis often refractory to drug treatment, sometimes requiring surgical resection.Within resected brain tissues from patients with TSC, detection of enlarged“balloon” cells is diagnostic for this disorder. Analysis of tubers andperituberal tissues indicates seizures in TSC originate in the perituberaltissues, and “balloon” cells may contain loss of heterozygosity (LOH) of TSC1/2compared to surrounding tissue. Though mutations in TSC1/2 lead to epilepsy andcause mTORC1 hyperactivation, unified criteria to identify balloon cells andinfer their lineage are lacking, and these diagnostic cells have not beenstudied across TSC cohorts at the protein level. In addition, how balloon cellsinfluence their microenvironment to produce epileptogenic foci is poorlyunderstood. High-dimensional approaches such as imaging mass cytometry(IMC) offer the opportunity to directly assess thirty (30+) proteins andsignaling events in single cells while documenting spatial relationships withinthe tissue. We developed a custom imaging panel, where each of thirty-five (35)antibodies was successfully tested on known positive and negative controls,including pharmacological manipulations of signaling proteins in human tissuesand cells. We developed a customized machine-learning workflow that identifiesprospective balloon cells with 93% precision and 69% efficiency within archivedcortical tubers. Currently, we are mapping the cytoarchitecture and signalingperturbations within these samples, with a specific focus on balloon cells andtheir immediate neighbors. These data will comprise a rich dataset forunderstanding the abundance, structure, and signaling activity of neuronal,glial, and immune cells within archived tubers and perituberal tissues,enabling quantitative comparison of TSC with other mTORopathies.

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Poster

351. Cell Lineage, Fate, and Migration

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Topic: A.01. Neurogenesis and Gliogenesis

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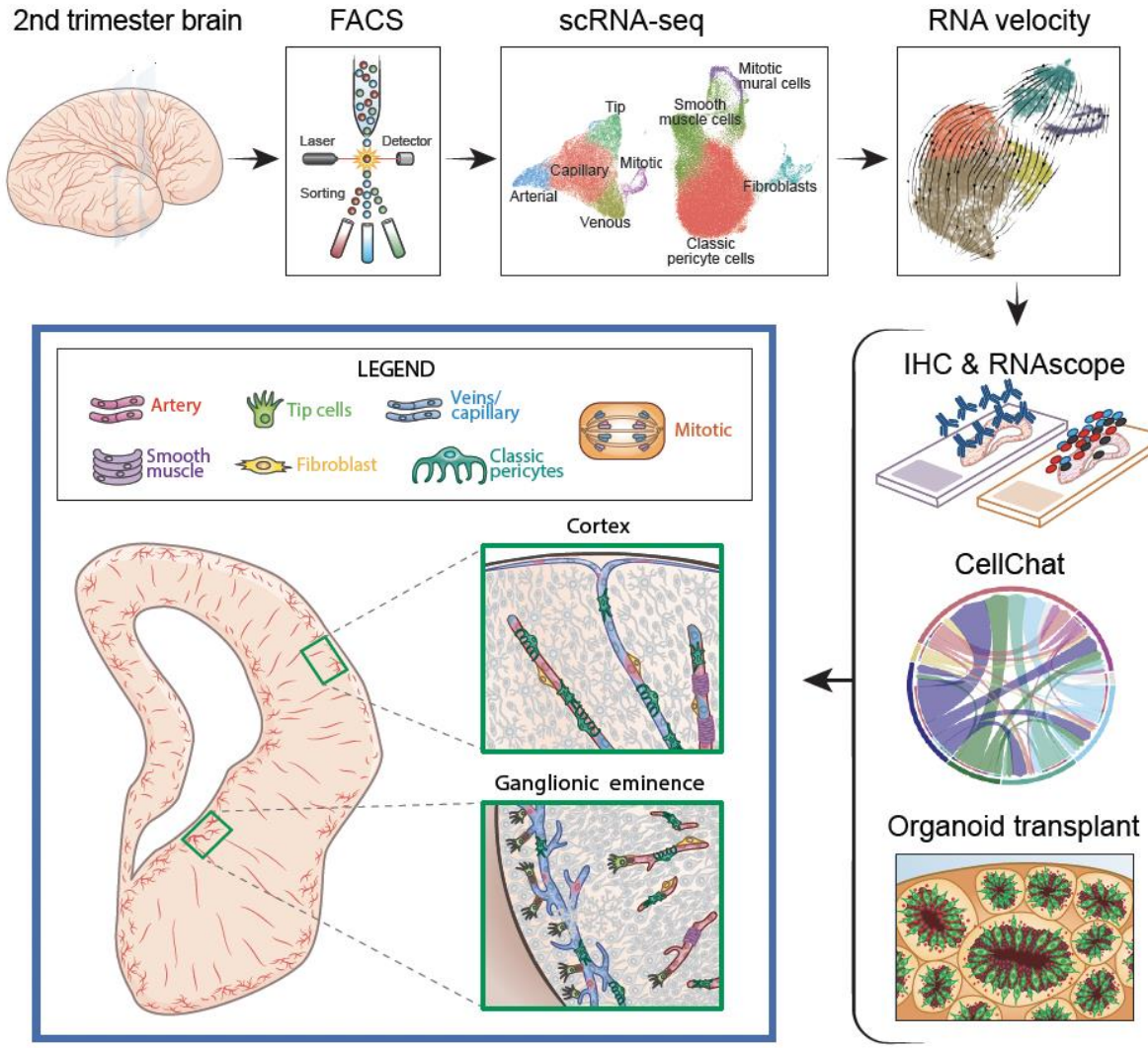
NIH K99 NS111731
NIH F31 F31NS117121
NIH K99 MH125329
SFSU EDUC2-12693

Title: Ensembles of endothelial and mural cells promote angiogenesis in prenatal human brain

Authors: *E. E. CROUCH[1], A. BHADURI[2], M. G. ANDREWS[3], A. CEBRIAN-SILLA[1], L. N. DIAFOS[1], J. OCHOA BIRRUETA[1], K. WEDDERBURN-PUGH[1], E. J. VALENZUELA[1], N. BENNETT[1], U. EZE[1], C. SANDOVAL-ESPINOSA[1], J. CHEN[1], C. N. MORA[1], J. M. ROSS[1], C. E. HOWARD[1], M. F. PAREDES[1], M. HAEUSSLER[4], K. NAKAMURA[1], A. ALVAREZ-BUYLLA[1], J. GARCIA-VERDUGO[5], A. R. KRIEGSTEIN[1], E. J. HUANG[1];
[1]UCSF, San Francisco, CA; [2]Univ. of California Los Angeles, MARINA DEL REY, CA; [3]Arizona State Univ., Tempe, AZ; [4]Univ. of California Santa Cruz, Santa Cruz, CA; [5]Univ. de València, Valencia, Spain

Abstract: Preterm infants born during the second trimester are prone to develop brain hemorrhage. Why the brain vasculature is vulnerable in this developmental window remains unclear, but infants with this condition are at risk for life-long neurodevelopmental disability. Using fluorescence-activated cell sorting, single-cell transcriptomics, and histological and ultrastructural analyses, we showed that an ensemble of endothelial and mural cell subtypes tiled the human prenatal brain vasculature during this critical period. These vascular cells followed distinct developmental trajectories and utilized diverse signaling mechanisms, most notably the extracellular matrix and growth factor Midkine, to facilitate cell-cell communication and maturation. Interestingly, our results revealed that tip cells, a subtype of endothelial cells, were highly enriched near the ventricular zone, the site of active neurogenesis. Indeed, when transplanted into cortical organoids, the prenatal vascular cells exhibited restricted lineage development that favored tip cells, promoted neurogenesis, and reduced cellular stress in neurons. Together, our results uncovered important mechanisms into vascular development and their implications for neural development during the second trimester.



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Poster

351. Cell Lineage, Fate, and Migration

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Program #/Poster #: 351.05

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant

Title: The fly larval nerve cord on a budget - preparing for the future while taking care of the present needs

Authors: *T. NGUYEN[1], R. VICIDOMINI[1], S. CHOUDHURY[2], T. HAN[1], T. BRODY[1], M. SERPE[1];
[1]NICHD/NIH, Bethesda, MD; [2]All India Inst. of Med. Sci. (AIIMS), New Delhi, India

Abstract: The insects ventral nerve cord (VNC), the equivalent of mammal the spinal cord, coordinates and integrates neural signaling from brain and periphery nervous system to produce a variety of locomotor outputs. Holometabolous insects, which undergo complete metamorphosis, need very different VNC functions, one for controlling crawling during the larval stages and another one for enabling the adult complex locomotor activities. How do insects develop and manage two different VNC? In flies, an atlas of the adult VNC has been assembled. However, the molecular characterization of the cells in third instar VNC remains to be determined.Here we use scRNA-seq to profile the transcriptome of 31,040 single cells from *Drosophila* third instar VNCs. Surprisingly, we found that almost 60% of these cells (17,920) are immature and undergoing a developmental program that will generate the adult VNC. These populations of cells include newborn adult interneurons (INs) and motor neurons (MNs), glial precursors and midline glia. The remaining cells are mature INs, MNs, and glia that fulfil various functions during the larval stages of development. Among the immature cells, we identified a transcription progression from neuroblasts to newborn adult neurons. We found that all 21 hemilineages (LINs) reported in adult VNCs have correspondent clusters of newborn neurons in the larval VNC. Moreover, each larval LIN has already acquired neurotransmitter identity and has its own repertoire of cell fate determinants. Almost 40% of the larval VNC cells are mature neurons and glia that control larval behavior and function. We identified over 40 subtypes of differentiated INs, each expressing unique combinations of transcription factors and neurotransmitters. Interestingly, larval INs are very different from the future adult neurons. We described the molecular signature of mature glia subtypes, including astrocytes, perineural glia, subperineurial glia, cortex glia and ensheating glia. Each glia type has unique features to support different aspects of neuronal development and function. The larval glia populations are very similar to the adult counterpart, highlighting the conserved nature of the glia functions. Finally, we identified and compared immature and mature MNs. In contrast with mature MNs, immature MNs have low level of glycolysis related genes, low level of genes encoding synaptic proteins, and express no BMP target genes. Our studies uncover a surprising partition of the larval VNC which prepares the cells for the adult VNC while ensuring that larval needs are met. This larval VNC atlas provide a valuable resource for future studies of neurodevelopment and behavior.

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Poster

351. Cell Lineage, Fate, and Migration

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Vision of Children
Fight for Sight Postdoctoral Fellowship
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Title: Defects in neurogenesis in the retinal ciliary margin alter the binocular circuit

Authors: N. SLAVI, R. BALASUBRAMANIAN, M. A. LEE, M. LIAPIN, ***C. MASON**; Zuckerman Inst., Columbia Univ., New York, NY

Abstract: In animals with frontally positioned eyes, partial decussation of retinal ganglion cell (RGC) axons in specific proportions at the optic chiasm forms the basis of stereopsis. The albino visual system is characterized by a reduction in the number of RGCs expressing Zic2, the transcription factor necessary for ipsilateral specification, and by abnormal distribution of ipsilateral and contralateral RGC axons. To uncover the developmental processes important for the establishment of ipsi- and contralateral RGC properties, and ultimately stereo vision, we examined neurogenesis and its regulation by CyclinD2 in the ciliary margin zone (CMZ), a neurogenic niche at the embryonic retina periphery that may be a specialized source of ipsilateral RGCs.

We combined genetic inducible fate mapping with birthdating to determine the number and production rate of CMZ-derived $Zic2^+$ RGCs. We performed dual pulse birthdating to monitor the cell cycle progression and exit of CMZ progenitors, and its regulation by CyclinD2 in pigmented, albino, and CyclinD2-deficient mice. We find that in the albino fewer $Zic2^+$ RGCs are generated from the CMZ, where there is reduced CyclinD2 expression and delayed cell cycle progression. In addition, deletion of CyclinD2 from the pigmented CMZ leads to a reduced number of progenitors reaching mitotic exit in time to express Zic2 and acquire ipsilateral RGC identity. In turn, upregulation of CyclinD2 via pharmacological stimulation of calcium channels in albino mice augments neurogenesis of ipsilateral RGCs.

To examine whether aberrations in the ipsi/contra RGC output lead to impaired depth perception, we compared the performance of pigmented, albino, and CyclinD2-deficient mice in a binocularly-driven visual cliff behavioral task. Both albino and CyclinD2-deficient mice failed to

reliably recognize the “cliff”, indicating reduced depth perception. Our study thus links proper RGC neurogenesis with the establishment of the circuit for binocular vision.

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Poster

351. Cell Lineage, Fate, and Migration

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NRF Korea Grant 2020R1C1C101024513
NRF Korea Grant 2020R1A3A300192913
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Title: Ano1 regulates neural stem cell fate and underlying physiological evidences in forebrain development

Authors: K. KIM[1], B. KANG[1], P. LEE[1], M.-S. KIM[1], H.-Y. KIM[1], *U. OH[2], G. HONG[3]; [1]Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of; [2]Korea Inst. of Sci. and Technol. (KIST), [3]Neurosci., Korea Inst. of Sci. and Technol. (KIST), Seoul, Korea, Republic of

Abstract: The mammalian forebrain is associated with motor function, emotion and cognition by processing high-level thinking. Malformation of the forebrain incurs disruption of neural circuits correlated with psychiatric and developmental disorders including anxiety, depression, schizophrenia, epilepsy, autism spectrum disorder and Timothy syndrome. The fine regulation of neurogenesis by neural stem cells (NSCs) are mediated by neurotrophic factors and genes including sonic hedgehog, Wnt proteins, glutamate, ATP, gamma-aminobutyric acid (GABA), epidermal growth factor (EGF) and fibroblast growth factor (FGF). Here, we report new findings of the molecular mechanism of anoctamin 1 (ANO1), a Ca^{2+}-activated Cl^- channel including activation mechanism of ANO1 and underlying other ion channel activity in specialized NSCs of the mouse embryonic ventricular zone. Further results of cell biological and physiological evidences from Ano1 knock-out mouse will be presented.

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Poster

351. Cell Lineage, Fate, and Migration

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant 1R01NS114578-01
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Jean and George Brumley Jr. Neonatal Perinatal Research Institute
AAP Marshall Klaus Perinatal Research Award 2020

Title: Breast milk-associated oxysterol reverses neonatal white matter injury through Gli2-dependent oligodendrogenesis

Authors: *A. S. CHAO[1], P. MATAK[1], K. PEGRAM[1], L. DUBOIS[2], J. W. THOMPSON[2], V. JAIN[3], N. YOUNGE[1], S. GREGORY[3], R. N. GOLDBERG[1], E. J. BENNER[1];
[1]Dept. of Pediatrics, Div. of Neonatology, Duke Univ. Sch. of Med., Durham, NC; [2]Duke Proteomics and Metabolomics Shared Resource, Dept. of Pharmacol. and Cancer Biol., [3]Duke Mol. Physiol. Inst., Duke Univ., Durham, NC

Abstract: White matter injury (WMI) is the most common brain injury leading to poor neurologic outcomes in premature infants. Intestinal perforation is a significant risk factor and there are no treatment options available. Developing novel drug therapies for neonates is challenged by appropriate concerns for safety. Thus using mass spectrometry, we discovered sonic hedgehog (Shh) agonist oxysterols in human maternal breast milk. We explored their therapeutic potential in directing neural stem cells (NSCs) into the oligodendrocyte (OL) lineage and in promoting oligodendrocyte progenitor cell (OPC) maturation. Oxysterol-induced oligodendrogenesis from subventricular zone-derived (SVZ) NSCs was assayed *in vitro* using immunocytochemistry, western blot, flow cytometry, and single cell sequencing (scRNA-seq), looking for markers of the OL lineage. Shh pathway activation was established by quantifying upregulation of target gene, Gli1, by western blot and RT-PCR. Shh-dependence was explored using multiple pharmacologic approaches and Gli1 and Gli2 (Shh effectors) knockout alleles. Maturation effects of oxysterols was analyzed in OPCs using Sholl analysis and scRNA-seq. Neonatal modeled intestinal perforation (MIP) leading to diffuse WMI was induced in mice on postnatal day 5 (P5). MIP mice were treated with vehicle alone or oxysterol (20-hydroxycholesterol, 20HC). Stereology was used to determine numbers of OPCs and mature OL numbers in the periventricular white matter. Nestin-CreER^{T2} alleles were used to lineage trace postnatal NSCs in treated and untreated mice post-MIP to identify oxysterol-induced oligodendrogenesis *in vivo*. Myelination was evaluated by performing g-ratios on electron microscopy images of the corpus callosum at P60. CatWalk gait analysis system analyzed motor function in mice at P60. We found that Gli2 is required for 20HC-induced oligodendrogenesis. Following neonatal WMI *in vivo*, 20HC treatment increased numbers of mature OLs, improved myelination and rescued motor deficits in mice. Lineage tracing showed that 20HC-mediated recovery of OL deficit is mediated in part through 20HC-induced SVZ-derived oligodendrogenesis. Additional recovery may be due to the impact of 20HC on OPC maturation. ScRNA-seq data on 20HC treated OPCs are currently undergoing analysis. Because oxysterols are found in human maternal breast milk, this approach may be a novel and safe therapeutic strategy to mitigate neonatal WMI.

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Poster

351. Cell Lineage, Fate, and Migration

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Program #/Poster #: 351.09

Topic: A.01. Neurogenesis and Gliogenesis

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National Science Foundation [DGE-1845298 (E.M.P.)]
Department of Veterans Affairs [RR&D Career Development Award IK2-RX003376 (J.C.O.)]

Title: A tissue-engineered rostral migratory stream as an in vitro platform to investigate subventricular zone-derived neuroblast migration

Authors: ***E. M. PURVIS**[1,2,3], A. D. GARCIA-EPELBOIM[1,2], J. C. O'DONNELL[1,2], D. K. CULLEN[1,2,4];
[1]Neurosurg., Ctr. for Brain Injury & Repair, Univ. of Pennsylvania, Philadelphia, PA; [2]Ctr. for Neurotrauma, Neurodegeneration & Restoration, Corporal Michael J. Crescenz Veterans Affairs Med. Ctr., Philadelphia, PA; [3]Neurosci., Perelman Sch. of Medicine, Univ. of Pennsylvania, Philadelphia, PA; [4]Bioengineering, Sch. of Engin. and Applied Sciences, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Brain injury can result in long-term neuronal loss that is exacerbated by the limited regenerative capacity of the central nervous system. The rostral migratory stream (RMS) facilitates neuroblast migration from the subventricular zone (SVZ) to the olfactory bulb throughout adulthood. Brain lesions attract neuroblast migration out of the RMS, but resultant regeneration is insufficient without intervention. Our lab has biofabricated a “living scaffold” that is implanted to enhance and redirect endogenous neuroblast migration from the subventricular zone to neuron-deficient brain regions. This approach utilizes the first biomimetic tissue-engineered RMS (TE-RMS), designed to leverage the brain’s natural mechanism for sustained neuronal replacement by replicating the native RMS to direct neuroblasts to distal sites of injury. Our previous work has characterized the structure of the TE-RMS fabricated from rat cortical astrocytes (rat TE-RMS) and human gingiva mesenchymal stem cell astrocytes (human

TE-RMS). In addition to a promising strategy for endogenous neuronal replacement, the TE-RMS is a powerful tool to unlock previously unanswered questions about adult neurogenesis. Here, we report recent advancements in rat TE-RMS fabrication techniques that promote robust TE-RMS health and structural stability up to 14 days *in vitro*. Additionally, we demonstrate that we can harvest neuronal progenitor cells (NPCs) from the SVZ of adult female rats and culture them as neurospheres. We have confirmed NPC phenotype with reliable positive expression of stem cell markers Nestin, PAX6, SOX2, and GFAP, and negative expression of mature neuronal marker MAP2 and mature oligodendrocyte marker MBP. Furthermore, following loading of SVZ-derived neurospheres into the end of TE-RMS constructs, individual neuroblasts migrate out of the neurosphere and throughout the length of 4 mm TE-RMS constructs. In contrast, acellular constructs did not facilitate neuroblast migration, suggesting active cell-cell signaling mechanisms that contribute to migration along engineered "living scaffolds". Current experiments are examining maturation and synaptic integration of SVZ-derived neuroblasts following migration through the TE-RMS and into destination cultures *in vitro*. Additionally, we are engineering this system with all human cell sources, thereby creating the first model system to investigate the process of adult neurogenesis in the human brain. Overall, the TE-RMS provides a unique *in vitro* platform to examine SVZ neuroblast migration and maturation, providing an avenue to investigate key chemical and molecular players in these processes.

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Poster

351. Cell Lineage, Fate, and Migration

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Topic: A.01. Neurogenesis and Gliogenesis

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USD Graduate Student and Creative Scholarship Grant

Title: Investigating the role of RNA localization in MAP1B's function in neuronal migration

Authors: *C. KITTOCK[1,2], L.-J. PILAZ[1,3];
[1]Pediatrics and Rare Dis. Group, Sanford Res., Sioux Falls, SD; [2]Basic Biomed. Sci., [3]Dept. of Pediatrics, Univ. of South Dakota Sanford Sch. of Med., Vermillion, SD

Abstract: RNA localization and subsequent local translation is animportant mechanism in maintaining polarized protein localization in matureneurons. However, the role of RNA localization in migrating neurons is unknown.We seek to uncover this process by using candidate RNA *Map1b*, coding for a microtubule associated protein important inbrain development, and an mRNA target of the RNA binding protein FMRP. Loss ofeither FMRP or MAP1B causes neuron migration defects in both humans and mice,and FMRP-mediated localization of *Map1b*is important in axon growth. Here we use Breasi-CRISPR and *in utero* electroporation (IUE) to investigate FMRP and MAP1Blocalization and dynamics in the developing mouse cortex. Breasi-CRISPRdemonstrates localization of FMRP and MAP1B to the leading process of migratingneurons. MAP1B is also very prevalent in axons. Live imaging of FMRP-EGFP showsthat in migrating neurons, FMRP is in the leading edge of migrating neurons,and is found at branch points along neurons. Follow up studies will includelive imaging of tagged *Map1b* mRNA inthe presence or absence of FMRP. Together, these data will improve ourunderstanding of the basic mechanisms of neuronal migration, and how theirperturbation leads to neuronal migration disorders.

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Poster

351. Cell Lineage, Fate, and Migration

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Topic: A.01. Neurogenesis and Gliogenesis

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Keio Gijuku Academic Development Funds
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The Uehara Memorial Foundation
SENSHIN Medical Research Foundation (SMRF)

Title: Elucidation of migration profiles of claustral neurons during brain development

Authors: *K. OSHIMA[1], S. YOSHINAGA[1,2], A. KITAZAWA[1,2], Y. HIROTA[1], K. NAKAJIMA[1], K.-I. KUBO[1,2];

[1]Dept. of Anat., Keio Univ. Sch. of Med., Tokyo, Japan; [2]Dept. of Anat., The Jikei Univ. Sch. of Med., Tokyo, Japan

Abstract: The claustrum is a cluster of neurons located between the insular cortex and the striatum. Many studies have shown that the claustrum plays an important role in higher brain function. Additionally, there is a growing body of evidence that claustral dysfunction is associated with neuropsychological symptoms. However, how the claustrum is formed during development is not fully understood. For instance, migration profiles of claustral neurons are largely unknown. In the present study, we tried to analyze the development of the mouse claustrum, especially focusing on the migration profiles of claustral neurons. First, we analyzed migration profiles of the claustral neurons by taking advantage of the FlashTag technology, in which fluorescent dyes were injected into the ventricle of the developing forebrains. Our analyses showed that the claustral neurons were mainly generated between embryonic day (E) 10.5 and E12.5 and that some claustral neurons first migrated radially outward and then changed their direction inward after reaching the surface. Next, we confirmed these unique migration profiles by performing time-lapse imaging of GFP-labeled cells. We considered that these migration behaviors of the claustral neurons showed a sharp contrast to those of neurons in the insular cortex, which migrate just outward. Lastly, we will discuss molecular mechanisms that underlie migration profiles of the claustral neurons.

Disclosures: K. Oshima: None. **S. Yoshinaga:** None. **A. Kitazawa:** None. **Y. Hirota:** None. **K. Nakajima:** None. **K. Kubo:** None.

Poster

351. Cell Lineage, Fate, and Migration

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 351.12

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH/NINDS 1R56NS094589
Rutgers Busch Biomedical Grant AWD00009650

Title: P75ntr prevents the onset of cerebellar granule cell migration via rhoa activation

Authors: *J. ZANIN[1], W. J. FRIEDMAN[2];
[1]Rutgers the State Univ., Newark, NJ; [2]Rutgers, The State Univ. of NJ - Newark, Newark, NJ

Abstract: Neuronal migration is a fundamental process during nervous system (NS) development. The assembly of functional neuronal circuits highly relies on it; thus, several neurodevelopmental disorders can be traced back to dysregulated migration. Although substantial efforts have been placed in identifying molecular signals that stimulate migration, little is known about potential mechanisms that restrict migration. Yet, restrictive mechanisms are also essential for proper nervous system development for several reasons. 1) It helps coordinate the timing for each neuronal population to arrive and establish proper connections. 2)

It contributes to maintaining a pool of progenitors by preventing migration away from a proliferative niche. 3) It prevents immature progenitors to start migrating earlier. Therefore, there is a need to study how the restrictive migratory mechanism works to complete our understanding of how neuronal migration is regulated. The external granule layer (EGL) of the cerebellum is a proliferative layer that gives rise to the cerebellar granule cells (CGNs) during the first two postnatal weeks in rodents. Upon cell cycle exit, the CGN migrates radially to establish the internal granule cell layer (IGL) of the cerebellum. The CGNs outnumber any other cell type in the brain. To achieve this massive number of neurons, a highly coordinated mechanism to regulate the proliferation/migration transition must be in place. The p75 neurotrophin receptor (p75NTR) is expressed in multiple neural populations, including the developing cerebellum. Depending on the cellular context p75NTR has been implicated in different aspects of nervous system development, including neuronal survival, proliferation, and apoptosis. In the present study, using *in vitro* and *in vivo* mouse and rat models, we show that proliferating granule cell precursors (GCPs) robustly express p75NTR in the EGL during postnatal development; however, migrating GCPs, either from the rhombic lip during embryogenesis or from the EGL during postnatal development, downregulate the expression of this receptor. The expression of p75NTR was sufficient to prevent the migration of the granule cells even in the presence of BDNF, a well-established chemotactic signal for this cell population. Additionally, we demonstrated that p75NTR prevented GCP migration by maintaining elevated levels of active RhoA. Our findings suggest that the expression of p75NTR might be a critical signal that stops and maintains the GCPs in the proliferative niche of the EGL, promoting the clonal expansion of cerebellar granule neurons.

Disclosures: J. Zanin: None. **W.J. Friedman:** None.

Poster

351. Cell Lineage, Fate, and Migration

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 351.13

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant NS089943

Title: PCDH12 regulates neuronal migration through ADAM10-mediated ectodomain shedding and membrane recruitment of cytoskeleton regulators

Authors: *M. EPSTEIN, J. RAKOTOMAMONJY, G. YANG, L. RYLAARSDAM, A. GUEMEZ-GAMBOA;
Neurosci., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL

Abstract: The *PCDH12* gene codes for the protocadherin-12 (PCDH12) transmembrane protein, a cellular adhesion molecule that is part of the protocadherin (PCDH) family. Bi-allelic loss of function variants in *PCDH12* result in impaired neural development and are implicated in several

neurodevelopmental disorders, including microcephaly, epilepsy, and developmental disability. Here, we investigated the effects of *PCDH12* deletion in neural progenitor cells (NPCs) derived from human stem cells. We showed that PCDH12 absence affects cell migration, as NPCs lacking the cadherin failed to migrate as far as wildtype (WT) cells. Previous studies have shown that ADAM10 (A Disintegrin and Metalloproteinase Domain 10) is involved in the cleavage of PCDH12. Moreover, the cleaved extracellular domain of PCDH12 has been detected both *in vitro* and in human samples including serum and urine. Because the cerebrospinal fluid (CSF) is one of the few sources of proteins derived from the brain of living humans, we searched for PCDH extracellular domains in a publicly available dataset. We found that human CSF is enriched with adhesion, synapse, and neurodevelopmental disorder risk molecules. Particularly, we found that the PCDH12 extracellular domain is highly abundant. Thus, we hypothesized that cleavage of PCDH12 by ADAM10 serves as a signal for neuronal migration. We used our neurosphere migration model to test the effect of pharmacological inhibition of ADAM10 with GI 254023X (GI) on neuronal migration. We found that *PCDH12*-WT neurospheres treated with GI exhibited decreased neuronal migration compared to controls. Finally, because migration depends on proper regulation of the actin cytoskeleton to be successful and the PCDH12 intracellular domain contains a binding motif for the WAVE Regulatory Complex (WRC), we tested whether PCDH12 promotes WRC membrane recruitment. We observed more WAVE in the plasma membrane fraction in *PCDH12*-WT compared to knockout (KO) NPCs, while protein levels were similar in cytosolic fractions and whole-cell lysates. This result confirms that PCDH12 recruits the WRC at the plasma membrane, possibly mediating cytoskeleton dynamics. Our data suggest that abnormal cell migration could be responsible for the disrupted cortical development observed in patients carrying homozygous *PCDH12* variants. These results provide insight into the cellular mechanisms regulated by PCDH12 during brain development.

Disclosures: M. Epstein: None. **J. Rakotomamonjy:** None. **G. Yang:** None. **L. Rylaarsdam:** None. **A. Guemez-Gamboa:** None.

Poster

351. Cell Lineage, Fate, and Migration

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 351.14

Topic: A.01. Neurogenesis and Gliogenesis

Support: Knowledge Foundation
The Zvi and Ofra Meitar Family Fund
Arsenov Foundation
Joint MRC / Wellcome Trust (grant # 099175/Z/12/Z)

Title: The first neurons of the human forebrain

Authors: *I. BYSTRON;
Univ. of Oxford, Oxford, United Kingdom

Abstract: Ectodermal stem cells become neural progenitors, epidermal progenitors, and neural plate border cells. Here, we provide evidence that some of the first neurons in dorsal diencephalon and adjacent ectoderm emerge from the edge of the fusing neural folds. We used a set of neuronal and proliferative markers to reveal the phenotypic characteristics and migratory potential of the earliest neurons of the human forebrain and adjacent cephalic ectoderm. We used a set of neuronal and proliferative markers to reveal the phenotypic characteristic and migratory potential of these precocious neurons. We were able to reconstruct cells by high-resolution volume rendering of multichannel 3D data sets from a Zeiss confocal microscope. Early embryonic tissue was obtained from the Human Developmental Biology Resource UK. The mode of neural tube closure in humans is different from that in other animal species, and neurogenesis is more advanced at the level of fusion of the neural folds in diencephalon at E31. Predecessor neurons, expressing neuron-specific beta 3 tubulin (TU-20 positive) which are born in dorsal diencephalon extent processes towards fusion line, and neurons with similar bipolar morphology are seen in adjacent cephalic ectoderm comprising premigratory neural crest cells. Moreover, expression of the neuronal marker was detected in some progenitos prior to cell delamination and migration from trigeminal and olfactory placodes and also from oral ectoderm. These bipolar TU-20-positive, GAP-43-negative precocious cells either leave the ectodermal layer through the basal lamina or migrate along cephalic ectoderm using somatic translocation. By E33 some of these unusual neurons migrate into periocular mesenchyme before formation of the trigeminal nerve. They extend non-axonal processes into the optic cup, through pigment retina and along the ventricular surface of the neural retina, among the apical processes of neural stem cells. Others invade the cerebral wall of the rostral telencephalon. These migratory cells are distinct from TBR1-positive olfactory pioneer neurons which penetrate the cerebral wall at E 35. Such a network of precocious neurons in the anterior human embryo has not been described in any other mammalian species. Considering that the neural border includes progenitors for the dorsal neural tube, the neural crest, placodes and ectoderm further work is needed to unravel the developmental relationship between predecessor neurons, and migratory neurons originating in the surface ectoderm and neurogenic placodes.

Disclosures: I. Bystron: None.

Poster

352. Postnatal Hippocampal Neurogenesis

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 352.01

Topic: A.02. Postnatal Neurogenesis

Support: R01NS124775

Title: Neural stem and progenitor cell derived vascular endothelial growth factor regulates the vascular niche in the adult mouse dentate gyrus

Authors: *T. J. DAUSE, E. D. KIRBY;
Psychology, Ohio State, Columbus, OH

Abstract: Neural stem and progenitor cells (NSPCs) reside in two primary neurogenic niches of the adult rodent brain, the subventricular zone (SVZ) and dentate gyrus of the hippocampus (DG), where they proliferate to produce new neurons throughout life. Recent research suggests that, in addition to creating new neurons, NSPCs regulate their niche through the expression of growth factors. We have previously found that, in the adult mouse DG, NSPCs express a significant portion of vascular endothelial growth factor (VEGF) an important vascular mitogen and chemoattractant. The vasculature is a key component of the neural stem cell niche. Vasculature is exceptionally dense in the neural stem cell niche and NSPCs reside in close contact with the endothelial cells that comprise blood vessels. Though this vascular niche is hypothesized to support NSPCs, there is almost nothing known about how it is maintained in adulthood. Given the well-established chemoattractive properties of VEGF for endothelia, we hypothesized that adult hippocampal NSPCs actively maintain and regulate their vascular niche through VEGF expression. Here, we used NestinCreER^{T2};VEGFlox/lox;ROSA-STOP-EYFP mice (iKD) to induce NSPC-specific VEGF knockdown and compared their hallmark vascular niche features to those of NestinCreER^{T2};VEGFwt/wt;ROSA-STOP-EYFP littermate controls. After tamoxifen treatment to induce VEGF knockdown in NSPCs adulthood, we found that radial glia like neural stem cells (RGLs) in iKD mice were significantly further from the DG vasculature and their radial process contacted the vasculature significantly less frequently than in WT mice. NSPC-derived VEGF knockdown also significantly increased the distance of intermediate progenitor cells (IPCs) from the vasculature. NSPC-derived VEGF knockdown had no effect on cell death, endothelial coverage or angiogenesis. Together these data show a dissociation of the DG NSPC vascular niche following NSPC-VEGF knockdown. Our findings imply that NSPCs actively maintain their own proximity to vasculature in adulthood via VEGF secretion. Future studies will address the role of NSPC-derived VEGF in NSPC-endothelia communication and vascular niche regulation. The results will provide a deeper understanding of stem cell-niche interactions in the adult brain, which is an important step to designing regenerative medicine strategies to support healthy brain function.

Disclosures: T.J. Dause: None. **E.D. Kirby:** None.

Poster

352. Postnatal Hippocampal Neurogenesis

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 352.02

Topic: A.02. Postnatal Neurogenesis

Support: NIH grant K01MH125144
NIH grant R35NS097370
NIH grant R35NS116843

Title: Milestones underlying the transition from developmental to adult neural stem cells in the dentate gyrus

Authors: *A. M. BOND, D. JIMENEZ-CYRUS, V. S. ADUSUMILLI, G.-L. MING, H. SONG; Univ. of Pennsylvania, Univ. of Pennsylvania, Philadelphia, PA

Abstract: The dentate gyrus region of the hippocampus is one of only two regions in the adult mammalian brain where substantial levels of neurogenesis occur throughout adulthood. Quiescent adult neural stem cells in the subgranular zone of the dentate gyrus occasionally become reactivated in adulthood to generate new neurons which integrate into the existing network and contribute an added layer of plasticity to the hippocampus. Previously, our lab showed that $Hopx^+$ embryonic neural stem cells which contribute to developmental cytogenesis in the mouse dentate gyrus transition into quiescent adult neural stem cells, suggesting that a common lineage of neural stem cells contributes to developmental and adult neurogenesis. However, the molecular mechanisms regulating this developmental transition remain completely unknown. Here we used single-cell RNA-sequencing of $Hopx^+$ dentate gyrus progenitors across multiple stages of early postnatal development in male and female mice to identify the molecular cascade associated with the transformation of developmental precursors to quiescent adult neural stem cells. We discover that early postnatal dentate gyrus neural stem cells exist in multiple heterogeneous states, and we identify a trajectory of cycling neural stem cells as they transition into a quiescent state. We also discover that dentate gyrus precursors gradually acquire an adult-like state after they exit cell cycle. This suggests that the developmental transition consists of two sequential steps: first precursors exit cell cycle to enter a quiescent state and then precursors acquire an adult-like state throughout a quiescence maturation period. We further identify transcription factor networks associated with each stage of the transition and biological processes that change over the course of the transition. Collectively, our work identifies molecular and cellular mechanisms associated with the transformation of developmental precursors to quiescent adult neural stem cells and serves as a foundation for future studies investigating mechanisms that regulate formation of the adult neural stem pool.

Disclosures: A.M. Bond: None. **D. Jimenez-Cyrus:** None. **V.S. Adusumilli:** None. **G. Ming:** None. **H. Song:** None.

Poster

352. Postnatal Hippocampal Neurogenesis

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 352.03

Topic: A.02. Postnatal Neurogenesis

Support: NIH Grant F30AG071144
NIH Grant AG060238
NIH Grant AG033570
NIH Grant AG062251

Title: Caveolin-1 regulates neuronal differentiation in adult hippocampal neurogenesis

Authors: *T. K. L. STEPHEN[1], E. QUIROZ[1], K. OWUSU-ANSAH[1], L. APONTE-COFRESI[1], A. SHETTI[1], J. BONDS[2], R. MINSHALL[1], O. LAZAROV[1];
[1]Univ. of Illinois at Chicago, Chicago, IL; [2]Univ. of California San Diego, San Diego, CA

Abstract: Adult hippocampal neurogenesis (AHN) arises from neural stem cells (NSCs) in the dentate gyrus of the hippocampus. Deficits in AHN have been found to underly memory impairments in several mouse models of Alzheimer's disease (AD) and risk factor like Type II diabetes (T2DM), yet the mechanisms regulating AHN are not fully understood. Our previous studies show that Caveolin-1 (Cav-1), a 22 kDa cell scaffolding and signaling protein, is associated with hippocampal memory deficits seen in T2DM. We hypothesize that Cav-1 plays a key role in cellular processes involved in hippocampal memory and thus, a potential regulator of AHN. To test this, we have generated a mouse model harboring conditional deletion of Cav-1 in NSCs ($NestinCreER^{T2}$;$Cav\text{-}1^{lox/lox}$). Isolation of NSCs from the hippocampus of these mice at 4-6 weeks of age revealed that deletion of Cav-1 reduced NSC proliferation levels. *In vivo* analysis revealed that Cav-1 deletion in NSCs reduced the number of proliferating NSCs without altering the total population of NSCs in the hippocampus at 3 and 6 months of age. Interestingly, Cav-1 deletion in NSCs increased the total number of neuroblasts and immature neurons in the dentate gyrus as well as improved AHN dependent memory performance at 6 months of age. Taken together, these studies indicate that Cav-1 is crucial for neuronal differentiation in AHN and AHN dependent memory function. Future studies will elucidate the contributions of Cav-1 in regulating AHN in mouse models of AD as well as risk factors of AD like aging and T2DM.

Disclosures: T.K.L. Stephen: None. **E. Quiroz:** None. **K. Owusu-Ansah:** None. **L. Aponte-Cofresi:** None. **A. Shetti:** None. **J. Bonds:** None. **R. Minshall:** None. **O. Lazarov:** None.

Poster

352. Postnatal Hippocampal Neurogenesis

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 352.04

Topic: A.02. Postnatal Neurogenesis

Support: NIH Grant NS086965
NIH Grant NS085171

Title: Identification of candidate key regulators of neural stem cell dynamics and neurogenesis in a transgenic mouse model of Alzheimer's disease

Authors: *Y. FURUTA, C.-H. FU, J. CHIN;
Baylor Col. of Med., Baylor Col. of Med., Houston, TX

Abstract: Neural stem cells (NSCs) ensure lifelong neurogenesis, and reduced neurogenesis is associated with cognitive decline in Alzheimer's disease (AD). Therefore, elucidation of

molecular mechanisms that regulate NSCs, and therapeutic intervention to preserve the NSC pool, may highlight novel strategies for AD. Using transgenic mice that express mutant human amyloid precursor protein (APP mice), our lab previously reported that early seizure activities that occur in AD affect gene regulation of mature granule cells and drive aberrant NSC division and neurogenesis, accelerating the depletion of a finite pool of NSCs in the dentate gyrus. However, the molecular mechanisms by which seizures disrupt NSCs and neurogenesis dynamics are not clear. To gain some perspective about molecules that could affect NSC and neurogenesis dynamics in AD, we examined a dentate gyrus RNA-seq dataset from our lab to identify genes differentially expressed between APP mice and nontransgenic littermate controls. We conducted literature research into the functions of about 200 of the most highly up/downregulated genes. Through this investigation, we found 13 candidate genes that may regulate NSCs and neurogenesis, and benchtop confirmed the expression changes identified in the RNA-seq dataset. One interesting molecule among these candidates is Follistatin, a secreted protein that antagonizes TGF-β superfamily proteins. Previous reports found that Follistatin inhibits NSC division and neurogenesis after acute excitotoxic neurodegeneration in the hippocampus. In our study, we found that the expression of Follistatin is significantly altered in APP mice. Furthermore, our data suggest that Follistatin may be under the regulation of seizure activities in APP mice. Together, these data indicate that the regulation of Follistatin expression may be one of the mechanisms by which seizure activities drive aberrant NSC and neurogenesis dynamics in APP mice.

Disclosures: **Y. Furuta:** None. **C. Fu:** None. **J. Chin:** None.

Poster

352. Postnatal Hippocampal Neurogenesis

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 352.05

Topic: A.02. Postnatal Neurogenesis

Support: NIH Grant R01 NS116914-03

Title: Modulation of adult dentate gyrus neurogenesis by cell type-specific Interleukin 1 receptor type 1 signaling

Authors: *M. I. SMIRNOVA[1,2], N. KOCAK[1,2], H. VAN PRAAG[1,3], D. P. NEMETH[1], N. QUAN[1,3,2];
[1]Stiles-Nicholson Brain Inst., [2]Charles E. Schmidt Col. of Sci., [3]Charles E. Schmidt Col. of Med., Florida Atlantic Univ., Jupiter, FL

Abstract: Adult neurogenesis in the granule cell layer of the dentate gyrus (DG) of the hippocampus is important for the formation of new memories, and its loss is implicated in various neurodegenerative diseases, such as Alzheimer's and Parkinson's, and affective disorders, such as depression and bipolar disorders. Many factors affect adult neurogenesis

including aging, oxidative stress, and neuroinflammation. Previous literature has shown that chronic stimulation by the pro-inflammatory cytokine Interleukin-1 (IL-1) decreases adult DG neurogenesis. However, other studies showed an increase in neurogenesis following IL-1 stimulation. This discrepancy might be accounted for by the different levels of IL-1 acting on different cell-type specific Interleukin 1 receptors (IL-1R1s). IL-1R1 is expressed by endothelial cells, neurons, myeloid cells, microglia, astrocytes, as well as ventricular cells near the DG. Our lab has developed a transgenic mouse model where IL-1R1 can be selectively expressed in specific cell types, allowing for the identification of the cell type(s) that mediates IL-1's effect. We have assessed neurogenesis in 10-week old female wildtype (Il-1r1$^{GR/GR}$), Tie2Cre-Il1r1$^{r/r}$ (endothelial IL-1R1), and LysMCre-Il1r1$^{r/r}$ (myeloid IL-1R1) mice. The mice were injected with a 5 x10^5 PFU/uL dose of adeno-IL-1 into the right DG, and with phosphate buffered saline (PBS) into the left DG as a control. Six days later, they were injected intraperitoneally with 5-Ethynyl-2'-deoxyuridine (EdU), a marker for proliferating cells, and perfusion-fixed four hours later. Sections were generated and immunostained with doublecortin, an immature neuronal marker. ClickIT Cell Proliferation Kit was used to stain EdU. Both dentate gyri were imaged. In Il1r1 GR/GR females, an increase of EdU labeling on the IL-1 injection side in the dentate gyrus was detected. No difference in EdU labeling was detected in IL-1 stimulated DG from the Tie2Cre-Il1r1 r/r and LysMCre-Il1r1 r/r Mice. Thus, this IL-1 stimulated proliferation of DG stem cells is not mediated by myeloid or endothelial IL-1R1.

Disclosures: **M.I. Smirnova:** None. **N. Kocak:** None. **H. van Praag:** None. **D.P. Nemeth:** None. **N. Quan:** None.

Poster

352. Postnatal Hippocampal Neurogenesis

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Program #/Poster #: 352.06

Topic: A.02. Postnatal Neurogenesis

Support: IZKF Advanced Medical Scientist Program (AMSP06)
DFG Wi830/12-2
ITN SmartAge 85989

Title: Conditional deletion of Cyclin D2 confirms critical functions in adult hippocampal neurogenesis

Authors: J. MÜCKE[1], M. THIELEMANN[1], O. AINHOA PULS[1], V. GOYAL[1], J. MÜLLER[1], C. BAYER[1], O. W. WITTE[1], G. ZIMMER-BENSCH[2], ***A. URBACH**[1];
[1]Neurol., Jena Univ. Hosp., Jena, Germany; [2]Neurobiologie und Biomed. Life Sci., RWTH Aachen, Aachen, Germany

Abstract: Cyclin D2 (D2) is one out of three homologous D-cyclins involved in cell cycle progression. Our previous studies suggest that D2 is a key regulator of adult hippocampal

neurogenesis (AHN) by controlling the proliferation of adult neural stem (aNSCs) and their progeny. However, available evidence is mainly based on conventional D2 knockout mice, which have several limitations including an impaired development of the aNSC pool. To bypass these limitations, we engineered a mouse line in which exons I and II of *ccnd2* are flanked by *loxP* sites (*ccnd2flox*). To validate the *ccnd2flox* line, we generated conditional null mutants (cD2KO^{null}) through cross-breeding with CreDeleter mice. *In situ* hybridization and Western blotting confirmed the lack of D2 in cD2KO^{null} mice, which displayed exactly the same phenotype as conventional D2KO mice (microcephaly, BrdU incorporation in the subgranular zone reduced by >90%). Next, we bred *ccnd2flox* mice to inducible Cre driver lines (Nestin-CreER^{T2}, Rosa26-CreER^{T2} or Glast-CreER^{T2}), which enable a spatiotemporally controlled deletion of D2 (iD2KO) during the postnatal period. The knockout was induced by administration of tamoxifen at an age of about seven weeks and newly born cells were labelled with bromodeoxyuridine (BrdU) thereafter. Acute and long-term effects of the iD2KO were assessed using multiple immunofluorescence at 6, 32 and 154 days after the first tamoxifen injection (1, 27, 149 days post BrdU). Depending on the Cre driver, we found a 50-90% reduction of BrdU-positive cells accompanied by a similar reduction in neuroblasts and newborn neurons, whereas neuronal differentiation was unaffected. Together, these data corroborate the importance of D2 for constitutive AHN. Whether D2 exerts this role through controlling the division of aNSCs or of their progeny is currently under investigation.

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Poster

352. Postnatal Hippocampal Neurogenesis

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 352.07

Topic: A.02. Postnatal Neurogenesis

Support: R01NS124775

Title: Investigating the role of adult mouse hippocampal neural stem cell expressed vascular endothelial growth factor on endothelial migration in vitro

Authors: *R. OSAP, T. J. DAUSE, E. D. KIRBY;
Psychology, Ohio State Univ., Columbus, OH

Abstract: The dentate gyrus (DG), a subregion of the hippocampus, is one of two primary niches in the adult mammalian brain where neural stem cells (NSCs) reside and proliferate throughout life to generate new neurons, a process known as neurogenesis. Many studies focus on how NSCs may aid hippocampal learning and memory function through the production of new neurons, but little is known about how NSCs may impact their microenvironment through

the production of secreted factors. Previously, we found that adult DG NSCs express a significant quantity of vascular endothelial growth factor (VEGF) as a part of their secretome. During development, VEGF from radial glial NSCs promote the migration of vascular endothelia into the brain. In the adult brain, DG NSCs reside close to endothelial-lined vasculature in a vascular niche. However, vasculature in the adult brain is generally considered to be more static than during development and the relationship of adult NSC expressed VEGF in regulating endothelia in the adult mouse vascular niche remains unclear. Our recent data derived from an adult NSC-specific VEGF knockdown mouse model show that NSC-expressed VEGF is essential for maintaining proximity of vascular endothelial cells in vivo. Here, we investigated whether this effect derives from VEGF-dependent changes in endothelial migration, NSC motility or both. To study NSC-endothelial interactions in isolation, we used mouse brain endothelial cells (bEnd.3s) and NSCs derived from adult mouse DG in a Boyden chamber assay. Our data thus far confirm that bEnd3 endothelial cells migrate towards a VEGF source. Ongoing work is assessing migration of bEnd.3 endothelial cells towards NSCs with or without a VEGF neutralizing antibody present to demonstrate the role of adult NSC-derived VEGF in driving endothelial migration. In future work, we will use the Boyden chamber assay to assess the migration of NSCs towards bEnd.3s with or without VEGF expression by NSCs intact. These data will show whether bEnd.3 secreted factors are able to attract NSCs and whether that attraction relies on an interchange with NSC-derived VEGF. We hypothesize that bEnd.3s will migrate towards NSCs due to their release of VEGF and that NSCs will be attracted to bEnd.3s but that that attraction will be VEGF-independent. These findings would suggest that NSC-endothelial affiliation is driven by attraction of endothelia to NSCs via VEGF. These studies will demonstrate the intercellular signaling mechanisms by which adult NSCs use secreted VEGF to modulate their vascular niche. The data will help advance understanding of the mechanisms supporting the maintenance of the microenvironment of adult stem cells.

Disclosures: R. Osap: None. **T.J. Dause:** None. **E.D. Kirby:** None.

Poster

352. Postnatal Hippocampal Neurogenesis

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 352.08

Topic: A.02. Postnatal Neurogenesis

Support: NIH Grant NS123797

Title: Glutamate Transport Through Excitatory Amino Acid Transporter 1 is Essential for Neural Stem Cell Maintenance

Authors: *I. ROSADO-BURGOS[1], J. RIESKAMP[1], E. D. KIRBY[2,3,4];
[1]Neurosci. Grad. Program, [2]Dept. of Psychology, [3]Dept. of Neurosci., [4]Chronic Brain Injury Program, The Ohio State Univ., Columbus, OH

Abstract: The birth of new neurons in the adult mammalian brain occurs in a small number of discrete areas, most notably the subgranular zone (SGZ) of the hippocampus and the subventricular zone. Generation of new neurons originates with neural stem cells (NSC), which can activate from quiescence to proliferate and create progenitors that go on to give rise to new neurons. In the SGZ, the neurotransmitter glutamate is well known to stimulate NSC proliferation and therefore neurogenesis. Even though the stimulatory role of glutamate for NSCs is well established, the mechanism of glutamate-induced NSC proliferation is unknown. Using isolated adult SGZ NSCs, we found that glutamate transport through the excitatory amino acid transporter 1 (EAAT1) into NSCs, and not stimulation of glutamate receptors, drove NSC proliferation. We therefore set out to test the hypothesis that EAAT1 is cell-autonomously essential for proliferation of NSCs the highly glutamatergic environment of the adult SGZ. To inhibit EAAT1 expression in adult SGZ NSCs, we infused a lentiviral vector expressing CRISPR interference (CRISPRi) targeted to the EAAT1 gene (or a non-targeted (NT) control) into the DG of adult mice. A T2A-linked GFP allowed us to differentiate CRISPRi knockdown NSCs from in-tact neighboring cells within the same mouse. We injected mice with EdU to label proliferating cells before euthanizing them 3 weeks after viral infusion. First, using immunofluorescent labeling, we found that the CRISPRI knockdown of EAAT1 protein was effective. We also found that total GFP+ NSCs and proliferating EdU+ GFP+ NSCs were reduced compared to GFP+ NSCs in NT-treated mice. The GFP- NSCs, however, were not reduced in EAAT1 knockdown vs NT mice. These findings imply that glutamate is not only involved in NSC proliferation, but it is also essential for NSC maintenance. Furthermore, it is EAAT1 that allows glutamate to preserve adult NSC. Ongoing data collection is focusing on quantifying NSC number and proliferation 1 week and 2 months after viral infusion to determine what cellular mechanism is causing the loss of GFP+ NSCs with EAAT1 knockdown and what its effect is on neurogenesis.

Disclosures: I. Rosado-Burgos: None. **J. Rieskamp:** None. **E.D. Kirby:** None.

Poster

352. Postnatal Hippocampal Neurogenesis

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 352.09

Topic: A.02. Postnatal Neurogenesis

Support: NINDS IRP

Title: Psychosis-associated valine to leucine mutation in Neuregulin1 disrupts expression of a schizophrenia susceptibility gene network and alters neuronal fate commitment in the dentate gyrus.

Authors: *P. RAJEBHOSALE[1], L. JIANG[2], L. W. ROLE[3], D. A. TALMAGE[4]; [1]NIH/ NINDS, Bethesda, MD; [2]NINDS, UC Irvine, Bethesda, MD; [3]NINDS, [4]NINDS, Bethesda, MD

Abstract: Neuregulin1-ErbB4 signaling plays a crucial role in synaptogenesis and has been implicated in schizophrenia. A psychosis-associated missense mutation in *NRG1* (rs74942016) is predicted to impair NRG1 nuclear back-signaling, thereby implicating this mode of signaling in the underlying cellular pathology. We generated a transgenic mouse harboring the rs74942016 mutation in *Nrg1* ($V_{321}L$ in Type III Nrg1). We performed transcriptomic analysis of the mutant dentate gyrus (DG) and identified ~1300 differentially expressed genes (DEGs) and developmental processes predicted to be altered compared to wildtype DG. We found that expression of typically DG-enriched genes was downregulated in the DG of mutant mice compared to WT with a concurrent gain in expression of genes that are typically depleted from the DG but enriched in pyramidal neuron-containing regions of the hippocampus. We confirmed that Prox1 and Calb1 were downregulated at the protein level in the mutant DG indicating potential alterations in committing to a DG granule cell (GC) specific transcriptome and alterations to GC maturation. In line with this we found that GCs in mutant mice had multiple dendrites sprouting at the soma, a feature not typically found in GCs but common to pyramidal neurons. We also found abnormal axonal targeting of mutant GCs by examining anatomy of the mossy fibers. DEGs in the mutant DG were enriched for regulatory sequences known to be bound by TFs which show striking overlap with TFs revealed to bind eQTLs in schizophrenia patient samples. Among these were members of the CTCF-cohesin complex involved in chromatin looping. We found that a subset of upregulated genes in the mutant DG shared binding sites for the CTCF-Cohesin complex and were located at boundaries of topologically associated domains using a hippocampal Hi-C dataset indicating potential alterations to higher order chromatin structure as a mechanism for altered fate specification. Ongoing experiments are aimed at uncovering potential alterations to genome structure as well as electrophysiological assessment of GCs.

Disclosures: P. Rajebhosale: None. **L. Jiang:** None. **L.W. Role:** None. **D.A. Talmage:** None.

Poster

352. Postnatal Hippocampal Neurogenesis

Location: SDCC Halls B-H

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Topic: A.02. Postnatal Neurogenesis

Support: NIH/NHGRI R35HG010718
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Institutional Funds

Title: Lack of the BMP antagonist Gremlin2 disrupts adult hippocampal neurogenesis and leads to heightened anxiety in mice.

Authors: *N. B. FRAZER[1], E. R. GAMAZON[2,3,5], A. K. HATZOPOULOS[1,4];
[1]Vanderbilt Univ. Sch. of Med., Vanderbilt Brain Inst., Nashville, TN; [2]Div. of Genet. Med.,

[3]Vanderbilt Genet. Inst., [4]Div. of Cardiovasc. Med., Vanderbilt Univ. Med. Ctr., Nashville, TN; [5]Clare Hall, Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Post-natal neurogenesis is critical for the success of current anti-anxiety medications. The Bone Morphogenic Protein (BMP) signaling pathway is vital in neural proliferation, differentiation, and cell specification, and is a recent target of interest in mood disorders. However, despite being the most potent natural inhibitor of BMP, the function of Gremlin2 (Grem2) in the brain is completely unknown. We created a strain of mice lacking Grem2 via homologous recombination ($Grem2^{-/-}$) and compared the hippocampi of these mice, aged 3-6 months, to wildtype (WT) counterparts using immunohistochemical and RNA markers of mature neurons, proliferation, and neuroblasts. Male and female mice were used and no sex differences were seen unless noted. We found that the CA3 within the dentate gyrus (DG) hilus is 15% (±3%) less dense in $Grem2^{-/-}$ mice, as marked by number and localization of neuronal nuclei (sections: n=29/29, mice: N=6/6; $Grem2^{-/-}$/WT). $Grem2^{-/-}$ mice also exhibited fewer proliferating cells in the DG as quantified by phosphohistone H3 staining ($Grem2^{-/-}$: 16.2 ±7.7, n=16, N=4; WT: 53.7 ±13.9, n=27, N=4). There was also a dramatic decrease in doublecortin (DCX) expressing immature neurons ($Grem2^{-/-}$: 11.8 ±8.0, n=69, N=3; WT: 24.3 ± 11.7, n=58, N=3). In addition, RNA analysis showed 2-fold decreases in markers of immature neuroblasts, DCX and nestin, as well as a 5-fold decrease in the neural stem cell-specific transcription factor sox2 (N=10/10). Due to the role of hippocampal neurogenesis in learning and neurological disorders, we tested mice on a battery of neurobehavioral tests. $Grem2^{-/-}$ mice (N=23) exhibited increased anxiety on the elevated zero maze (EZM) - spending 63% (±15%) of time in the closed arm, compared to 55% (±20%) in WT mice (N=21). In addition, mice underwent acute (1hr) and chronic (7 days, 1hr/day) restraint stress followed by EZM tests (N=14/15). With acute stress, $Grem2^{-/-}$ mice spent significantly more time in the closed arms (79% ±7%) than WT mice (69% ±12%). Chronic stress also increased closed arm time to 91% (±6%) in $Grem2^{-/-}$ mice and only 85% (±9%) in WT mice, suggesting Grem2 is critical in mitigating anxiety following a stressor. A 2-way ANOVA revealed significant interaction of sex and genotype ($p = 0.04$) in the chronic stress condition, with male mice experiencing the greatest deficit without Grem2. These results suggest Grem2 inhibition of BMP signaling is vital in maintaining homeostasis of adult hippocampal neurogenesis, and changes in Grem2 contribute to the development and progression of neurogenesis-related disorders such as anxiety. These findings open up exciting new avenues for the development of treatments for such disorders.

Disclosures: **N.B. Frazer:** None. **E.R. Gamazon:** None. **A.K. Hatzopoulos:** None.

Poster

352. Postnatal Hippocampal Neurogenesis

Location: SDCC Halls B-H

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Program #/Poster #: 352.11

Topic: A.02. Postnatal Neurogenesis

Support: JSPS KAKENHI Grant Number 20J20975
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Title: Decreased Setd8 expression mediates early NSC aging in the adult hippocampus.

Authors: *S. MATSUBARA[1], T. MATSUDA[1], H. SEKIRYU[1], H. DOI[1], T. NAKAGAWA[1], H. ODA[2], K. NAKASHIMA[1];
[1]Dept. of Stem Cell Biol. and Med., Grad. Sch. of Med. Sciences, Kyushu Univ., Fukuoka-shi, Japan; [2]Dept. of Cancer Genome Med., Saiseikai Kumamoto Hospital, Div. of Integrative Med. Oncology, Kumamoto-shi, Japan

Abstract: Neural stem cells (NSCs) in the adult mouse hippocampus generate new neurons, which integrate into existing neural circuits and consequently support learning and memory. Hippocampal NSCs maintain their populations by controlling their activity to proliferate or to stay quiescent. During the aging process, NSCs gradually weaken proliferative capability and fall into a deeper quiescent state, resulting in declining hippocampal neurogenesis and cognition. Recent studies have reported that alteration of microenvironmental niche affects NSC property. However, cell-intrinsic mechanisms underlying the age-related property change of NSCs remain largely unknown. Here, we show that the reduction of *Setd8*, a sole enzyme that catalyzes mono-methylation of histone H4 at lysine 20 (H4K20me1), underlies the age-related alteration of NSC properties in the hippocampus. To identify the factors associated with NSC dysfunction with age, we performed single-cell RNA-seq of EGFP-positive cells isolated from the hippocampal dentate gyrus of Nestin-EGFP reporter mice at different time points (postnatal days 5, 12 weeks, and 24 weeks) and found that *Setd8* was gradually downregulated in NSCs with age. Conditional knockout of *Setd8* in the adult NSCs decreased H4K20me1 levels, inducing deeper dormancy of NSCs accompanied by impaired neurogenesis and lost NSC pool in the adult hippocampus. Moreover, *Setd8* conditional knockout mice showed impaired performance on the novel place recognition task compared with control mice, suggesting disrupted hippocampal-dependent memory function. We then performed RNA-seq analysis of control and Setd8-knocked down NSCs *in vitro*. Up- or down-regulated genes by Setd8-knocked down displayed statistically significant overlaps with age-dependently up- or down-regulated genes in hippocampal NSCs, respectively. We also observed the argumentation of gene expression associated with NSC quiescence in Setd8-downregulated cells. Taken together, it is conceivable that the reduction of *Setd8* expression accelerates age-dependent gene expression alteration associated with NSC quiescence and consequently induces deeper dormancy of NSCs, impairing neurogenesis in the hippocampus and spatial learning and memory during the aging process.

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Poster

352. Postnatal Hippocampal Neurogenesis

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Program #/Poster #: 352.12

Topic: A.02. Postnatal Neurogenesis

Support: NIH/NIA R01AG061382
NIH/NIA RF1AG072300

Title: Timp2-mediated remodeling of the extracellular matrix regulates hippocampus-dependent cognitive function and plasticity

Authors: *A. C. FERREIRA, B. M. HEMMER, S. M. PHILIPPI, H. LIU, J. ROSENSTADT, Y. WANG, J. D. ZHU, M. VARGHESE, P. HOF, J. M. CASTELLANO;
Icahn Sch. Med. at Mount Sinai, New York, NY

Abstract: Aging is the major risk factor for neurological disorders such as Alzheimer's disease (AD), and exposure to youthful-blood factors counteracts age-related decline. The blood-borne youth-associated factor, tissue inhibitor of metalloproteinases-2 (TIMP2), was shown to revitalize aged mouse hippocampus, while its depletion impairs long-term potentiation, yet its mechanism of action and how its function relates to age-related disorders remains unclear. To define how TIMP2 regulates hippocampus-dependent function, we first characterized its expression pattern and putative cellular targets. We find high levels of TIMP2 in mouse brain interstitial fluid by in vivo microdialysis, along with high expression in hippocampal neurons. Differential gene expression following TIMP2 deletion revealed changes in genes related to synapse organization, memory, and neurogenesis. Indeed, we find that TIMP2 knockout mice exhibit impaired dendritic spine complexity and reduced adult hippocampal neurogenesis, with concomitant deficits in hippocampus-dependent cognition. We further find that TIMP2-deficient hippocampi exhibit altered levels of TIMP2's target MMP2 with a corresponding accumulation of extracellular matrix (ECM) proteins in contact with synapses, reflecting dysregulated ECM turnover adjacent to synapses. We report that migration of immature neuroblasts is also impaired in the absence of TIMP2, likely as a result of stiffness imparted by dysregulated ECM. We further corroborated the major functional phenotypes using a conditional TIMP2 KO model in which neuronal TIMP2 was targeted, which we generated using CRISPR technology. Finally, peripheral and hippocampal TIMP2 levels are decreased in mouse models of AD pathology, phenocopying deficits observed in aging and suggesting interactions with pathology. Using several additional tools we developed, we further characterize changes in APP models following perturbations in TIMP2 metabolism, which may help define mechanisms through which TIMP2 regulates hippocampus-dependent function to inform novel therapies for aging and AD.

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Poster

352. Postnatal Hippocampal Neurogenesis

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Topic: A.02. Postnatal Neurogenesis

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Title: Cyclin D2-dependent formation of the adult stem cell pool in the early postnatal dentate gyrus - emergence of a discrete cellular entity

Authors: A. SYEDA ZAHRA[1], O. PASTOR-ALONSO[2], E. BOCKELMANN[1], V. KNÖLKER[1], B. KASKE[1], F. TETZLAFF[1], F. GARCÍA-MORENO[2], *O. W. WITTE[1], J. M. ENCINAS[2], A. URBACH[1];
[1]Neurol., Jena Univ. Hosp., Jena, Germany; [2]Achucarro Basque Ctr. for Neurosci., Leioa, Spain

Abstract: The subgranular zone (SGZ) of the adult dentate gyrus harbors a pool of quiescent neural stem cells (aNSCs) which produce new granule neurons throughout life. However, their origin and the mechanisms leading to their establishment are still unclear. Here, we used mutant mice that lack cyclin D2 (D2KO) and express a green fluorescent protein under control of the nestin reporter, *in vitro* assays, confocal immunofluorescence and targeted *in vivo* retroviral injections to investigate when, how and where the long-lived, quiescent aNSC pool is formed. In D2 wildtype mice, we observed a transient D2-expressing NSC population that emerged in the first postnatal week and disappeared from postnatal day 14 (P14) on. In parallel, but with a slight delay, the number of D2-negative NSCs increased and expanded until the end of the second postnatal week. The D2KO impaired the postnatal formation of the quiescent aNSC pool, leading to a smaller SGZ devoid of radial glia-like NSCs with self-renewing potential in adults. Retroviral fate mapping revealed that aNSCs are born from precursors dividing inside the postnatal dentate gyrus. Hence, although aNSCs are a functionally heterogeneous population (Petrik et al., 2022), the finding that they are formed on-site, in a discrete time window and in a strictly D2-dependent manner suggests that these cells constitute an entity distinct from their developmental precursors and that AHN is not a mere continuation of development.

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Poster

352. Postnatal Hippocampal Neurogenesis

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Title: Functional rejuvenation of aged neural stem cells by Plagl2 and anti-Dyrk1a activity

Authors: *T. KAISE[1], M. FUKUI[1], R. SUEDA[2], W. PIAO[3], M. YAMADA[3], T. KOBAYASHI[3], I. IMAYOSHI[3], R. KAGEYAMA[1];
[1]RIKEN Brain Sci. Inst. - Wako, WAKO, Japan; [2]Univ. Col. London, London, United Kingdom; [3]Kyoto Univ., Kyoto, Japan

Abstract: In the hippocampus of the aged mouse brain, not only the number of neural stem cells (NSCs) but also their activation rate and/or neurogenic potential significantly decline, leading to cognitive dysfunctions. This decline involves up-regulation of senescence-associated genes, but inactivation of such genes failed to reverse aging of hippocampal NSCs. Because many genes are up-regulated or down-regulated during aging, manipulation of single genes would be insufficient to reverse aging. Here we searched for a gene combination that can rejuvenate NSCs in the aged mouse brain from nuclear factors differentially expressed between embryonic and adult NSCs and their modulators. We found that a combination of inducing the zinc finger transcription factor gene Plagl2 and inhibiting Dyrk1a, a gene associated with Down syndrome (a genetic disorder known to accelerate aging), rejuvenated aged hippocampal NSCs, which already lost proliferative and neurogenic potential. Such rejuvenated NSCs proliferated and produced new neurons continuously at the level observed in juvenile hippocampi, leading to improved hippocampus-dependent learning and cognition. Live-imaging analysis showed that quiescent NSCs start to express proneural gene Ascl1 in oscillatory manner, a hallmark feature of active NSCs, by inducing Plagl2 and inhibiting Dyrk1a. Epigenome and transcriptome analyses indicated that this gene combination induces up-regulation of embryo-associated genes and down-regulation of age-associated genes by changing their chromatin accessibility, thereby rejuvenating aged dormant NSCs to function like juvenile active NSCs. Thus, aging of NSCs can be reversed to induce functional neurogenesis continuously, offering a way to treat age-related neurological disorders.

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Poster

352. Postnatal Hippocampal Neurogenesis

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Topic: A.02. Postnatal Neurogenesis

Support: NSF GRFP
NSF Brain Initiative NeuroNex

Title: Synaptic development on dentate gyrus granule cell dendrites

Authors: *A. SOROKINA[1], V. SAMPATHKUMAR[2], N. B. KASTHURI[2];
[2]Neurobio., [1]Univ. of Chicago, Chicago, IL

Abstract: The hippocampal dentate gyrus (DG) is one of few places in the mammalian brain that undergoes adult neurogenesis. While adult-born granule cells functionally integrate into the existing circuit, the exact mechanism for how this integration occurs remains unknown. We used large volume serial electron microscopy (EM) to address this gap. We reconstructed synapses and circuits in DG at two time points: in younger adult mice (P56) undergoing higher rates of neurogenesis, and compared these reconstructions to older adults (P115) where neurogenesis is purportedly reduced. We first traced the dendritic arbors of granule cells from the hilus to the outer molecular layer to capture dendritic morphology. Next, we collected higher resolution EM of the previously traced dendrites to analyze their synaptic connectivity. Consistent with reduced neurogenesis, we find that neuronal density decreases across these ages but surprisingly, despite this reduced density, we find most measures of synapses are similar across these ages: both datasets have comparable rates of postsynaptic densities and axon boutons. One current hypothesis about formation of new synapses on adult-born neurons is that multi-synaptic boutons (MSBs) are an intermediary step in this process. However, we find little difference in the rate of MSBs in P56 vs. P115 suggesting a role for MSBs beyond adult-born granule cell integration in this circuit. Secondly, when comparing individual cells to each other, we find that dendrites in P56 have similar spine densities, while dendrites in P115 range from sparse to dense spines, with some cells having 4x the average spine/micron as P56 cells. To investigate this further, we plan to couple high-resolution EM analyses with a post-embedding BrdU label to accurately birth date adult-born neurons in our datasets.

Disclosures: A. Sorokina: None. **V. Sampathkumar:** None. **N.B. Kasthuri:** None.

Poster

352. Postnatal Hippocampal Neurogenesis

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Topic: A.02. Postnatal Neurogenesis

Support: NIH Grant ZIAMH002784

Title: Effects of acute and chronic food restriction on adult hippocampal neurogenesis in male and female rats

Authors: *N. FREEDGOOD, H. CAMERON;
Natl. Inst. of Hlth., Bethesda, MD

Abstract: Food restriction (FR) is commonly used in behavioral experiments to increase motivation to work for food rewards. A variety of FR protocols have been examined but with mixed results. Several studies have found beneficial effects of FR on adult neurogenesis using protocols aimed to maintain healthy body weight in aging animals (Mattson et al., 2003; Park & Lee, 2011; Murphy et al., 2014). However, other studies have shown negative effects (Cardoso et al., 2016; Huggins & Curlik, 2019) and no study has examined the effects in both males and females. We assessed the effects of one-week FR, commonly used before starting behavior testing, and early-onset FR used in our laboratory for weight maintenance and behavior testing on adult hippocampal neurogenesis in both male and female rats. The FR groups were given 50% of the average ad libitum food consumption for their sex (10g/day for females and 15g/day for males) with the one-week FR beginning at 11 weeks and the early-onset FR beginning at 4 weeks. All rats were injected with bromodeoxyuridine (BrdU) at 11 weeks of age and perfused one week later. One-week FR in females resulted in a decrease in the survival of new neurons measured by BrdU, but the early-onset FR females showed an increase in proliferation measured by PCNA. Both early-onset and one-week FR in males resulted in a significant decrease in proliferation. However, only the early-onset FR males had a significant decrease in the number of immature doublecortin positive neurons. An additional cohort testing 30% restriction in males (20g/day) was added to see if a less restrictive protocol might prevent the decreased neurogenesis seen in the 50% FR males. Indeed, no significant changes in any markers were seen in the early or late 30% FR males relative to their ad-lib controls. Overall, the findings suggest that 50% FR in males has detrimental effects on adult hippocampal neurogenesis, with one-week of FR being potentially less detrimental than the longer-term early-onset FR. A 30% FR protocol may be better for behavioral testing in males because it does not decrease adult neurogenesis. In females, the 50% FR had some beneficial effects on neurogenesis, and the decrease in survival with one-week FR could have been caused by the stress of the change in diet. Behavioral testing will be conducted in order to determine whether the 30% FR paradigm produces different effects on motivation and anxiety-like behaviors compared to the 50% FR paradigm.

Disclosures: **N. Freedgood:** None. **H. Cameron:** None.

Poster

352. Postnatal Hippocampal Neurogenesis

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Topic: A.02. Postnatal Neurogenesis

Support: NIAAA R01AA027462
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Title: Influence of diet on maternal ethanol consumption, blood ethanol concentration and the neurogenic response to enriched environment in a mouse model of prenatal alcohol exposure

Authors: L. LI, D. JIMENEZ, M. MURPHY, A. M. RODRIGUEZ, D. L. LINSENBARDT, C. F. VALENZUELA, *L. A. CUNNINGHAM;
Neurosciences, Univ. of New Mexico Hlth. Sci. Ctr., Albuquerque, NM

Abstract: Fetal Alcohol Spectrum Disorders (FASDs) are associated with impaired hippocampal function. We previously demonstrated impaired adult hippocampal neurogenesis in response to enriched environment (EE) in a mouse model of moderate prenatal alcohol exposure (PAE), which was associated with impaired pattern discrimination, broadened behavioral activation of the dentate gyrus, and impaired dendritic branching of adult-generated dentate granule cells (aDGCs). Based on recent studies demonstrating significant effects of laboratory diet on voluntary ethanol consumption, and a potential impact on adult neurogenesis, we compared two commercially available diets on maternal drinking and the neurogenic response to EE in PAE offspring. Experiments were performed using Nestin-CreER^{T2}:tdTomato reporter mice to allow for visualization of aDGCs. Dams were maintained on either Teklad 2920X (TL20; Envigo®; extruded) or LabDiet 5001 (LD01, LabDiet®; compressed) diet from weaning and throughout mating and pregnancy. PAE offspring were generated using a limited access "drinking-in-the-dark" (DID) gestational exposure paradigm in which dams were offered 10% EtOH/0.066% saccharin or saccharin alone (SAC, control) for 4 hr per day using electronically monitored volumetric sippers. PAE and SAC offspring were gender segregated at weaning and maintained under standard (SH) or EE (toys and running wheels) for 10 weeks until sacrifice (maintained on their respective diets). aDGCs were labeled by administration of tamoxifen for 5 consecutive days at 1 wk post-weaning. We found no effect of diet on average daily EtOH consumed (3.19 $\pm$ 0.13 *vs.* 3.63 $\pm$ 0.27 gm EtOH consumed/kg/day, mean $\pm$ SEM, TL20 *vs.* LD01, n=12/group). However, blood EtOH concentrations (BECs) were approximately 2-fold higher in the LD01 *vs.* TL20 diet group; (132 $\pm$ 67 mg/dL *vs.* 70 $\pm$ 52 mg/dL, respectively, p=0.02, n=12/group). Interestingly, BECs were directly correlated with alcohol consumption for the TL20 group (R^2=0.4), but not for the LD01 group (R^2=0.07). In male offspring (females TBD), there was no effect of diet on baseline or EE-mediated neurogenesis in the SAC control group. Furthermore, EE-mediated neurogenesis in TL20 PAE offspring was impaired despite lower average maternal BECs, as previously demonstrated. Ongoing analysis will determine whether PAE similarly impaired EE-mediated neurogenesis in LD01 offspring. In sum, our observations thus far demonstrate a significant effect of diet on maternal BEC, but no effect on baseline or EE-mediated adult hippocampal neurogenesis in offspring.

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Poster

352. Postnatal Hippocampal Neurogenesis

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Program #/Poster #: 352.18

Topic: A.02. Postnatal Neurogenesis

Support: NIH INBRE Grant 2P20RR016462

Title: Effects of testosterone and its metabolites on stages of hippocampal neurogenesis in adult male rats

Authors: *H. BARR[1], E. A. ROY[1], C. MICHAELCHECK[1], L. PANELLA[1], A. QIAN[1], H. H. M. NGUYEN[1], D. XU[1], J. M. BARKER[2], M. D. SPRITZER[1];
[1]Biol. and Neurosci., Middlebury Col., Middlebury, VT; [2]Dept. of Psychology and Grad. Program in Neurosci., Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Within the mammalian brain, neurogenesis occurs throughout adulthood along the subgranular zone of the dentate gyrus. Testosterone influences levels of hippocampal neurogenesis in male rats, but its effects seem to depend on the timing of testosterone exposure relative to the stage of neural development. Additionally, it remains unclear which of testosterone's major metabolites, estradiol or 5α-dihydrotestosterone (DHT), influences neurogenesis in males. Therefore, we tested the effects of testosterone, estradiol, and DHT administered during three different stages of neural development. Adult male rats (n = 5-8/group) were bilaterally castrated and given a single injection of bromodeoxyuridine (BrdU; 200 mg/kg) on the first day of the experiment to label actively dividing cells. Subjects were euthanized sixteen days later to assess BrdU labeling. All subjects received five consecutive days of injections during one of three stages of neural development: days 1-5 (cell proliferation and migration), days 6-10 (neurite growth), or days 11-15 (neuron maturation). In three separate experiments, subjects were injected during these time periods with either testosterone propionate (0.250 or 0.500 mg/rat), DHT (0.250 or 0.500 mg/rat), or estradiol benzoate (1.0 or 10 µg/rat). Each experiment also included a control group injected with sesame oil (0.1 ml/rat). Rats were transcardially perfused, brains sectioned (40 µm), and peroxidase immunohistochemistry was used to visualize BrdU-labeled cells. Light microscopy was used to count all labeled cells in every 10th section throughout the dentate gyrus. Testosterone injections had a significant effect on the number of BrdU-labeled cells ($p = 0.022$), with the 0.500 mg/rat dose causing a significant increase in the number of BrdU-labeled cells compared to the 0.250 mg/rat dose and the control group. Among the three time periods, only the later stage (11-15 days) showed a significant effect of testosterone ($p = 0.006$). In contrast, neither DHT ($p = 0.67$) nor estradiol ($p = 0.62$) injections had any significant effects on number of BrdU-labeled cells during any stage of development. Fluorescent double-labeling of tissue (BrdU and NeuN) assessed by confocal microscopy is currently in progress to determine if any of the treatments influenced the probability that newly proliferated cells developed into neurons. Our results add to past evidence that testosterone increases neurogenesis in adult males and that later stages of neural development are most sensitive to these effects. Additionally, both metabolites of testosterone (estradiol and DHT) may be necessary for testosterone to enhance adult neurogenesis.

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Poster

352. Postnatal Hippocampal Neurogenesis

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Program #/Poster #: 352.19

Topic: A.02. Postnatal Neurogenesis

Support: NIH Grant 21AMH002784

Title: A novel role for adult neurogenesis in a two-armed bandit reversal learning task

Authors: *K. B. HUNTZICKER[1,2], R.-M. KARLSSON[1], H. A. CAMERON[1];
[1]Natl. Inst. of Mental Hlth., Bethesda, MD; [2]Neurosci., Brown Univ., Providence, RI

Abstract: Although neurogenesis in the mammalian hippocampus persists throughout adulthood, the function of newly-born hippocampal cells remains largely unknown. This study employs a pharmacogenetic method of neurogenesis ablation to determine the influence of adult neurogenesis on decision-making. Rats expressing herpes simplex virus thymidine kinase (HSV-TK) under the control of the human glial fibrillary acidic protein (GFAP) promoter were given an antiviral drug in adulthood to induce complete ablation of hippocampal neurogenesis. This method is highly specific, inhibiting neurogenesis only upon drug administration and affecting only neural progenitors.

Treated transgenic (TK) rats, despite lacking all newly-born neurons, do not exhibit deficits in many standard learning tasks. However, our lab has previously found that TK rats respond differently than wild-type (WT) controls when faced with conflicting or ambiguous threat cues. To test whether adult neurogenesis also affects behavioral responses to ambiguity under non-threatening conditions, we trained rats on an operant two-armed bandit reversal learning paradigm.

In this task, rats learn that one lever produces a food reward 80 percent of the time, and the other, 20 percent. At several points during the session, the lever identities switch. This type of probabilistic learning – and subsequent reversal learning when lever outcomes are swapped – provide rats with many instances of ambiguous feedback. For example, a previously lucrative lever failing to deliver a reward could represent either a reversal or the 20 percent chance that the correct lever does not produce a reward. We found that male and female TK rats exhibit higher win-stay ratios and earn more rewards than WT controls, suggesting that TK rats employ different decision-making strategies than their WT counterparts. This genotype effect is apparent after as little as one week of testing and persists for the duration of the experiment (28 days). Notably, the performance effect becomes less pronounced with increasing task difficulty, but persists when lever outcomes are deterministic. Using Bayesian reinforcement learning models, we can further quantify the observed behavioral differences to dissect how exactly new neurons influence reversal learning and, more broadly, responses to situational uncertainty in general.

Disclosures: **K.B. Huntzicker:** None. **R. Karlsson:** None. **H.A. Cameron:** None.

Poster

352. Postnatal Hippocampal Neurogenesis

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 352.20

Title: WITHDRAWN

Poster

352. Postnatal Hippocampal Neurogenesis

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 352.21

Topic: A.02. Postnatal Neurogenesis

Support: CIHR

Title: Distinct effects of chronic neurostimulation (TMS and ECS) on hippocampal new neurons in males and females

Authors: *T. ZHANG, B. ASKARI, A. KESICI, E. M. GUILHERME, F. VILA-RODRIGUEZ, J. S. SNYDER;
Univ. of British Columbia, Vancouver, BC, Canada

Abstract: The hippocampus is a brain region linked with the presentation of depression and antidepressant therapeutic action. Hippocampal neurogenesis, the continuous addition of new neurons throughout adulthood, is a unique plasticity process that is affected by depression and its treatment. Non-invasive stimulation therapies such as electroconvulsive therapy (ECT) or transcranial magnetic stimulation (TMS) are becoming increasingly mainstream for their high efficacy on drug treatment-resistant depression. In our previous study, we directly compared hippocampal neurogenesis as induced by acute electroconvulsive shock (ECS), the animal analog of ECT, and different forms of TMS and found that ECT increased neurogenesis significantly more than either forms of TMS (Zhang et al., 2021). A newer form of TMS called intermittent theta-burst stimulation (iTBS) showed a greater neurogenic potential than the traditional repetitive TMS (rTMS) in our acute study, therefore we conducted the first study examining neurogenesis following chronic iTBS. As ECS is so potent in inducing adult neurogenesis, we also wanted to examine whether new neurons compete for synaptic connections or even replace older, developmentally-born neurons. Thus we also applied chronic ECS and examined hippocampal neurons of different ages. We found that chronic iTBS did not significantly increase the amount of neurogenesis or affect the gross dendritic morphology of new neurons.

However, iTBS did increase presynaptic mossy fiber terminal size on adult-born neurons in males, but not females. iTBS also increased the number of terminal-associated filopodia, putative synapses onto inhibitory interneurons in male mice. Our results suggest iTBS may invoke distinct hippocampal mechanisms in males and females by accelerating the maturation of efferent pathways emerging from newborn neurons in a sex-specific fashion. When we applied chronic ECS, we found a robust increase in neurogenesis markers. We found that the generation of new-born neurons was not accompanied by the death of developmentally-born neurons. However, as adult-born and developmentally-born neurons may compete for synaptic connections, we further looked at synaptic terminals of these neurons. Our preliminary findings suggest that ECS increases adult-born neuron mushroom spine density, mossy fiber bouton area, and filopodia, possibly indicating a bias in synaptic transmission towards adult-born neurons. In summary, chronic ECS had a greater neurogenic effect than chronic iTBS and increases in mossy fiber bouton size and number of efferent filopodia may be a converging response of stimulation modalities.

Disclosures: **T. Zhang:** None. **B. Askari:** None. **A. Kesici:** None. **E.M. Guilherme:** None. **F. Vila-Rodriguez:** None. **J.S. Snyder:** None.

Poster

352. Postnatal Hippocampal Neurogenesis

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 352.22

Topic: A.02. Postnatal Neurogenesis

Title: The temporal origin of dentate granule neurons affects their axonal architecture

Authors: *P. MORTESSAGNE[1], T. KERLOCH[1], F. FARRUGIA[1], J. TEILLON[2], D. ABROUS[1], E. PACARY[1];
[1]Neurocentre Magendie, "Neurogenesis and Pathophysiology" Group, INSERM U1215, Bordeaux CEDEX, France; [2]UMS 3420, US 4 F-33000 Bordeaux, France, Univ. Bordeaux, CNRS, INSERM, Bordeaux Imaging Center, BIC, Bordeaux CEDEX, France

Abstract: In the dentate gyrus (DG) of the hippocampus, the generation of dentate granule neurons (DGNs) starts during late embryogenesis, peaks around birth and continues at low levels during adulthood. The DG is therefore a peculiar brain structure composed of DGNs of different temporal origins constituting subpopulations of DGNs that might play different roles in hippocampal physiology. Surprisingly, this hypothesis has received little attention and, although the morpho-functional properties of adult-born DGNs (Adu-DGNs) have been extensively studied, very little is known about the developmentally-generated ones. In this context, we have undertaken to analyse the morphological characteristics of these different subpopulations of DGNs, in a mouse model. For this purpose, we targeted DGNs generated at E14.5 and P0 using in vivo electroporation of a GFP expressing plasmid and Adu-DGNs using stereotactic injection of a GFP encoding retrovirus. Using these strategies, we have already shown that

developmentally-born DGNs (Dev-DGNs), especially embryonically-born cells, and Adu-DGNs display distinct dendritic arbors once mature (Kerloch et al., *Cerebral Cortex* 2019). Interestingly, we have also found striking differences at the axonal level. In the hilus and CA3, the size of the mossy fiber boutons and the number of filopodia emerging from these boutons are different according to the birth date of DGNs. Indeed, the later the DGNs are generated, the bigger their boutons are and the more filopodia they have. Furthermore, our data show that the projection sites of their axons in CA3/CA2 as well as the length and position of their axonal initial segment are also dependent on their ontogenetic origin. Altogether our data demonstrate that the temporal origin of DGNs dictates their morphological properties and thus potentially their functions in the hippocampal network.

Disclosures: P. Mortessagne: None. **T. Kerloch:** None. **F. Farrugia:** None. **J. Teillon:** None. **D. Abrous:** None. **E. Pacary:** None.

Poster

352. Postnatal Hippocampal Neurogenesis

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 352.23

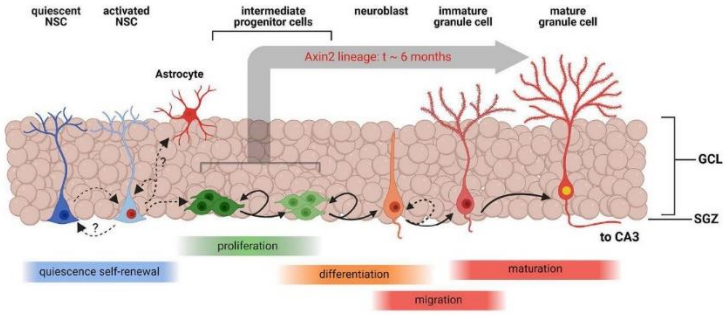
Topic: A.02. Postnatal Neurogenesis

Support: Focused Ultrasound Foundation Grant (to PT)

Title: Prolonged Integration of Adult-born Axin2 Cell Lineage into Granule Neurons of the Dentate Gyrus

Authors: *K. SHARIFI[1], F. FARZAD[2], S. SOLDOZY1[2], R. PRICE[3], M. S. KALANI[4], P. TVRDIK[2];
[1]Univ. of Virginia, [3]Biomed. Engin., [2]Univ. of Virginia, Charlottesville, VA; [4]St. John's Neurosci. Inst., Tulsa, OK

Abstract: The Wnt pathway plays many roles during adult neurogenesis. The expression of Axin2 is induced by Wnt/β-catenin signaling, making this gene a sensitive indicator of canonical Wnt activity. We have employed pulse-chase genetic lineage tracing with the Axin2-CreER allele to follow the fate of *Axin2*-positive cells in the hippocampus. Simultaneously with tamoxifen induction of *Axin2* fate mapping, the dividing cells were marked with 5-ethynyl-2´-deoxyuridine (EdU). Tamoxifen induction resulted in significant increase of dentate gyrus granule cells three months later; however, none of these neurons contained EdU signal. Conversely, six months after the tamoxifen/EdU pulse-chase labeling, EdU-positive granule neurons were identified in each animal. Our data implies that *Axin2* is expressed in several different stages of adult granule neuron differentiation, including the dividing neural stem cells. However, our data also indicates that the process of integration of the adult-born neurons from certain cell lineages may take longer than previously thought.



Schematic representation of Wnt-associated subgranular neurogenesis
A summary diagram of subgranular neurogenesis from quiescent neuronal stem cells (NSCs) to mature granule cells. Quiescent NSCs are multipotent undifferentiated cells. Once induced, quiescent NSCs differentiate into activated NSCs, which may replenish the pool of quiescent NSCs, or commit to the astrocyte maturation pathway. Activated NSCs also develop into multipolar intermediate progenitor cells and neuroblasts which continue to proliferate and eventually migrate inside the granular layer and enter the granule cell maturation pathway. *Axin2*-positive NSCs and progenitors represent a sub-lineage of the cell lines, contributing to subgranular neurogenesis. In this study, we have unequivocally detected *Axin2* lineage markers in the intermediate progenitors and neuroblasts, which are amenable to mitotic labeling, and post-mitotic mature neurons. Our data suggest that the process of integration of progenitors into mature granule cells can take as long as six months in this cell lineage.

Disclosures: **K. Sharifi:** None. **F. Farzad:** None. **S. Soldozy1:** None. **R. Price:** None. **M. S. Kalani:** None. **P. Tvrdik:** None.

Poster

353. Stem Cell Models for Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 353.01

Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant K08NS119747

Title: Using SCN8A mutant hippocampal organoids as a model to study pathogenesis of developmental epileptic encephalopathy-13 (DEE-13)

Authors: *K. LOZANO[1], E.-A. BUTLIG[2], N. FOTION[2], I. MODY[3], B. NOVITCH[2], R. SAMARASINGHE[2];
[1]UCLA, UCLA, Woodland Hills, CA; [2]UCLA, Los Angeles, CA; [3]Dept Neurol., UCLA Sch. Med., Los Angeles, CA

Abstract: Epilepsy is a neurological disorder most commonly characterized by sudden, recurrent seizures. Disease pathology has revealed a correlation to the abnormal development of neural networks. One region of particular interest is the hippocampus since hippocampal abnormalities are seen in multiple types of epilepsy. The hippocampus is located in the mesial region of the temporal lobe and plays a key role in the regulation, encoding, and consolidation of memory. We focused on the gain of function mutation in the SCN8A gene since it is associated with a severe childhood epilepsy known as developmental epileptic encephalopathy-13 (DEE-13). The study of neural development has traditionally been performed using animal models, typically rodents, however, these models fall short when it comes to encapsulating the complexity of the human brain development. Cerebral organoids show promise in changing our understanding by better recapitulating normal and abnormal early embryonic development to study pathogenesis. Here, we generated hippocampus-like (Hc), cortex-like (Cx) and ganglionic eminence-like (GE) organoids from a CRISPR/Cas9-corrected isogenic control and a patient derived human induced pluripotent stem cell line containing a pathogenic SCN8A mutation. We fused Hc and GE and Cx and GE organoids in order to resemble the proper mix of inhibitory and excitatory neurons observed in vivo. Mutant and iso-control fusions were then compared between each other in order to assess differences in cell expression and subjected to local field potential (LFPs) in order to examine the electrophysiological activity. Our hypothesis is that immunohistochemistry (IHC) experiments may reveal that the mutation causes significant changes to the expression of cells affecting important structures of the Hc such as dentate gyrus and cornu ammonis, leading to the effects observed in DEE13. Electrophysiological experiments may also reveal a difference between the iso-control and mutant fusion organoids in the presence or absence of important oscillatory features such as sharp wave ripples (SWRs).

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Poster

353. Stem Cell Models for Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 353.02

Topic: A.03. Stem Cells and Reprogramming

Support: NIH NINDS F31 NS129377 to J.P.M.
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Title: Targeted pharmacologic blockade of aberrant sodium current in a human induced pluripotent stem cell-derived neuron model of SCN3A neurodevelopmental disorder

Authors: *J. P. MERCHANT[1], G. QU[5], J. CLATOT[5,6], M. SALVATORE[7], J. LI[7], D. J. FREDERICK, Jr[7], L. M. DEFLITCH[5], J. MAGUIRE[8], D. L. FRENCH[8,2], S. A. ANDERSON[7,3], E. M. GOLDBERG[5,6,4,1];
[1]Dept. of Neurosci., [2]Departments of Pathology and Lab. Med., [3]Dept. of Psychiatry, [4]Dept. of Neurol., The Univ. of Pennsylvania Perelman Sch. of Med., Philadelphia, PA; [5]Div. of Neurology, Dept. of Pediatrics, [6]The Epilepsy NeuroGenetics Initiative, [7]Dept. of Psychiatry, [8]Ctr. for Cell. and Mol. Therapeut., The Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: Genetic variation in *SCN3A*, which encodes the voltage-gated sodium (Na^+) channel α subunit Nav1.3, is associated with *SCN3A* neurodevelopmental disorders (*SCN3A*-NDD), a spectrum that includes epilepsy and malformation of cortical development (MCD). *SCN3A* is expressed highly in the embryonic forebrain, yet it remains unclear how genetic variation in *SCN3A* leads to epilepsy and other pathology, in part due to lack of model systems available for study.
To investigate the mechanisms of *SCN3A*-NDD, we generated an induced pluripotent stem cell (iPSC) line from a human patient with the recurrent *de novo* heterozygous disease-causing missense variant *SCN3A*-c.2624T>C (p.Ile875Thr) as well as a CRISPR/Cas9-corrected isogenic control line; we also modified a control iPSC line via CRISPR to generate a separate cell line harboring the same variant. Using the Ngn2 rapid induction protocol, we generated glutamatergic forebrain-like neurons (iNeurons) from all four lines and confirmed by RT-PCR that Na^+ channel subunit transcript expression in iNeurons mirrors that seen in early human brain development. We then performed whole-cell patch-clamp recordings to determine the effect of the *SCN3A*-p.Ile875Thr variant on endogenous Na^+ current and cellular excitability in iNeurons. We found that iNeurons generated from both of the variant-expressing lines exhibited markedly increased slowly-inactivating/persistent Na^+ current relative to corrected-patient and control lines, which was partially but specifically blocked by the Nav1.3-selective antagonist ICA-121431 (ICA). *SCN3A*-p.Ile875Thr iNeurons displayed a more hyperpolarized voltage threshold for action potential generation - consistent with increased persistent current - which was reversibly increased by ICA at sub-micromolar concentrations. A prominent subset of *SCN3A*-p.Ile875Thr iNeurons displayed irregular firing patterns with paroxysmal bursting and plateau-like potentials with action potential failure. Paradoxically, ICA blocked these plateau-like potentials and led to an increase in maximal steady-state firing frequency in neurons exhibiting this feature. However, more consistent with action as a Na^+ channel blocker, ICA decreased excitability of corrected-patient and control iNeurons. Our study demonstrates that an iPSC-derived neuronal system models the trajectory of Na^+ channel expression in the developing brain. *SCN3A*-p.Ile875Thr iPSC-derived neurons exhibit increased persistent current and electrophysiological properties consistent with this abnormality, and selective blockade of Nav1.3 can normalize paroxysmal activity observed in *SCN3A*-p.Ile875Thr iNeurons.

Disclosures: J.P. Merchant: None. **G. Qu:** None. **J. Clatot:** None. **M. Salvatore:** None. **J. Li:** None. **D.J. Frederick:** None. **L.M. DeFlitch:** None. **J. Maguire:** None. **D.L. French:** None. **S.A. Anderson:** None. **E.M. Goldberg:** None.

Poster

353. Stem Cell Models for Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 353.03

Topic: A.03. Stem Cells and Reprogramming

Title: High-throughput functional screening to develop novel therapies for CDKL5-deficiency disorder using human iPSC-derived cortical organoids

Authors: *M. V. GREEN[1], T. ALSTAT[1], H. AHMED[2], K. PRUM[1], C. CARROMEU[2], A. LACROIX[1], R. FREMEAU[2];
[1]Vyantbio, VyantBio, Maple Grove, MN; [2]VyantBio, San Diego, CA

Abstract: CDKL5-deficiency disorder (CDD) is a rare genetic neurodevelopmental disorder that causes seizures, developmental delay, and severe intellectual disability. CDKL5 is an X-linked gene expressed highly in the brain that codes for a serine/threonine kinase with a limited number of defined substrates localized in various subcellular compartments. Mutations in CDKL5 that cause loss of enzymatic function produce aberrant hyperexcitable neuronal function. Consistent with the observed seizure activity in CDD patients, CDKL5-knockout animals display a hyperexcitable and hypersynchronous neuronal phenotype. However, these models do not always recapitulate the most prominent features of CDD, including epilepsy, and may not be the best predictors for pre-clinical drug discovery. Here, we describe a patient-derived CDKL5-deficient line of human induced pluripotent stem cells (hiPSCs) and their potential for high-throughput pre-clinical screening. hiPSCs are differentiated into neural progenitor cells (NPCs) that are used to create neuron and astrocyte co-cultures in both 2D monolayer and 3D cortical organoid format. Characterization of the cultures in both 2D and 3D cortical organoid format show hyperexcitability as measured by an increase in calcium peak frequency in the CDD line compared to control cell lines. We establish the robust reproducibility of the disease phenotype using calcium imaging via FLIPR and highlight their potential for high-throughput functional screening. Consistent with the hyperexcitable phenotype, immunocytochemistry staining in 2D monolayers shows an increase in synaptic puncta and single-nuclei RNA transcriptomic analyses of the cortical organoids show an increased expression of genes related to glutamatergic signaling in CDD organoids compared to control lines. Finally, we used this CDD organoid model to screen a library of compounds, and identified promising molecules that rescued the hyperexcitable FLIPR peak frequency. A “human first” high-throughput screening strategy using patient-derived iPSC organoids may accelerate and de-risk the drug discovery process by establishing human disease efficacy early in a program reducing the failure rate.

Disclosures: **M.V. Green:** A. Employment/Salary (full or part-time):; VyantBio. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); VyantBio. **T. Alstat:** A. Employment/Salary (full or part-time):; VyantBio. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); VyantBio. **H. Ahmed:** A. Employment/Salary (full or part-time):; VyantBio. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); VyantBio. **K. Prum:** A. Employment/Salary (full or part-time):; VyantBio. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); VyantBio. **C. Carromeu:** A. Employment/Salary (full or

part-time):; VyantBio. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); VyantBio. **A. LaCroix:** A. Employment/Salary (full or part-time):; VyantBio. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); VyantBio. **R. Fremeau:** A. Employment/Salary (full or part-time):; VyantBio. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); VyantBio.

Poster

353. Stem Cell Models for Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 353.04

Topic: A.03. Stem Cells and Reprogramming

Support: NIH BRAINS Award MH107800
NYSCF Robertson Stem Cell Investigator Award
Kwan Research Fund
Coates Foundation
Senkut Foundation
Uytengsu Research Fund
Chan Zuckerberg Initiative Ben Barres Investigator Award

Title: Human cortico-striatal assembloids from human pluripotent stem cells to study human forebrain circuits development and disease

Authors: *Y. MIURA[1], M.-Y. LI[1], F. BIREY[2], K. IKEDA[1], O. REVAH[1], M. V. THETE[1], J.-Y. PARK[1], A. PUNO[1], S. H. LEE[1], M. H. PORTEUS[1], S. P. PASCA[1];
[1]Stanford Univ., Stanford, CA; [2]Emory Univ., Atlanta, GA

Abstract: Cortico-striatal projections in the forebrain are critical components of cortico-basal ganglia circuits that regulate motivated behaviors and movement. A major challenge in understanding the developmental assembly of human cortico-basal ganglia circuit and how dysfunction in this pathway leads to neuropsychiatric disease is the lack of access to functional human brain tissue. Here, we generate three-dimensional regionalized neural organoids from human pluripotent stem cells that transcriptionally resemble human developing striatum and show how they can be assembled with cortical organoids to form cortico-striatal circuits *in vitro*. To investigate and manipulate human cortico-striatal circuits, we implement Cre recombination and G-deleted rabies viruses to identify what cortical neurons project and connect in cortico-striatal assembloids and we apply optogenetic stimulation to analyze neuronal activity using genetically encoded calcium indicators. These methods in combination with electrophysiology demonstrate the assembly of functional human cortico-striatal circuits from pluripotent stem cells. Moreover, we found disease-related synaptic defects in cortico-striatal assembloids derived from patients with a neurodevelopmental disorder caused by a deletion on chromosome 22q13.3.

We anticipate that this approach can be used to study human cortico-basal ganglia circuits development and disease modeling.

Disclosures: **Y. Miura:** None. **M. Li:** None. **F. Birey:** None. **K. Ikeda:** A. Employment/Salary (full or part-time):; Daiichi Sankyo. **O. Revah:** None. **M.V. Thete:** None. **J. Park:** None. **A. Puno:** None. **S.H. Lee:** None. **M.H. Porteus:** Other; CRISPR Therapeutics. **S.P. Pasca:** None.

Poster

353. Stem Cell Models for Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 353.05

Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant NS111965
NIH Grant NS111986
Eagles Autism Foundation
Schlumberger Foundation

Title: Overexpression of UBE3A is necessary, but not sufficient, for the development of the cellular phenotypes of the autism- and epilepsy-related Dup15q syndrome

Authors: *M. ELAMIN[1], A. DUMARCHEY[2], C. STODDARD[2], T. M. ROBINSON[1], C. COWIE[1], D. GORKA[2], S. CHAMBERLAIN[2], E. S. LEVINE[1];
[1]Neurosci., [2]Genet. and Genome Sci., Univ. of Connecticut Sch. of Med., Farmington, CT

Abstract: Dup15q is a neurodevelopmental disorder caused by maternal duplications of the 11.2-13.1 region of the long arm of chromosome 15. Children with an isodicentric supernumerary chromosome that carries two extra copies of the 15q11.2-q13.1 suffer from profound autism and refractory epileptic seizures among other symptoms. Because paternal duplications do not cause the syndrome, UBE3A, which encodes an E3 ubiquitin ligase, is likely a major driver of Dup15q because it is the only imprinted gene in the region expressed solely from the maternal allele. Nevertheless, the exact role of UBE3A has not been determined. To establish whether UBE3A overexpression is required for Dup15q neuronal deficits, we used CRISPR Cas9 technology to remove the extra isodicentric chromosome and generate an isogenic control line for a Dup15q patient-derived induced pluripotent stem cell line. Patch-clamp recordings of Dup15q neurons reveal hyperexcitability phenotypes characterized by increased maximum action potential firing frequency and increased inward and outward current densities. Calcium imaging experiments demonstrated increased spontaneous firing frequency in Dup15q cells compared to the corrected controls. With the exception of a partial effect on miniature excitatory postsynaptic currents (mEPSC), these phenotypes were generally prevented by normalizing UBE3A levels using antisense oligonucleotides. Overexpression of UBE3A in a line with paternal duplication in the same region resulted in a profile similar to that of Dup15q neurons, except for the altered synaptic transmission. These results indicate that UBE3A

overexpression is necessary for most Dup15q cellular phenotypes, but also suggest it is insufficient to cause the development of all cellular phenotypes, specifically, the synaptic phenotypes. This supports the hypothesis that other non-imprinted genes play a role in the disorder.

Disclosures: **M. Elamin:** None. **A. Dumarchey:** None. **C. Stoddard:** None. **T.M. Robinson:** None. **C. Cowie:** None. **D. Gorka:** None. **S. Chamberlain:** None. **E.S. Levine:** None.

Poster

353. Stem Cell Models for Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 353.06

Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant NS111986
NIH Grant NS125850
Eagles Autism Foundation

Title: Contribution of GABA-A receptor subunits to human Angelman syndrome and Dup15q syndrome neuronal phenotypes

Authors: *T. M. ROBINSON, D. ANJAN KUMAR, E. S. LEVINE;
Neurosci., Univ. of Connecticut Sch. of Med., Farmington, CT

Abstract: Maternal deletion or duplication of chromosomal region 15q11-q13 causes Angelman syndrome (AS) and Chromosome 15q11-q13 duplication syndrome (Dup15q), respectively. These neurogenetic disorders are characterized by developmental delay, language impairment, intellectual deficits, ataxia, and seizures. The primary driver gene for these syndromes is *UBE3A*, which encodes an ubiquitin protein ligase. This gene is normally imprinted in the brain, and expression in neurons is only from the maternal allele. Thus, a maternal deletion or mutation results in complete loss of neuronal *UBE3A* expression, and maternal, but not paternal, duplication results in *UBE3A* overexpression in neurons. Several lines of evidence suggest that the altered expression of other genes in the region, including a cluster of GABA-A receptor subunits, contributes to AS and Dup15q pathophysiology. In these studies, we used patient-derived induced pluripotent stem cell (iPSC) lines along with isogenic corrected lines to examine the contributions of *UBE3A* and the GABA-A receptor subunits to neuronal phenotypes. For AS, we compared neurons derived from a patient with a *UBE3A* mutation to patients with a full AS deletion as well as unaffected controls. Behavioral phenotypes of individuals with a *UBE3A* mutation are typically less severe than individuals with a 15q11-q13 AS deletion. Resting membrane potential and action potential maturation were affected in *UBE3A* mutation neurons to a similar extent as AS deletion neurons, suggesting *UBE3A* loss is sufficient for these phenotypes. *UBE3A* mutation neurons also had increased excitability, which was greater in AS deletion neurons, and inhibitory synaptic activity was not altered in *UBE3A* mutation neurons, in

contrast to AS deletion neurons, suggesting roles of other genes in these phenotypes. Ongoing experiments are exploring whether decreasing the expression of GABA-A receptor subunit genes in *UBE3A* mutation neurons will mimic the cellular phenotypes in AS deletion neurons. We also examined the role of GABA-A receptor subunit overexpression in Dup15q neuronal phenotypes. Normalizing the expression of the *GABRB3* subunit caused a significant depolarization of action potential threshold, reversing the phenotype observed in Dup15q neurons. Knocking down GABRB3 also caused a significant decrease in inhibitory synaptic activity. These results suggest a contributory role for GABA-A receptor overexpression in Dup15q phenotypes. Defining the roles of non-UBE3A genes in AS and Dup15q will help identify novel therapeutic targets and will inform the development of mouse models for studying behavioral phenotypes.

Disclosures: T.M. Robinson: None. **D. Anjan Kumar:** None. **E.S. Levine:** None.

Poster

353. Stem Cell Models for Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 353.07

Topic: A.03. Stem Cells and Reprogramming

Title: Transcriptional dysregulation and impaired activity in FMR1 knock-out IPSC-derived models

Authors: *G. MAUSSION[1], C. ROCHA[1], N. ABDIAN[1], D. YANG[1], L. PIMENTEL[1], C. X.-Q. CHEN[1], S. HO[2], S. HIGGINS[3], D. CARRILLO VALENZUELA[1], R. SCHUBERT[2], T. M. DURCAN[1];
[1]The Neuro's Early Drug Discovery Unit (EDDU), Montreal Neurolog. Institute, McGill Univ., Montreal, QC, Canada; [2]Roche Sequencing, Res. and Early Develop., Roche Mol. Systems, Pleasanton, CA; [3]Roche Sequencing, Computat. Sci. & Informatics, Roche Mol. Systems, Santa Clara, CA

Abstract: RNA binding proteins such as the Fragile X Mental Retardation Protein (FMRP) are crucial for the neuronal development, the synapse formation, maturation and maintenance. FMRP is involved in processes that range from RNA processing to control of local protein translation. Moreover, the lack of FMRP causes the Fragile X syndrome (FXS) which is the most prevalent form of syndromic autisms. The absence of FMRP protein, responsible for the FXS, results from the transcriptional repression of *FMR1* gene caused by CGG expansion in the 5'UTR region.
Taking advantage of CRISPR-Cas9 genome editing, we have generated an *FMR1* knock-out to assess how the absence of the functional *FMR1* gene affects the transcriptional and functional activity within IPSC-derived cortical neuronal progenitors and neurons.
Long read RNA sequencing and MEA recordings have been performed on both knockout and wild-type cells and work to date have helped to identify differential expression of transcriptional splice variants that corroborate changes in neuronal differentiation and activity in *FMR1* lacking

cells.
These findings will provide information on how altered RNA processing and the local regulation of translation affects neuronal activity and differentiation in the context of Fragile X syndrome.

Disclosures: **G. Maussion:** None. **C. Rocha:** None. **N. Abdian:** None. **D. Yang:** None. **L. Pimentel:** None. **C.X. Chen:** None. **S. Ho:** None. **S. Higgins:** None. **D. Carrillo Valenzuela:** None. **R. Schubert:** None. **T.M. Durcan:** None.

Poster

353. Stem Cell Models for Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 353.08

Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant R03 MH115426-01

Title: Examining AUTS2 deficiency on human neural progenitor cells and its consequence in neuronal differentiation

Authors: *V. MONTEIRO SAIA CEREDA[1], A. SHARMA[2], J. WHITLEY[3], C. MARCHETTO[4];
[1]UCSD, San Diego, CA; [2]Salk Inst. for Biol. Sci., San Diego, CA; [3]Columbia Univ., New York, NY; [4]LOGG, Salk Inst., La Jolla, CA

Abstract: The Autism susceptibility candidate 2 (AUTS2) gene has been implicated in neurodevelopment and as a candidate gene for numerous neurological disorders, including autism spectrum disorders (ASD) and intellectual disability (ID). Individuals with mutations in this gene commonly show microcephalic features, craniofacial abnormalities, and repetitive behaviors. Additionally, the genomic structural variants and single nucleotide polymorphisms in the AUTS2 locus are also associated with a wide range of other neurological disorders such as epilepsy, schizophrenia, attention deficit hyperactivity disorder, dyslexia and depression as well as addiction-related behaviors, implicating that AUTS2 is broadly involved in neurodevelopment. While AUTS2 is often disrupted in patients with neurodevelopmental conditions abovementioned, the mechanisms underlying the neuronal pathogenesis in developing human neurons are still unclear. To bridge this gap, we integrated pluripotent stem cells and CRISPR/Cas9 gene editing technology to investigate the biological consequences of knocking down AUTS2 gene on human stem cell lines. We studied the effect of decreased AUTS2 expression on transcriptomics and neuronal function. Our initial data analysis shows that axon development and neurogenesis pathways are significantly affected on AUTS2 deficient human neuronal progenitors. We will present data characterizing unique phenotypical properties of AUTS2 knockdown in human neural progenitors and its consequences on pathways related to neuronal differentiation. Understanding the cellular disfunction and molecular pathways

involved in AUTS2 deficiency could shed a light on the neuropathology of neurodevelopmental conditions such as ASD, ID and others.

Disclosures: V. Monteiro Saia Cereda: None. **A. Sharma:** None. **J. Whitley:** None. **C. Marchetto:** None.

Poster

353. Stem Cell Models for Neurodevelopmental Disorders

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Program #/Poster #: 353.09

Topic: A.03. Stem Cells and Reprogramming

Support: RDM Positive Impact Foundation 3-39D54
LaMPT32 Fellowship

Title: Crispr activation of syngap1 haploinsufficiency

Authors: *P. VIJ[1,2,3,4,5], P. DENG[1,2,3,4,5], J. WALDO[1,2,3,4,5], J. CARTER[1,2,3,4,5], C. GONZALEZ[1,2,3,4,5], R. NAPOLIELLO[1,2,3,4,5], K. LUCOT[1,2,3,4,5], J. HALMAI[1,2,3,4,5], K. FINK[1,2,3,4,5];
[1]Ctr. for Interventional Genet., Sacramento, CA; [2]MIND Inst., Sacramento, CA; [3]Stem Cell Program and Gene Therapy Ctr., Sacramento, CA; [4]Inst. for Regenerative Cures, Sacramento, CA; [5]Neurol., Univ. of California Davis Hlth. Systems, Sacramento, CA

Abstract: SYNGAP1 is a gene that encodes the cytosolic protein SYNGAP1 (SYNaptic GTPase Activating Protein 1), which is essential for synaptic development, structure, function, and plasticity. De novo mutations in this gene result in loss of functions and cause haploinsufficiency, leading to the MRD5 phenotype that comprises intellectual disability, developmental delay, autism, and epilepsy. A concept of possible therapeutic relevance is ‘reversal of haploinsufficiency’, which is the increase of expression levels of the normal gene copy by directly targeting the regulatory elements that control it. Therefore, the purpose of our study is to use a genome modifying mechanism, CRISPR-mediated activation (CRISPRa), which targets a transcriptional activator to the gene’s promoter as a viable approach that could potentially restore SYNGAP1 expression to healthy levels and rescue the haploinsufficiency. Due to the important role of SYNGAP1 in neurodevelopment, elucidating more details and filling the gaps in its neurobiology will help to gain a better understanding of normal and abnormal development. Thus, we will develop and test a platform, establish a disease model by characterizing a patient-derived iPSC neuronal model, and perform rescue experiments to better understand SYNGAP1 haploinsufficiency phenotype. In regards to developing and testing our platform, preliminary data from our lab has identified a lead guide RNA in the SYNGAP1 promoter that resulted in increased expression of RNA in HEK293 and Neuronal Stem Cells (NSCs) following RNA guide and effector domain screening. Our future directions are to validate these RNA guides in healthy iPSC-derived NSCs and perform molecular and

morphological characterizations, as well as rescue experiments in the patient-derived iPSC neurons.

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Poster

353. Stem Cell Models for Neurodevelopmental Disorders

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Topic: A.03. Stem Cells and Reprogramming

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Edward Mallinckrodt Jr. Foundation and National Science Foundation 5351784498
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Title: The Autism-associated Gene SYNGAP1 Regulates Human Cortical Neurogenesis

Authors: *A. DEL DOSSO[1], M. BIRTELE[1], T. XU[1], T. NGUYEN[1], B. WILKINSON[1], J.-P. URENDA[1], G. KNIGHT[3], R. ASHTON[3], E. J. HUANG[4], M. P. COBA[1], G. QUADRATO[2]; [2]Dept. of Stem Cell Biol. & Regenerative Med., [1]USC, Los Angeles, CA; [3]Biomed. Engin., Univ. of Wisconsin-Madison, Madison, WI; [4]Pathology, Univ. of California San Francisco, San Francisco, CA

Abstract: Autism spectrum disorder (ASD) is a genetically heterogeneous disorder linked with rare, inherited and *de novo* mutations occurring in two main functional gene categories: gene expression regulation and synaptic function. Accumulating evidence points to dysregulation in cortical neurogenesis as a convergent mechanism in ASD pathophysiology. While asynchronous development has been identified as a shared feature among ASD-risk genes in the category of gene expression regulation, it remains unknown whether this phenotype is also associated with ASD-risk genes in the synaptic function category. Here we show for the first time the expression of the synaptic Ras GTP-ase activating protein 1 (SYNGAP1), one of the top ASD risk genes, in human cortical progenitors (hCPs). Interestingly, we found that multiple components of the postsynaptic density (PSD) of excitatory synapses, of which SYNGAP1 is one of the most abundant components, are enriched in the proteome of hCPs. Specifically, we discover that SYNGAP1 is expressed within the apical domain of human radial glia cells (hRGCs) where it lines the wall of the developing cortical ventricular zone colocalizing with the tight junction-associated protein and MAGUK family member TJP1. In a cortical organoid model of SYNGAP1 haploinsufficiency, we show dysregulated cytoskeletal dynamics that impair the scaffolding and division plane of hRGCs, resulting in disrupted lamination of the cortical plate

and accelerated maturation of cortical projection neurons. Overall, the dual function of SYNGAP1 in neuronal synapses and progenitor cells reframes our understanding of the pathophysiology of SYNGAP1-related disorders and, more broadly, underscores the importance of dissecting the role of synaptic genes associated with neurodevelopmental disorders in distinct cell types across developmental stages.

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Poster

353. Stem Cell Models for Neurodevelopmental Disorders

Location: SDCC Halls B-H

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Topic: A.03. Stem Cells and Reprogramming

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National Council for Scientific and Technological Development (CNPq)

Title: How similar are proteomics signatures of schizophrenia in postmorten brain, iPSC-derived neural cells, and brain organoids?

Authors: *J. M. NASCIMENTO[1,2], V. M. SAIA-CEREDA[1], G. S. ZUCCOLI[1], G. REIS-DE-OLIVEIRA[1], S. K. REHEN[3,2], D. MARTINS-DE-SOUZA[1];
[1]Univ. of Campinas (UNICAMP), Campinas, Brazil; [2]D'Or Inst. of Res. and Educ., Rio de Janeiro, Brazil; [3]UFRJ, Rio de Janeiro, Brazil

Abstract: As a complex and severe neuropsychiatric disorder, schizophrenia has a wide range of debilitating symptoms. Including several multifactorial aspects of its complexity that are still unknown, with some accepted to be an early developmental deficiency and a neurodevelopmental origin. Disturbances during neural cell differentiation processes could be understood by looking at the time and neural paths of differentiation, which could lead to insight into the development of the disorder. In this context, human brain organoids and neural cells differentiated from patient-derived induced pluripotent stem cells are of great interest as a model to study the developmental origins of the disease. Here we evaluated the differential expression of proteins of schizophrenia patient-derived in three moments: neural progenitors, early neurons, and brain organoids, comparing those with *postmortem* brains. Using bottom-up shotgun proteomics with a label-free approach for quantitative analysis, 535 proteins were found differentially expressed in organoids, 364 in neural progenitor cells (NPCs), and 264 in immature neurons. Multiple dysregulated proteins were found in pathways related to synapses, in line with *postmortem* tissue studies of schizophrenia patients. However, organoids and immature neurons

exhibit impairments in pathways never found in patient-derived induced pluripotent stem cell studies, such as spliceosomes and amino acid metabolism. In conclusion, here we provide comprehensive, large-scale, protein-level data that may uncover underlying mechanisms of the developmental origins of schizophrenia.

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Poster

353. Stem Cell Models for Neurodevelopmental Disorders

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 353.12

Topic: A.03. Stem Cells and Reprogramming

Support: NIH R01_MH125528

Title: Modeling Schizophrenia associated gene SETD1A loss-of-function using human neurons

Authors: *X. SU[1,2], Y. HONG[3], L. WANG[2], S. ZHANG[4], H. ZHANG[4], H. SONG[3], J. DUAN[5,4], G.-L. MING[3], Z. P. PANG[2];
[1]Rutgers Univ. Grad. Program In Cell Develop. and Biol., New Brunswick, NJ; [2]Dept. of Neurosci. and Child Hlth. Inst. of New Jersey, Rutgers Robert Wood Johnson Med. Sch., New Brunswick, NJ; [3]Dept. of Neuroscience, Perelman Sch. of Med., Univ. of Pennsylvania, Philadelphia, PA; [4]Ctr. for Psychiatric Genet., NorthShore Univ. Hlth. Syst., Evanston, IL; [5]Univ. of Chicago Pritzker Sch. of Med., Univ. of Chicago Pritzker Sch. of Med., Evanston, IL

Abstract: Rare loss-of-function (LoF) mutations in *SETD1A* are strongly associated with schizophrenia (SZ), adebilitating mental disorder affecting 1% of the population, and other severe neurodevelopmental disorders. *SETD1A* encodes a component of the histone methyltransferase complex producing mono-, di,and trimethylated histone H3 at Lysine 4 (H3K4). H3K4 trimethylation (H3K4me3) and H3K4me1 are epigenomic marks of active gene transcriptional promoters and enhancers, respectively. Interestingly, histone methylation has also been suggested as one of the most enriched gene pathways in common variant-based genome-wide associations studies (GWAS) of major psychiatric disorders. However, the detailed molecular mechanism by which it causes neuronal dysfunction is still unclear. Recent advances in stem cell biology have allowed the efficient conversion of human stem cells into defined neuron subtypes allows to address this question. Using CRISPR/Cas9 gene editing, we have generated isogenic hiPSC lines carrying heterozygous LoF mutations on different genetic backgrounds of *SETD1A*. Preliminary results showed that mutant lines were defective in neuronal development with premature neuronal differentiation at early developmental stages. Furthermore, morphological, electrophysiological and transcriptomic analyses of hiPSC-derived induced neurons carrying *SETD1A* LoF mutation showed defective synaptic neurotransmission. Ongoing experiments are evaluating *SETD1A* LoF with functional, morphological, biochemical

and genomic parameters to understand the cellular mechanisms that how *SETD1A* LoF contributes to the pathogenesis of SZ. The study enables us to perform a well-controlled assessment of the impact of *SETD1A* LoF mutations on the molecular and cellular mechanisms underlying deficits in early neurodevelopment and synaptic properties.

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Poster

353. Stem Cell Models for Neurodevelopmental Disorders

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Program #/Poster #: 353.13

Topic: A.03. Stem Cells and Reprogramming

Support: Hungarian National Brain Research Program (Grant NAP-B KTIA_NAP_13-2014-0011)
Hungarian National Brain Research Program (Grant 2017-1.2.1-NKP-2017-00002)

Title: Investigation of de novo mutations in schizophrenia by induced pluripotent stem cell based disease modeling and CRISPR genome editing

Authors: *J. RÉTHELYI[1], C. TORDAI[1], K. VINCZE[1], Á. PÓTI[2], D. SZÜTS[2], Á. APÁTI[2]; [1]Semmelweis Univ., Budapest, Hungary; [2]Inst. of Enzymology, Res. Ctr. for Natural Sci., Budapest, Hungary

Abstract: Schizophrenia (SCZ) is a severe neuropsychiatric disorder of complex, poorly understood etiology. Both genetic and environmental factors play a role in the development of SCZ. De novo mutations (DNMs) represent a recently described new source of genetic variation in the background of SCZ. While several mutations have been associated with SCZ, in most cases their biological significance remains unclear. The aim of this study was to investigate the biological mechanisms connected to DNMs in SCZ by combining induced pluripotent stem cell (IPSC) based disease modeling and CRISPR based genome editing. To this end we selected a SCZ case-parent trio, where the affected patient carries a potentially disease causing DNMs. Based on exome sequencing studies we chose a patient harboring a zinc finger MYND domain-containing protein 11 (ZMYND11) 1495C>T nonsense DNM resulting in a R399X stop codon. ZMYND11 encodes a chromatin reader protein that specifically binds H3.3K36me3, co-localizes with highly expressed genes and promotes intron retention.
We used RNA sequencing, morphological, and functional assays to test for transcriptomic and functional differences between patient-derived and healthy control cell lines at the neuronal progenitor cell (NPC) and mature neuron stages. In the CRISPR experiments we introduced monoallelic or biallelic frameshift mutations into a control wild type IPSC line using CRISPR non-homologous end joining (NHEJ). Next, IPSC lines were generated from each member of the

case-parent trio using Sendai virus based reprogramming. The investigated ZMYND11 mutation was corrected using CRISPR homology-directed repair (HDR) in the affected IPSC line. These isogenic IPSC lines were taken forward to neuronal differentiation experiments using the dentate gyrus differentiation protocol.
Results showed distinct molecular alterations characteristic for schizophrenia-derived NPCs and neurons. RNASeq analyses at the NPC and neuronal stage showed the massive upregulation of neuronal differentiation genes in the mutant cell lines, and downregulation of cell adhesion genes. Calcium-imaging and multi-electrode array experiments indicated impaired neuronal function. These approaches can shed light on the molecular disease pathway underlying schizophrenia.

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Poster

353. Stem Cell Models for Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 353.14

Topic: A.03. Stem Cells and Reprogramming

Support: INI Fellowship
Nellie Ball
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Title: In vitro cerebellar development and axonal pathfinding in schizophrenia

Authors: *D. A. MADENCIOGLU[1,2,3,4], A. J. WILLIAMS[1,2,3,4];
[1]Dept. of Psychiatry, Univ. of Iowa, Iowa City, IA; [2]Iowa Neurosci. Inst., Iowa City, IA; [3]Pappajohn Biomed. Inst., Iowa City, IA; [4]Carver Col. of Med., Iowa City, IA

Abstract: Schizophrenia is a neurodevelopmental disorder caused by environmental and genetic risk factors, and is characterized by symptoms that include delusions, disorganized speech, social withdrawal, as well as cognitive and motor impairments. Risk genes that are associated with schizophrenia are highly expressed during development, especially during the critical window of neurogenesis and axonal pathfinding. One of the major brain regions that is implicated in the pathophysiology of schizophrenia is the cerebellum, showing altered connectivity and activity. Yet, these changes are observed in adult patients, and the initial changes that have occurred during development remain unknown. Studies investigating human cerebellar development has only been possible through neuroimaging or post-mortem studies and studying the molecular and cellular changes during this critical timepoint, especially in disease context, has been challenging. With the ability to reprogram somatic cells to induced pluripotent stem cells (iPSCs) and further re-differentiate them into neurons has made it possible to model human brain

development *in vitro*. To investigate cerebellar development in schizophrenia, we generated cerebellar neurons from iPSCs that were reprogrammed from fibroblasts, collected from healthy controls and patients diagnosed with schizophrenia. We employed a cerebellar differentiation protocol by inducing cerebellar fate with FGF2 and insulin that generates a 2D monolayer of cells. At day 35 of differentiation, both healthy controls and schizophrenia cells express cerebellar markers including ATOH1, PTF1α, PAX6, EN2, and KIRREL2, indicating that glutamatergic and GABAergic cerebellar neuronal precursors as well as Purkinje cell progenitors are generated. Additionally, we confirmed the expression of axonal guidance molecules relevant for cerebellar development and connectivity, such as semaphorin-4C, plexin-B2, and neuropilin-1. Moreover, our data suggests that axonal guidance molecules are differentially expressed in cells generated from patients diagnosed with schizophrenia. These findings may enhance our understanding of the developmental aspect of schizophrenia and how disruption in axonal guidance pathways in the cerebellum contribute to the miswiring of neuronal circuits.

Disclosures: D.A. Madencioglu: None. **A.J. Williams:** None.

Poster

353. Stem Cell Models for Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 353.15

Topic: A.03. Stem Cells and Reprogramming

Title: Evidence that Inflammation may initiate psychosis

Authors: *Z. LIU, C. A. TAMMINGA;
UT Southwestern Med. Ctr., Dallas, TX

Abstract: The role of inflammation in psychosis has long been suspected, and antipsychotics are known to suppress inflammation. To explore how, we used RNA-seq to assess the gene expression in the postmortem hippocampal subfields, DG, CA3, and CA1 from healthy controls and volunteers with psychosis both ON- and OFF-antipsychotics. We found that inflammatory biomarkers were significantly activated in CA3, but not CA1 or DG, from psychosis OFF-antipsychotics (N=6) relative to psychosis ON-antipsychotic (N=7), and that anti-apoptotic biomarkers were robustly upregulated in CA3 from psychosis ON-antipsychotics compared to either psychosis OFF-antipsychotics or healthy controls (N=13). These data are consistent with the hypothesis that inflammation may precipitate psychosis via activating apoptosis, and that antipsychotics improve psychosis by diminishing the inflammatory and apoptotic pathways. At this point, we generated neural progenitor cells (NPCs) from induced pluripotent stem cells (iPSCs) derived from both patients with psychosis (N=5) and controls (N=4). Even though the inflammatory biomarkers were no different between psychosis- and control-derived lines at the iPSC stage, the RNA-seq data revealed that inflammatory biomarkers were significantly higher in psychosis-lines relative to control-lines at the NPC stage, suggesting that inflammation may have a role in both early neurodevelopment and early psychosis in psychotic people. Together,

our data recommend that inflammatory biomarkers can possibly initiate and precipitate psychosis, at least in some individuals mediated by some of these biomarkers, expanding our idea of a potential molecular basis and understanding of the pathogenesis of psychosis.

Disclosures: Z. liu: None. **C.A. Tamminga:** None.

Poster

353. Stem Cell Models for Neurodevelopmental Disorders

Location: SDCC Halls B-H

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Program #/Poster #: 353.16

Topic: A.03. Stem Cells and Reprogramming

Support: National Centre for the Replacement, Refinement and Reduction of Animals in Research NC/S001506/1

Title: Il-6 induces cell-specific transcriptional and morphological changes associated with neurodevelopmental disorders

Authors: *A. C. M. COUCH, S. SOLOMON, A. MARROCU, R. R. R. DUARTE, L. SICHLINGER, R. MATULEVICIUTE, S. KORDASTI, D. P. SRIVASTAVA, A. C. VERNON; King's Col. London, London, United Kingdom

Abstract: Irrespective of species, elevated interleukin (IL-)6 is consistently associated with an increased likelihood for neurodevelopmental disorders (NDDs), such as schizophrenia (SZ), autism spectrum disorder (ASD) and depression. While we know much about IL-6 effects in rodent models, our understanding of these IL-6 effects in human microglia and neurons is incomplete. Human induced pluripotent stem cell (hiPSC)-derived microglia-like cell (MGL) and cortical neural progenitor cell (NPC) co-culture models are ideal to study effect of IL-6 on human neurodevelopment, given the neurodevelopmental differences between human and rodents. Here we have characterised the response of these cells to IL-6. MGLs and NPCs were generated from three healthy male donors, with three clone cultures per donor. We measured expression of relevant receptor machinery for IL-6 signaling by qPCR and quantified their responses to various doses of IL-6 by immunoblot and qPCR. The MGL transcriptomic response to IL-6 was further quantified by RNAseq. Based on these data, we additionally characterized the impact of IL-6 on cell motility and morphology by live imaging and assessed the secretome of treated cells by ELISA and immunoblot. We observed that NPCs did not respond to IL-6 in monoculture at both a protein and transcript level because they did not express the essential receptor machinery *IL-6Ra*. MGLs, but not NPCs, secreted the soluble IL-6Ra (sIL-6Ra) and this secretion was unaffected by IL-6 treatment. Only IL-6 exposed MGLs but not NPCs resulted in STAT3 phosphorylation within 15min and increased *IL-6, JMJD3, IRF8* and *IL-10* expression after 3h. This confirms classical activation of the IL-6 signalling pathway in MGLs only. RNAseq identified 156 upregulated genes after MGL 3h IL-6 exposure, which significantly overlapped with an upregulated gene set from post-mortem schizophrenia patient tissue, but not

with the genes downregulated in cases. IL-6 MGL 3h exposure significantly induced motility and morphological changes suggesting a gain of surveillance function that corresponded with gene ontology pathways identified in the RNAseq dataset. Given the inability of NPCs to respond to IL-6 in monoculture, the increased likelihood for NDDs posed to developing NPCs by IL-6 requires an additional cell type to facilitate IL-6 trans-signaling to the NPCs. This additional cell type could be microglia releasing sIL-6R and shifting towards an increased surveillance state. These findings suggest IL-6 may increase likelihood for NDDs in a cell-specific manner and highlight the requirement for both cell types to be present in co-culture when investigating the human molecular basis for NDDs.

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Poster

353. Stem Cell Models for Neurodevelopmental Disorders

Location: SDCC Halls B-H

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Program #/Poster #: 353.17

Topic: A.03. Stem Cells and Reprogramming

Support: R01MH110438
R01NS100808

Title: Modeling psychiatric disorders using human pluripotent stem cells

Authors: *D. DESAI, A. VASUDEVAN;
Angiogenesis and Brain Develop., Huntington Med. Res. Inst., Pasadena, CA

Abstract: One in four people worldwide is affected by some form of psychiatric disorder. These disorders negatively affect an individual in terms of thinking, feeling, and behaving, and are commonly characterized by a combination of abnormal thoughts, perceptions, emotions, behavior, and relationships. Current treatments for psychiatric disorders focus on alleviating some of the symptoms, but there is no cure. Abnormalities in brain cells/neurons have been extensively implicated in psychiatric disorders, and a growing field of research is tapping into the potential of iPSC technology for human psychiatric disease modeling and treatment. Our previous research identified a specialized embryonic forebrain endothelial cell population that can directly contribute to psychiatric disorders, such as schizophrenia, anxiety, and depression, and we have translated this discovery from mouse to human. We have developed a protocol to generate human embryonic forebrain-like endothelial cells from control and patient-specific human induced pluripotent stem (iPS) cells. These embryonic forebrain-like endothelial cells can be used not only for cell-based therapies, but also for understanding the basic biology of the disease. Our protocol involves the use of two crucial molecules - GABA and WNT7A, that stimulate specific aspects of angiogenesis, and the cell surface receptor - GABRB3, that serves

as a distinct marker for endothelial cells that are forebrain-like. Following the use of these molecules, the $CD31^+$ and $GABRB3^+$ cells were isolated to generate pure populations of human embryonic forebrain-like endothelial cells. Differences in morphology and marker expression (GABA, GABRB3, PAX6, WNT7A, CLAUDIN5, and ZO1) were observed between the control and the patient-specific embryonic forebrain-like endothelial cells. Additionally, there were distinct phenotypic differences seen in the functionality of the patient-specific endothelial cells. Endothelial cells generated from patient iPS cells demonstrated abnormal tube formation, along with a decreased rate of proliferation, and migration. Based on these results, we wish to delineate novel fundamental neurovascular mechanisms that are altered in psychiatric disorders.

Disclosures: D. Desai: None. **A. Vasudevan:** None.

Poster

353. Stem Cell Models for Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 353.18

Topic: A.03. Stem Cells and Reprogramming

Support: NIH R01 NS109540

Title: Alteration in mRNA translation of Tuberous Sclerosis Complex patient-derived neural progenitor cells is only partially reversed by rapamycin

Authors: I. S. AKSOYLU[1], **P. MARTIN**[2], F. ROBERT[3], K. J. SZKOP[1], N. E. REDMOND[2], S. CHEN[1], R. L. BEAUCHAMP[2], I. NOBELI[4], O. LARSSON[1], J. PELLETIER[3], *V. RAMESH[2]; [1]Dept. of Oncology-Pathology, Sci. for Life Lab., Karolinska Inst., Stockholm, Sweden; [2]Ctr. for Genomic Med., Massachusetts Gen. Hosp., Boston, MA; [3]Dept. of Biochem. and Goodman Cancer Res. Ctr., McGill Univ., Montreal, QC, Canada; [4]Dept. of Biol. Sciences, Inst. of Structural and Mol. Biology, Birkbeck, Univ. of London, London, United Kingdom

Abstract: Tuberous sclerosis complex (TSC) is an inherited multi-system disorder caused by mutations in the *TSC1* or *TSC2* gene. TSC patients are often diagnosed with a range of neurodevelopmental (ND) manifestations termed TSC-associated neuropsychiatric disorders (TAND) including autism spectrum disorder (ASD), intellectual disability (ID), anxiety and mood disorders. Hamartin (TSC1) and tuberin (TSC2) proteins form a complex inhibiting mechanistic target of rapamycin complex 1 (mTORC1) kinase signaling. Loss of TSC1 or TSC2 activates mTORC1 that, among several targets, controls protein synthesis by inhibiting translational repressor eIF4E-binding proteins. Using neural progenitor cells (NPCs) from patient-derived induced pluripotent stem cells (iPSCs), we recently reported early ND phenotypic changes, including increased cell proliferation and altered neurite outgrowth in CRISPR-modified *TSC1*-null NPCs, which were unaffected by mTORC1 inhibition by rapamycin, the only approved therapy for TSC. Here, to assess TSC1-dependent gene expression programs in NPCs, we used polysome-profiling, which quantifies changes in mRNA abundance

and translational efficiencies at a transcriptome-wide level. In addition to changes in mRNA abundance, this revealed numerous genotype-dependent alterations in translational efficiencies depending on expression of TSC1. TSC1-sensitive translation associated with 5'UTR features including length and GC content, known to underlie mTOR-sensitive translation. To assess the relevance of these gene expression alterations we performed polysome-profiling in post-mortem brains originating from ASD donors and matched controls. Strikingly, TSC1-dependent alterations in mRNA translation observed in NPCs were largely recapitulated in human brains. Furthermore, although polysome-profiling revealed a partial reversal of TSC1-associated gene expression alterations following rapamycin treatment, most genes related to neural activity/synaptic regulation or ASD that showed TSC1-dependent translation were rapamycin-insensitive. Therefore, we also examined whether early ND rapamycin-insensitive phenotypes in *TSC1*-null NPCs could be rescued by a third-generation bi-steric, mTORC1-selective inhibitor RMC-6272 (Revolution Medicines, Inc.). Unlike rapamycin, RMC-6272 strongly inhibited translation and reversed TSC1-associated proliferation and neurite outgrowth phenotypes. In summary, we reveal ample translational alterations in TSC1 patient-derived NPCs recapitulating human brain expression profiles and potential implications for treatment of TAND.
The first 4 authors contributed equally.

Disclosures: I.S. Aksoylu: None. **P. Martin:** None. **F. Robert:** None. **K.J. Szkop:** None. **N.E. Redmond:** None. **S. Chen:** None. **R.L. Beauchamp:** None. **I. Nobeli:** None. **O. Larsson:** None. **J. Pelletier:** None. **V. Ramesh:** None.

Poster

354. Autism: Synaptic Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 354.01

Topic: A.07. Developmental Disorders

Support: R37 NS-071785-013

Title: Hippocampal network dysfunction in null Cntnap2 mouse model of autism spectrum disorder

Authors: *R. PATERNO, J. R. MARAFIGA, S. C. BARABAN;
Epilepsy Res. Lab. and Weill Inst., San Francisco, CA

Abstract: Mutation of the CNTNAP2 gene is a risk factor for development of autism spectrum disorder (ASD). Our recent work showed that Cntnap2 KO animals exhibit a loss of parvalbumin (PV)+ interneurons, a decreased perisomatic inhibition in hippocampal area CA1 as well as impaired spatial object recognition task performance (Paterno et al. Cell Rep. 2021). We also reported a complex disruption of theta-gamma oscillations along CA1 and reduced sharp wave ripple power. To investigate how a deficit in PV+ INs alter CA1 pyramidal cells (PC) sub-circuits and hippocampal-dependent behaviors, here we use customized 32-channel silicon

probes, comprised of two shanks with 20-µm vertical spacing between electrodes, to simultaneously record in vivo local field potentials (LFPs) and single units from CA1 superficial and deep pyramidal cells. Recordings were performed while the mice were navigating an open field, a linear track (100 cm long), a delayed spatial alternation task, as well as during sleep states in the home cage before and after task performance. We tested place cell features and place field stability as well as hippocampal place cells ability to remap in a new environment. Furthermore, we identified CA1 putative interneurons classified into PV-like and SOM-like interneurons based upon burst index and refractory period, we analyzed their firing properties and their correlation with theta phase in both WT and Cntnap2 null mice. Firing patterns of pyramidal cells and PV+ interneurons correlated with pathological information processing in CA1. These data suggest altered hippocampal circuit dynamics play a role in the autism phenotype.Support: NIH/NINDS grant #R37 NS-071785-013 (to S.C.B.)

Disclosures: R. Paterno: None. **J.R. Marafiga:** None. **S.C. Baraban:** None.

Poster

354. Autism: Synaptic Mechanisms

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 354.02

Topic: A.07. Developmental Disorders

Support: SFARI Pilot Award #724187
NIH Grant R01MH124870

Title: The development of excitatory and inhibitory neocortical circuits in a mouse model of autism spectrum disorder

Authors: D. J. BOYLE, M. HANSON, D. NAGARAJAN, J. FITZGERALD, E. GAITTEN, A. MARSHALL, O. KOKIKO-COCHRAN, B. GU, ***J. C. WESTER**;
Neurosci., Ohio State Univ., Columbus, OH

Abstract: An imbalance in the ratio of excitation to inhibition (E/I) is a potential pathophysiological circuit mechanism of autism spectrum disorder (ASD). However, excitatory and inhibitory neuronal subtypes are diverse, and their relative contributions to abnormal circuit development are largely unknown. *Arid1b* is a top 25 ASD risk gene expressed during early development in both excitatory and inhibitory neurons. Here, we used Emx1-IRES-Cre and Nkx2.1-Cre mice to conditionally knockout one copy of *Arid1b* from either excitatory projection neurons or inhibitory interneurons, respectively, starting from embryonic day 10.5. We then performed paired whole-cell patch clamp experiments in slices of primary visual cortex from mature mice to investigate synaptic connectivity and physiology. We parsed excitatory cells based on their projection class as intratelencephalic (IT) or pyramidal tract (PT), and inhibitory neurons based on their firing properties as fast spiking (FS) perisomatic-targeting or non-FS dendrite-targeting cells. Among excitatory neurons, *Arid1b* haploinsufficiency resulted in

increased synaptic connectivity rate among IT-type cells, with no homeostatic compensation in synaptic input from FS interneurons. Furthermore, we observed hyperpolarized spike threshold in a subset of cells. This suggests pathology in excitatory neurons contributes to E/I imbalance. However, we found weak behavioral and seizure phenotypes; thus, these cellular and synaptic changes are insufficient to account for autistic behavioral abnormalities. Alternatively, inhibitory interneuron dysfunction may be a primary cause. Indeed, previous work found that conditional *Arid1b* haploinsufficiency in interneurons reproduces ASD behaviors observed in germline mutants. We found that interneuron *Arid1b* haploinsufficiency resulted in fewer FS interneurons in mature mice, with a concomitant reduction in the rate of their synaptic connectivity to PT-type excitatory projection neurons in paired recordings. Furthermore, we observed evidence for disrupted feedback inhibition from non-FS dendrite targeting cells to IT-type excitatory neurons. Our data support an emerging hypothesis that developmental disorders such as ASD may be "interneuronopathies".

Disclosures: **D.J. Boyle:** None. **M. Hanson:** None. **D. Nagarajan:** None. **J. Fitzgerald:** None. **E. Gaitten:** None. **A. Marshall:** None. **O. Kokiko-Cochran:** None. **B. Gu:** None. **J.C. Wester:** None.

Poster

354. Autism: Synaptic Mechanisms

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Program #/Poster #: 354.03

Topic: A.07. Developmental Disorders

Support: R01NS105333

Title: Impaired homeostatic plasticity of PV inhibitory circuits in 2 mouse models of autism

Authors: *H. R. MONDAY[1], K. W. WONG[2], F. D. BOLIO[2], D. E. FELDMAN[3];
[1]Helen Wills Neurosci. Inst., [3]UC Berkeley, [2]UC Berkeley, Berkeley, CA

Abstract: Parvalbumin (PV) neuron hypofunction in sensory cortex is thought to contribute to abnormal sensory processing in Autism Spectrum Disorders (ASDs), but the functional origins and molecular mechanisms of PV hypofunction remain unclear. We hypothesize that PV hypofunction is associated with impaired homeostatic plasticity within PV circuits, which normally acts to maintain cortical excitability during periods of shifting sensory input. In L2/3 of whisker somatosensory cortex (S1), PV circuit homeostasis occurs in response to brief (1 day) whisker deprivation and involves rapid reduction of intrinsic excitability of PV neurons, decreasing feedforward inhibition. We assayed PV circuit homeostasis in *Fmr1-/y* and *Tsc2+/-* mouse models of ASD by testing for deprivation-induced reduction in L4-evoked feedforward IPSCs in L2/3 pyramidal (PYR) cells. *Fmr1-/y* and *Tsc2+/-* models share PV hypofunction but differ in other molecular and synaptic phenotypes, making them a powerful test for common impairment of PV homeostasis in ASD. Deprivation reduced feedforward IPSCs in WT mice,

but not in *Fmr1-/y* or *Tsc2+/-* mice, indicating an impairment in PV circuit homeostasis. To probe the mechanisms of PV homeostasis, we identified molecular players that could mediate deprivation-induced weakening of PV intrinsic excitability, which is known to involve increased expression of Kv1 potassium currents. In PV cells, activity regulates Kv1.1 expression via the transcription factor ER81, whose synthesis is activity-dependent. Using immunohistochemistry, we found that 1 day of deprivation upregulated ER81 and Kv1.1 in L2/3 PV cells in WT mice, but this upregulation failed to occur after deprivation in *Fmr1-/y* or *Tsc2+/-* mice. Thus, PV homeostasis involves activity-dependent regulation of ER81 and Kv1.1 proteins, and these molecular signatures of homeostasis are absent in ASD model mice. This homeostatic deficit may be related to the general dysregulation of activity-dependent protein synthesis in both *Fmr1-/y* and *Tsc2+/-* mice. The common impairment in adaptive homeostatic plasticity in PV cells in 2 mouse models of autism suggests that rescue of PV homeostasis could be a useful therapeutic target for ASD.

Disclosures: H.R. Monday: None. **K.W. Wong:** None. **F.D. Bolio:** None. **D.E. Feldman:** None.

Poster

354. Autism: Synaptic Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 354.04

Topic: A.07. Developmental Disorders

Support: Telethon-Italy GGP11043

Title: Impaired excitatory and inhibitory synaptic plasticity in the NLG3-R451C mouse model of autism spectrum disorder

Authors: *M. BRUNO[1,2], S. STANCHEVA[1], L. MANNINO[1], M. RUBEN[1], A. POLENGHI[1], F. COLACI[1], E. PETRINI[1], A. BARBERIS[1];
[1]Neurosci. Area, Inst. Italiano di Tecnologia, Genova, Italy; [2]Dinogmi, Univ. degli Studi di Genova, Genova, Italy

Abstract: Autistic spectrum disorder (ASD) has been associated to genetic alterations of proteins involved in synaptic function such as the point mutation R451C in neuroligin 3 (NLG3), a postsynaptic adhesion molecule which binds its presynaptic partner neurexin at both excitatory and inhibitory synapses. In the present study, by exploiting the transgenic NLG3-R451C knock-in (KI) mice as an ASD animal model, we aimed at investigating the role of this mutation in the coordination of both excitatory and inhibitory synaptic plasticity. We found that, with respect to WT condition, the $NLG3^{R451C}$ protein was less expressed at the neuronal surface and showed increased lateral diffusion at GABAergic synapses. However, we observed comparable molecular and functional properties of excitatory and inhibitory synapses in WT and KI neurons, as documented by electrophysiological recordings and immunocytochemistry experiments

whereby key molecular synaptic components were probed. Next, we tested WT and KI neurons for the expression of synaptic plasticity. In WT animals, we observed that, in response to a chemical protocol for the induction of plasticity (NMDA treatment), excitatory synaptic currents were depressed (LTD) whereas the inhibitory ones were potentiated (iLTP). Interestingly, such opposed synaptic plasticity was abolished in KI neurons. These effects were paralleled by changes in synaptic receptors and scaffold proteins at both excitatory and inhibitory synapses in WT and KI neurons. Indeed, after the NMDA stimulation, WT mice showed increased immunoreactivity for GABAAR and the scaffold protein gephyrin at synapses. In contrast, no significant changes were observed in KI mice. Along the same line, NMDA treatment induced the decrease of the GluA1 and GluA2 subunits of AMPA receptors in WT mice, whereas the same protocol left AMPARs unchanged in KI mice. In addition, the quantification of NLG3 clusters fluorescence intensity revealed that, after the NMDA treatment, surface NLG3 decreased in WT neurons while it was unaffected in KI neurons. Moreover, in KI conditions, we found changes in the expression of the NR2B subunit of NMDA receptor that may determine the altered coordination of excitatory and inhibitory plasticity through the perturbation of intracellular spatio-temporal calcium dynamics. Collectively, our results reveal that the perturbed synaptic molecular composition of both glutamatergic and GABAergic synapses induced by the NLG3-R451C mutation disrupts the coordination of excitatory and inhibitory synaptic plasticity thus potentially contributing to the pathophysiology of ASD.

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Poster

354. Autism: Synaptic Mechanisms

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Program #/Poster #: 354.05

Topic: A.07. Developmental Disorders

Support: SFARI Bridge to Independence award 479737

Title: Syngap1 haploinsufficiency in inhibitory interneurons impairs sensory-guided goal-directed learning in mice

Authors: *M. ZHAO, S. E. KWON;
Univ. of Michigan, Ann Arbor, MI

Abstract: SynGAP (Synaptic Ras-GTPase-activating protein) is a synaptic protein causally linked to neurodevelopmental disorders including autism, with common symptoms of cognitive impairments and sensory processing deficits. Although *SYNGAP1* is predominantly expressed in excitatory neurons of forebrain structures including the cerebral cortex and hippocampus, its expression is also detected in cortical inhibitory interneurons. Whether and to what extent SynGAP functions in the cortical inhibitory circuit has remained relatively unexplored. In this

study, we created mice with inhibitory neuron-specific SynGAP haploinsufficiency (SynGAP het) and tested its impact on neocortical circuit function using the whisker somatosensory system. We recorded the activity of layer 2/3 neurons in whisker somatosensory cortex (wS1) with two-photon calcium imaging during a whisker-guided tactile detection task. Each trial of the task begins with a simple auditory tone, followed by whisker deflection on 60% of trials. Licking in response to whisker deflection results in water reward delivery whereas premature licking before the whisker stimulus onset results in trial abortion. SynGAP het mice displayed impaired behavioral performance accompanied by abnormal neuronal responses, compared to wild-type mice. First, SynGAP het mice displayed a lower fraction of correct trials and less efficient licking during the response window. After analyzing the real-time neural activities, we found pairwise noise correlation between individual neurons was higher and more widely distributed in SynGAP het mice. Analytically removing those noise correlations by shuffling improved population decoding of stimulus more in SynGAP het mice than in wild-type mice, which indicates that groups of neurons in SynGAP het mice with abnormally elevated correlations may contribute to the impaired behavioral performance. Second, trial abortion rate was significantly elevated in SynGAP het mice due to increased number of premature licks in response to the auditory tone rather than the whisker stimulus. An increased fraction of tone-responding neurons and a greater magnitude of tone-evoked responses were observed in SynGAP het mice, which may be highly associated with the increased number of premature licks and aborted trials. Collectively, our results reveal that SynGAP expression in inhibitory neurons is important for encoding of task-relevant sensory input and cortical filtering of irrelevant sensory cue.

Disclosures: M. Zhao: None. **S.E. Kwon:** None.

Poster

354. Autism: Synaptic Mechanisms

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Program #/Poster #: 354.06

Topic: A.07. Developmental Disorders

Support: NIH IPR Grant ZIAMH002959

Title: Reduction of SYNGAP1 in Parvalbumin (PV)-positive GABAergic neurons impairs long-range cortico-cortical connectivity

Authors: *S. NASKAR[1], P. STEVENSON[3], J. QI[4], S. LEE[2];
[2]NIMH, [1]NIH, Bethesda, MD; [3]NIH, Natl. Inst. of Mental Hlth., Washington, MD; [4]NATIONAL INSTITUTES OF HEALTH, Baltimore, MD

Abstract: Impairment of the communication between cortical areas, including feedback interaction, have been attributed to a wide range of neurodevelopmental disorders (NDDs). Previously, we reported that functional connectivity of diverse long-range inputs from different brain areas preferentially recruit specific types of GABAergic interneurons (INs) in the primary

somatosensory cortex (S1). This input-area-dependent recruitment of specific GABAergic INs plays an important role in active sensory processing. Abnormal sensory perception is common in NDDs, and the mechanisms that underlie impaired sensory processing associated with NDDs are not well studied. Haploinsufficiency of the *SYNGAP1* (Synaptic Ras GTPase Activating Protein1) gene, one of the key postsynaptic density proteins, has been shown to impair sensory processing and cognition in both humans and mice. Reduction of SYNGAP1 in mice causes cognitive deficits by enhancing glutamatergic transmission and increasing excitatory connectivity. While the importance of *SYNGAP1* in the excitatory neurons has been reported, the role of *SYNGAP1* in cortical GABAergic neurons is largely unknown. Here, we asked whether and how the disruptions of *SYNGAP1*, one of NDD risk genes, in different GABAergic IN subtypes lead to the impairment of input-area-dependent corticocortical communication. We first confirmed that cortical GABAergic neurons do express *SYNGAP1* gene. Using optogenetics and *ex vivo* whole-cell patch-clamp recordings with specific deletion of *SYNGAP1* in different types of GABAergic neurons, we found that the long-range cortical inputs to different types of GABAergic INs in S1 are altered. In PVCre:*Syngap1*$^{fl/w}$ (PVCre:*Syngap1* cKO) mice, PV neurons in S1 receive abnormally strong excitatory inputs from the whisker-related primary motor cortex (wM1). This result contrasts with our earlier report where PV INs in S1 do not receive strong excitatory inputs from wM1. Since it is known that *SYNGAP1* regulates the level of AMPA receptors at the postsynaptic membrane of excitatory neurons, we asked whether the NMDA and AMPA conductance are altered for wM1 inputs to S1 in PVCre:*Syngap1*$^{fl/w}$ mice. We found that the NMDA/AMPA ratio from PV INs was significantly higher in PVCre:*Syngap1*$^{fl/w}$ mice compared to that of control. Together, our study suggests that *SYNGAP1* plays a critical role in regulating the excitatory synaptic strength from long-range inputs to cortical GABAergic neurons.

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Poster

354. Autism: Synaptic Mechanisms

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Program #/Poster #: 354.07

Topic: A.07. Developmental Disorders

Support: FRQS fellowship for Postdoctoral Training (Citizens of other countries), Numero dossier 287538

Title: Conditional deletion of Syngap1 in Nkx2.1-expressing MGE-derived interneurons decreases AMPA-mediated synaptic transmission and thalamocortical input onto Parvalbumin-positive interneurons in mouse auditory cortex.

Authors: *R. FRANCAVILLA[1], B. CHATTOPADHYAYA[2], J. L. MICHAUD[3], G. DI CRISTO[4];
[1]Departments of Neurosciences, Ctr. de Recherche du Chu Sainte-Justine, Montréal, QC,

Canada; [2]CHU Sainte-Justine Res. Ctr., Montreal, QC, Canada; [3]Dept. of Pediatrics, Hôpital Sainte-Justine, Montreal, QC, Canada; [4]Departments of Neurosciences and Pediatrics, CHU Ste.justine-University of Montreal, Montreal, QC, Canada

Abstract: Intellectual disability (ID) and autism spectrum disorder (ASD) are among the most common neurodevelopmental disorders observed in childhood. In addition to cognitive and behavioural impairments, neurodevelopmental disorders often result in sensory processing deficits. Nevertheless, the biological mechanisms that underlie impaired sensory processing associated with neurodevelopmental disorders are poorly understood. *SYNGAP1* haploinsufficiency-related intellectual disability (*SYNGAP1*-ID) is characterized by moderate to severe ID, generalized epilepsy, ASD and other behavioral abnormalities. To date hundreds of individuals with *SYNGAP1*-ID have been reported worldwide through genetic sequencing and this number is rising rapidly due to more widespread genetic testing. We have recently found that auditory sensory processing and associated neuronal oscillations in the gamma band are altered in *SYNGAP1*-ID patients and *Syngap1* haploinsufficient mice, however the underling cellular mechanisms are currently unknown. To investigate the role of *Syngap1* deletion in parvalbumin-positive (PV+) cell circuits *in vitro*, we generated mice that were heterozygous for the *Syngap1*^{flox} allele and hemizygous for the Tg(*Nkx2.1-Cre*) transgene. In mouse, Nkx2.1-expressing medial ganglionic eminence (MGE) precursors produce most of PV+ and Somatostatin cortical interneurons. Here, using Tg(*Nkx2.1Cre; Syngap1*$^{lox/+}$) conditional mice, we examined the cell-specific effects of conditional deletion of *Syngap1* on AMPA and GABAergic-mediated synaptic transmission onto PV+, fast spiking GABAergic interneurons from layer IV of mouse auditory cortex, using whole-cell voltage clamp recording in combination with electrical stimulation of thalamic fibers. We found that the amplitude of AMPA-mediated spontaneous excitatory postsynaptic currents (EPSCs) was decreased in PV cells from Tg(*Nkx2.1Cre; Syngap1*$^{lox/+}$) conditional mice compared to control littermates. On the other hand, no change was found in the spontaneous inhibitory postsynaptic currents (IPSCs). Furthermore, we found that the AMPA component of thalamocortical evoked-EPSC was decreased in PV cells from mutant mice. Taken together, these data suggest that, in Tg(*Nkx2.1Cre; Syngap1*$^{lox/+}$) conditional mice, unaltered inhibition and a decrease in the spontaneous and thalamocortical AMPA component could lead to a reduced recruitment of PV cells. The latest could contribute to the increased baseline cortical gamma rhythm, a phenotype observed in both mutant mice and *SYNGAP1*-ID patients.

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Poster

354. Autism: Synaptic Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 354.08

Topic: A.07. Developmental Disorders

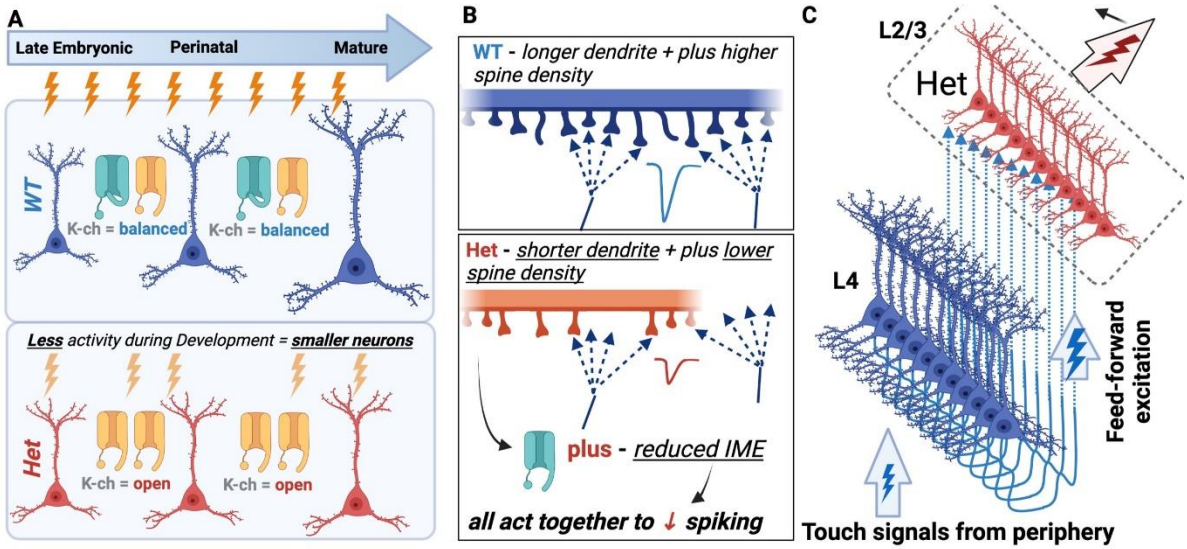
Support: Leon and Friends Foundation Grant

Title: Syngap1 Assembles Cortical Circuits by Regulating Intrinsic Membrane Excitability

Authors: *V. ARORA[1], M. ACETI[2], S. MICHAELSON[1], M. KLINIK[3], C. MILLER[1], G. RUMBAUGH[1];
[1]UF SCRIPPS BIOMEDICAL RESEARCH, UF SCRIPPS BIOMEDICAL RESEARCH, JUPITER, FL; [2]AstraZeneca, Cambridge, United Kingdom; [3]Alkahest Inc, San Francisco, CA

Abstract: *SYNGAP1* haploinsufficiency in humans causes a neurodevelopmental disorder defined by cognitive impairment and behavioral maladaptations. However, it remains unclear how expression of this gene during development promotes the functioning of neural systems controlling behavior and cognition. We demonstrate in mice that a consequence of *Syngap1* expression in development is to control cortical neuron intrinsic membrane excitability (IME). *Syngap1* promotes IME in upper-lamina sensory cortex neurons by suppressing potassium conductances. It is also required for homeostatic plasticity of IME. Targeting alternatively spliced *Syngap1* isoforms revealed that regulation of IME was dissociable from synaptic scaling, indicating that it regulates these processes through distinct mechanisms. *Syngap1* regulation of IME was required for the *in vivo* assembly of excitatory cortical circuit motifs implicated in perceptual decision making. Restoring IME in embryonic *Syngap1* mutant mice unleashed activity-dependent dendritic morphogenesis and prevented excitatory circuit assembly impairments. Thus, *Syngap1* expression naturally shapes the assembly of cortical circuit motifs through control of IME. **Highlights** 1. Novel *Syngap1* function*;* regulation of pyramidal neuron intrinsic membrane excitability. 2. It sets resting membrane potential and enables homeostatic plasticity. 3. Achieved through regulation of neuronal potassium currents. 4. Unleashes dendritic morphogenesis required for excitatory circuit assembly.

Model of how *Syngap1* promotes assembly of feed-forward motifs in sensory cortex



Disclosures: **V. Arora:** None. **M. Aceti:** None. **S. Michaelson:** None. **M. Klinik:** None. **C. Miller:** None. **G. Rumbaugh:** None.

Poster

354. Autism: Synaptic Mechanisms

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Program #/Poster #: 354.09

Topic: A.07. Developmental Disorders

Support: Jerome Lejeune Foundation
Colorado State University

Title: Autism-associated δ-catenin G34S mutation disrupts social behavior by GSK3b-mediated degradation

Authors: *H. MENDEZ-VAZQUEZ, K. NIP, R. LEE, M. C. MOSELEY, M. SATHLER, R. DANZMAN, S. KIM;
Colorado State Univ., Colorado State Univ., Fort Collins, CO

Abstract: Social impairment is a core symptom in several mental disorders, including autism spectrum disorder (ASD), depression, and schizophrenia. However, the physiological, cellular, and molecular factors underlying social behavior are poorly understood. In humans, mutations in the δ-catenin gene are linked to severe ASD. δ-catenin is a post synaptic scaffolding protein and is important for AMPA receptor (AMPAR) GluA2 subunit localization and functions. δ-catenin KO mice exhibit social dysfunction with decreased synaptic GluA2 levels in the cortex. This suggests that δ-catenin deficiency induces social deficits by impairing AMPAR-mediated synaptic functions. Yet, the link between δ-catenin function and social behavior is largely unknown. A glycine to serine mutation at residue 34 (G34S) in the amino-terminal region of δ-catenin is identified in humans as one of several ASD-associated missense mutations. The G34S mutation significantly reduces excitatory synapse density in cultured neurons, indicating a loss of δ-catenin function. Glycogen synthase kinase 3 beta (GSK3β) phosphorylates δ-catenin, causing ubiquitination and degradation by proteasome. The Group-based Prediction System predicts that the G34S mutation in the amino-terminus of δ-catenin may provide an extra site for GSK3β-mediated phosphorylation of δ-catenin. We indeed find that the G34S mutation promotes δ-catenin degradation via GSK3β-mediated phosphorylation. A δ-catenin G34S knock-in (KI) mouse model was further used to determine if the G34S δ-catenin mutation reduced synaptic AMPAR levels and induced social dysfunction. We found a significant reduction in synaptic δ-catenin and GluA2 expression in the cortex of G34S animals, suggesting potential alteration of AMPAR activity. Finally, we used the three-chamber sociability test in male and female δ-catenin KI mice and WT littermates and found disruptions in sociability and social novelty in the mutant mice. Taken together, we reveal that G34S δ-catenin significantly reduces synaptic δ-catenin levels by GSK3β-mediated phosphorylation, which likely changes AMPAR activity in

the cortex, leading to impaired sociability and social novelty. Therefore, GSK3β inhibition is likely neuroprotective in ASD-associated δ-catenin G34S mutants.

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Poster

354. Autism: Synaptic Mechanisms

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Program #/Poster #: 354.10

Topic: A.07. Developmental Disorders

Support: Jerome Lejeune Foundation
Colorado State University

Title: Loss of δ-catenin function impairs social behavior

Authors: *R. L. ROACH, H. MENDEZ-VAZQUEZ, R. LEE, M. C. MOSELEY, M. SATHLER, R. A. DANZMAN, S. KIM;
Colorado State Univ., Colorado State Univ., Fort Collins, CO

Abstract: Normal social behavior is vital to many species for survival. Therefore, its underlying mechanisms have long been a focus for research. However, our comprehension of the physiological, cellular, and molecular mechanisms of social behavior is still limited. Moreover, there are several neurological/psychiatric disorders such as autism spectrum disorder (ASD) that have social dysfunction as a common characteristic. With this, expanded knowledge of the mechanisms mediating social behavior will improve our understanding of such diseases. Importantly, genetic alterations in the δ-catenin gene in humans are associated with severely affected ASD patients from multiple families. δ-catenin is a postsynaptic scaffolding protein and is important for AMPA receptor (AMPAR) GluA2 subunit localization and functions in several regions of the brain. Some ASD-associated δ-catenin mutations significantly reduce excitatory synapse density in cultured neurons, indicating a loss of δ-catenin function. Moreover, our preliminary findings demonstrate that δ-catenin knockout (KO) mice exhibit social dysfunction. We also show that synaptic AMPAR subunit GluA2 levels in cortical areas are significantly lower in δ-catenin KO mice than in their wild-type (WT) littermates. This indicates that a loss of δ-catenin functions induces social deficits via impairing AMPAR-mediated synaptic functions. Additional new data show that δ-catenin KO increases glutamatergic excitation in cultured cortical excitatory cells, whereas it is decreased in inhibitory cells. This suggests that δ-catenin deficiency likely disrupts the cellular E/I balance, which will in turn disturb the neural activity in the brain, particularly in the medial prefrontal cortex (mPFC) that is known to regulate social behavior. These joint findings lead us to hypothesize that normal social behavior relies on δ-catenin-mediated prefrontal activity at the cellular and network levels.

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Poster

354. Autism: Synaptic Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 354.11

Topic: A.07. Developmental Disorders

Support: NRF Grant 2019H1A2A1076692
NRF Grant 2020M3E5D9080794
IBS-R002-D1

Title: Adult re-expresssion of BBB-penetrant IRSp53 rescues NMDA receptor function and social behavior in IRSp53-mutant mice

Authors: *Y. NOH[1], C. YOOK[2], J. KANG[2], S. LEE[1], Y. KIM[1], E. YANG[3], H. KIM[3], E. KIM[1]; [1]Korea Advanced Inst. of Sci. and Technol., Korea Advanced Inst. of Sci. and Technol., Daejeon, Korea, Republic of; [2]Ctr. for Synaptic Brain Dysfunctions, Inst. for Basic Sci. (IBS), Daejeon, Korea, Republic of; [3]Dept. of Anat. and BK21 Grad. Program, Biomed. Sciences, Col. of Medicine, Korea Univ., Seoul, Korea, Republic of

Abstract: IRSp53 (or BAIAP2) is an abundant excitatory postsynaptic scaffolding/adaptor protein that is involved in actin regulation and has been implicated in autism spectrum disorders, schizophrenia, and attention-deficit/hyperactivity disorder. IRSp53 deletion in mice leads to enhanced NMDA receptor (NMDAR) function and social deficits that are responsive to NMDAR inhibition. However, it remains unclear whether IRSp53 re-expression in the adult IRSp53-mutant mouse brain after the completion of brain development could reverse these synaptic and behavioral dysfunctions. Here we employed a brain-blood barrier (BBB)-penetrant adeno-associated virus (AAV) known as PHP.eB to drive adult IRSp53 re-expression in IRSp53-mutant mice. The adult IRSp53 re-expression normalized social deficits without affecting hyperactivity or anxiety-like behavior. In addition, adult IRSp53 re-expression normalized NMDAR-mediated excitatory synaptic transmission in the medial prefrontal cortex. Our results suggest that adult IRSp53 re-expression can normalize synaptic and behavioral deficits in IRSp53-mutant mice and that BBB-penetrant adult gene re-expression has therapeutic potential.

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Poster

354. Autism: Synaptic Mechanisms

Location: SDCC Halls B-H

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Topic: A.07. Developmental Disorders

Support: MOST 110-2320-B-A49A-503
MOST 110-2320-B-A49A-504

Title: Characterization of differential GABAergic deficits in the generational pathophysiology of social deficit

Authors: *M. CHU[1], C.-W. LEE[1], C.-H. CHANG[1], T.-J. YANG[1], T.-N. PENG[1], H. CHI[1], Y.-C. LIN[1], H.-C. LIN[1,2];
[1]Inst. of Physiology, Natl. Yang Ming Chiao Tung Univ., Taipei, Taiwan; [2]Brain Res. Center, Natl. Yang Ming Chiao Tung Univ., Taipei, Taiwan

Abstract: Disruption of normal brain development is implicated in numerous psychiatric disorders with neurodevelopmental origins, including autism spectrum disorder (ASD). Widespread abnormalities in brain structure and functions caused by dysregulations of neurodevelopmental processes has been recently shown to exert adverse effects across generations. An imbalance between excitatory/inhibitory (E/I) transmission is the putative hypothesis of ASD pathogenesis, supporting by the specific implications of inhibitory gamma-aminobutyric acid (GABA)ergic system in autistic individuals and animal models of ASD. However, the contribution of GABAergic system in the neuropathophysiology across generations of ASD is still unknown. Here, we uncover profound alterations in the expression and function of $GABA_A$ receptors ($GABA_A$Rs) in the amygdala across generations of the VPA-induced animal model of ASD. The F2 generation was produced by mating an F1 VPA-induced male offspring with naïve females after a single injection of VPA on embryonic day (E12.5) in F0. Autism-like behaviors were demonstrated across two generations of the VPA-induced offspring. Decreased synaptic $GABA_A$R and gephyrin levels, and inhibitory transmission were found in the amygdala from two generations of the VPA-induced offspring with greater reductions in the F2 generation. Weaker association of gephyrin with $GABA_A$R was shown in the F2 generation than the F1 generation. Moreover, dysregulated NMDA-induced accumulations of gephyrin and $GABA_A$R at the synapse in the VPA-induced offspring was worsened in the F2 generation than the F1 generation. Taken together, these findings revealed the inhibitory synaptic abnormalities in the amygdala from two generations of the VPA-induced offspring with GABAergic deteriorations in the F2 generation, suggesting a potential therapeutic role of the GABAergic system to generational pathophysiology of ASD.

Disclosures: M. Chu: None. **C. Lee:** None. **C. Chang:** None. **T. Yang:** None. **T. Peng:** None. **H. Chi:** None. **Y. Lin:** None. **H. Lin:** None.

Poster

354. Autism: Synaptic Mechanisms

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Topic: A.07. Developmental Disorders

Support: NIMH: 5R01MH094681
NIMH: 5T32MH073124

Title: Clinical Correlates of GABAergic Interneuron Pathology in Autism

Authors: *B. DUFOUR[1], E. L. MCBRIDE[2], T. BARTLEY[2], P. JUAREZ[3], V. MARTINEZ-CERDENO[2];
[1]Psychiatry and Behavioral Sci. / MIND Inst., [2]Pathology, [3]Integrative Pathobiology Grad. Program, UC Davis, Sacramento, CA

Abstract: Autism Spectrum Disorder (ASD) is a prevalent and debilitating neurodevelopmental disorder, characterized by social communication deficits and restricted repetitive behaviors. Intellectual disability (ID) and epilepsy are common comorbidities. While a variety of pathological changes have been identified in the human ASD brain, it remains largely unclear how specific neuropathological changes correspond with ASD symptomology. Here, in an exploratory retrospective study, we assessed the relationship between GABAergic interneuron pathology and ASD symptom severity and comorbidity. Clinical records were collected from Autism (n=20) and control (n=19) brain donors, from whom we previously identified a reduction in parvalbumin+ Chandelier Cells (ChCs), a specific type of GABAergic interneuron, in the ASD prefrontal cortex (BA9, 46, and 47). The relationship between ASD core symptom severity, as indicated by ADI-R scores, and ChC pathology was assessed using Pearson Correlation. The relationship between ASD comorbidities and GABAergic interneuron cell counts (including parvalbumin+ [PV], calbindin+ [CB], and calretinin+ [CR] interneurons) were assessed using repeated measures ANOVA. We found a significant correlation between the severity of ChC loss in the Orbitofrontal Cortex (BA47) with the severity of stereotypic motor behaviors in ASD subjects. This finding was replicated in two sets of subjects measured with two separate indicators of ChC loss - reductions in PV+ interneurons in one cohort ($r= -0.722$, $p=.043$), and reduced GAT1+ cartridges in a second cohort ($r= -0.710$, $p=.049$), both corresponding to increased severity of stereotypic motor behavior. Patterns of interneuron loss in the prefrontal cortex differed between ASD subjects with Intellectual Disability (ID, IQ<70) and ASD subjects without ID. While all ASD subjects showed a significant reduction in PV+ interneurons ($p<.05$), ASD with ID exclusively showed a reduction in total interneuron number (PV+, CB+, CR+) relative to non-ID ASD (-38.7%, $p<.05$), and also relative to controls (-36.6%, $p<.01$). We identified that distinct patterns of GABAergic interneuron pathology in the ASD prefrontal cortex are associated with specific types of ASD symptomology and comorbidity. These findings highlight the importance of accounting for clinical subtypes when investigating neuropathological changes in the ASD brain, emphasize the importance of GABAergic dysfunction to the neurobiology of ASD, and thus provide strong rationale for further investigation.

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Poster

354. Autism: Synaptic Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 354.14

Topic: A.07. Developmental Disorders

Title: Effects of Global and Astrocyte-Specific Deletion of FMR1 on tonic GABA currents in Hippocampus CA1 Pyramidal Neurons

Authors: *S. MAPLES[1], V. WAGNER[2], A. KULINICH[3], I. ETHELL[3], P. HICKMOTT[4]; [1]Univ. of California, Riverside, Univ. of California, Riverside, Chino, CA; [2]Neurosci., [3]Biomed. Sci., [4]Psychology, Univ. of California, Riverside, Riverside, CA

Abstract: Knockout of the FMR1 protein in mice serves as an important model system for human fragile-X syndrome (FXS) and other autism-spectrum disorders (ASD). There is increasing evidence that hyperexcitability of neural circuits caused by decreased inhibition is a common deficit in both FXS and ASD. Studies of inhibition have primarily focused on synaptic inhibition, however, there is also significant tonic inhibition caused by ambient GABA acting at extra-synaptic receptors. Tonic inhibition is thought to play important roles in regulating overall inhibitory tone and may increase when synaptic inhibition is decreased. We have recent evidence that suggests that GABA release from astrocytes may be upregulated in FMR1 KO; we will use standard whole-cell recordings from global and astrocyte-specific FMR1 KO mice to assess the physiological relevance of this observation. Tonic GABA currents will be investigated using whole-cell recordings from Hippocampus CA1 pyramidal neurons from mice aged p21-p28. Bath-application of 100μM bicuculline methiodide (BMI) is used to block GABAA receptors, thus revealing the tonic GABA currents as a change in the baseline holding current when the cell is voltage-clamped to 0mV (to suppress excitatory currents). GABA currents will be examined in both a global FMR1 knockout (KO) and an astrocyte-specific one. Both male and female mice will be used, resulting in a full KO condition (males) and a partial KO condition (females), for both a global FMR1 KO and a Hippocampus-astrocytic FMR1 KO, compared to male and female wildtypes. The data will be collected in a double-blind study, with identification made post-hoc through genotyping. The data presented will showcase a comparison of Hippocampus astrocytic-specific FMR1 deletion, partial astrocytic deletion, general deletion, and gender effects. Based on prior research, it is predicted that the complete astrocytic-specific FMR1 KO condition will have the greatest increase in tonic GABA currents resulting in higher baseline excitation of the pyramidal neurons. The partial astrocytic FMR1 KO will have enhanced tonic GABA currents but not as much as the complete astrocytic FMR1 KO, yet significantly more so than the wild type. The general FMR1 KO should be similar to the complete astrocytic FMR1 KO.

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Poster

354. Autism: Synaptic Mechanisms

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Program #/Poster #: 354.15

Topic: A.07. Developmental Disorders

Support: NRF-2017R1A5A2015391
NRF-2020M3E5D9080794
IBS-R002-A1
IBS-R002-D1

Title: Early postnatal synaptic correction leads to long-lasting correction of synaptic and behavioral phenotypes in Arid1b-haploinsufficient mice

Authors: *H. KIM[1], D. KIM[2], Y. CHO[3], K. KIM[2], J. D. ROH[2], Y. KIM[4], E. YANG[5], S. KIM[6], S. AHN[6], H. KIM[5], H. KANG[7], Y. BAE[3], E. KIM[2];
[1]Dept. of Biol. Sci., Korea Advanced Inst. for Sci. and Technol. (KAIST), Yuseong-gu, Korea, Republic of; [2]IBS, Daejeon, Korea, Republic of; [3]Sch. of Dentistry, Kyungpook Natl. Univ., Daegu, Korea, Republic of; [4]Grad. Sch. of Biomed. Engin., KAIST, Daejeon, Korea, Republic of; [5]Korea Univ. Col. Med., Seoul, Korea, Republic of; [6]KRICT, Daejeon, Korea, Republic of; [7]KISTI, Daejeon, Korea, Republic of

Abstract: ARID1B, also known as BAF250B, is a subunit of the SWI/SNF chromatin remodeling complex and has been implicated in intellectual disability, autism spectrum disorders (ASD), and Coffin-Siris syndrome. We characterized Arid1b-haploinsufficient mice and found that these mice display autistic-like behaviors, including social impairments and repetitive behaviors. Electrophysiological analyses indicate that mutant cortical neurons display synaptic deficits at juvenile and adult stages, which accompanies transcriptional changes in synapse-associated genes. Pharmacological corrections of these synaptic deficits at early postnatal stages prevent both synaptic and behavioral deficits in adult mice, highlighting the importance of early postnatal corrections of mechanistic deviations for long-lasting effects.

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Poster

354. Autism: Synaptic Mechanisms

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Program #/Poster #: 354.16

Topic: A.07. Developmental Disorders

Support: NIH Grant 1F31MH123008-01A1

Title: The autism spectrum disorder gene NEXMIF affects neural dynamics in the hippocampal CA1 in mice

Authors: R. MOUNT[1], ***M. ATHIF**[1], M. O'CONNOR[2], H. MAN[2], X. HAN[1];
[1]Dept. of Biomed. Engin., [2]Dept. of Biol., Boston Univ., Boston, MA

Abstract: The genetic bases of autism spectrum disorder (ASD) are heterogeneous and polygenic, and many ASD risk genes have been identified over the years. While their molecular and biochemical functions have been examined, it remains unclear how mutations in these genes influence neural circuit dynamics. To understand how these mutations lead to neural circuit deficits, we performed in vivo calcium imaging in a transgenic mouse model with the total knockout of the neurite extension and migration factor (NEXMIF) gene. NEXMIF is an X-linked ASD risk gene identified through genomic screening of human patients with autistic phenotypes. Patients carrying NEXMIF mutations exhibit intellectual deficiency, language impairments, repetitive behavior, and seizures. NEXMIF mRNA is strongly expressed in the mouse hippocampus, and NEXMIF-knockout mice exhibit a variety of behavioral deficits, including profound learning and memory deficits.
We performed large-scale calcium imaging from hundreds of individual CA1 neurons simultaneously in NEXMIF KO mice and their wildtype littermates during voluntary locomotion. We found that individual calcium events in neurons from NEXMIF KO mice are narrower compared to WT, suggesting that NEXMIF is important in regulating cellular calcium dynamics. Interestingly, both during rest and running, neurons from KO mice showed significantly higher calcium event rates than WT mice. Further, a larger fraction of neurons in KO mice responded to movement compared to WT mice. The elevated response during both rest and running supports increased neuronal excitability in KO mice during behavioral conditions, consistent with the synaptic level observation of increased E/I balance. To understand how changes in individual neurons relate to population circuit dynamics, we examined the Pearson correlation between neuron pairs. In WT mice, the correlation coefficients between significantly correlated neuron pairs were significantly higher during rest than running, suggesting a desynchronization of the CA1 network during running. But in KO mice, the correlations were significantly higher during running than in rest, suggesting a lack of circuit desynchronization that naturally occurs in the CA1 network. Furthermore, a significantly larger fraction of movement-responsive cells in KO mice are significantly correlated, suggesting that increased network synchronization is related to pathological behavior. These results provide in vivo evidence that NEXMIF gene mutations that increase synaptic E/I balance can lead to over-excitability at the individual neuron level, which leads to pathological network synchronization during behavior.

Disclosures: R. Mount: None. **M. Athif:** None. **M. O'Connor:** None. **H. Man:** None. **X. Han:** None.

Poster

354. Autism: Synaptic Mechanisms

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Topic: A.07. Developmental Disorders

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NRF-2018R1A5A2025964
NRF-2017M3C7A1029611
NRF-2018H1A2A1061381

Title: The activity of NAcSh-projecting IL neurons is critical for social recognition in mice

Authors: *G. PARK[1], C. RYU[2], S. KIM[3], M. YOO[3], S. KIM[4], Y.-S. LEE[4];
[1]Seoul Natl. Univ. Col. of Med., Seoul, Korea, Republic of; [2]Seoul Natl. Univ. Col. of Med., Seoul Natl. Univ. Col. of Med., Jongno-Gu, Seoul, Korea, Republic of; [3]Seoul Natl. Univ., [4]Seoul Natl. Univ. Col. of Med., Seoul Natl. Univ. Col. of Med., Seoul, Korea, Republic of

Abstract: The medial prefrontal cortex (mPFC) plays important roles in social behaviors. However, it is not clear how the mPFC contributes to the distinct aspects of social behaviors. We took an advantage of a social isolation-regrouping protocol which induces selective deficit in social recognition without affecting social interaction to investigate the neurobiological mechanism underlying social recognition in mice. We found that mice which had experienced 8 weeks of single housing (SH) show impaired social recognition, which symptom being sustained after 4 weeks of re-socialization. In SH mice, prefrontal infralimbic (IL) neurons projecting to the shell region of nucleus accumbens (NAcSh) show decreased excitability compared to those of group housed (GH) mice. These neurons are activated when GH mice encounter a familiar conspecific, suggesting that the activity of this neuron is critical for social recognition. Chemogenetic inhibition of NAcSh-projecting IL neurons in mice which never experienced social isolation impaired social recognition without affecting social preference. Consistently, activation of these neurons reverses the social recognition deficit in SH mice. Our findings demonstrate that early social experience critically affects the mPFC IL-NAcSh projection, the activation of which is required for the social recognition.

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Poster

354. Autism: Synaptic Mechanisms

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Program #/Poster #: 354.18

Topic: A.07. Developmental Disorders

Support: Simons Foundation Autism Research Initiative-Research Award

Title: Developmental progression of prefrontal circuit dysfunction in a SHANK3$^{-/-}$ mouse model of Autism Spectrum Disorder

Authors: *G. DEVIENNE[1], G. VANTOMME[2], J. VISWANATHAN[2], J. HUGUENARD[2];
[1]Wu Tsai Neurosciences Inst. Stanford Neurosciences Building, Stanford Univ., Stanford, CA;
[2]Stanford Univ., Stanford Univ., Palo Alto, CA

Abstract: Autism Spectrum Disorders (ASDs) are phenotypically and genetically heterogeneous behavioral disorders of neural development. Despite numerous studies focusing on ASD-related pathophysiological circuit mechanisms in mature mice, little is known regarding ASD onset and its evolution through development. Using the Shank3 complete knockout (Shank3$^{-/-}$) ASD mouse model our team aims to fill this knowledge gap. The medial prefrontal cortex (mPFC) circuit plays an important role in social and cognitive behavior. We studied mPFC circuitry in mice at two developmental stages: adults and 14 days old, the latter at a developmental period in which Shank3 protein expression is transiently reduced. We used linear silicon arrays to characterize mPFC function in acute brain slices of adult wild-type (WT) and Shank3$^{-/-}$ mice. This allowed recording of local field potential (LFP) across the entire cortical column. Intracortical electrical stimulation resulted in a distinct LFP laminar profile, which was deconstructed via current source density (CSD) analysis. Pharmacological dissection isolated distinct presynaptic and postsynaptic sources/sinks. We identified a global mPFC hyperfunction in adults, with the greatest deficits seen in deeper neocortex (by ~2x at maximum stimulus). Furthermore, pyramidal cells intracellular recordings revealed a significant difference in resting membrane potential (RMP: -65 +/- 1.5 mV vs -69 +/- 1.8 mV) and action potential properties of layer 5 pyramidal cells. Intrinsic excitability differences were limited to mPFC L5 while superficial L2/3 pyramidal neurons were unaffected. Strikingly at P14, our LFP results show reduction of CSD signal in Shank3$^{-/-}$ in both pre- and post-synaptic upper mPFC layers signals without any differences affecting the deeper layers. Of note, Shank3$^{-/-}$ L5 pyramidal cells at this developmental stage exhibit a depolarized RMP. In contrast, preliminary intracellular data in L2/3 suggest hypoexcitable pyramidal cells at P14. To summarize our study shows a novel developmental transient change in mPFC excitability. This suggests that early circuit hypofunction may lead to the later observed mPFC hyperexcitability network and ASD development. Interestingly, the adult's hyperexcitability seems to be linked to a L5 specific alteration of pyramidal cells intrinsic excitability, which is in line with other studies. Building on these findings we hypothesize that the output synapses from the prefrontal cortex are affected in SHANK3$^{-/-}$ mouse. Therefore, we plan to examine mPFC-subcortical targets synapses in order to better understand the downstream effects of mPFC alteration during postnatal development.

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Poster

354. Autism: Synaptic Mechanisms

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Program #/Poster #: 354.19

Title: WITHDRAWN

Poster

354. Autism: Synaptic Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 354.20

Topic: A.07. Developmental Disorders

Support: SFARI Pilot Award #724187
NIH Grant R01MH124870

Title: Arid1b haploinsufficiency in interneurons alters hippocampal early network activity

Authors: *A. H. MARSHALL, M. HANSON, D. NAGARAJAN, N. BIBI, J. C. WESTER; Neurosci., Ohio State Univ., Columbus, OH

Abstract: Autism spectrum disorder (ASD) is hypothesized to be caused by pathological development of excitatory and inhibitory neural circuits. However, the developmental stage during which these circuit deficits occur is unknown. Here, we studied early postnatal circuit development in a mouse model of ASD, Arid1b haploinsufficiency. In mice, cortical circuits spontaneously generate synchronized network activity known as giant depolarizing potentials (GDPs) at the end of the first postnatal week. GDPs promote synapse development and are crucial to ensure proper circuit formation in the adult brain. GDPs are generated through the synergistic depolarizing action of both glutamatergic and GABAergic currents due to high intracellular chloride during early development. Arid1b haploinsufficiency results in reduced density of interneurons due to disruptions in neuronal proliferation, thus we hypothesized this will alter GDPs during early development. We used a transgenic mouse model which leads to Arid1b haploinsufficiency in a population of interneurons known to be essential for GDP initiation and propagation. To assay early network activity, we performed dual whole-cell patch clamp recordings of an interneuron and neighboring pyramidal cell to monitor GDPs in slices of mouse hippocampus. We assessed GDP frequency, synaptic charge transfer (CT), and temporal synchrony of GDPs observed in paired recordings. Our preliminary data show that GDPs occur with high temporal synchrony in interneurons and pyramidal cells in both control and mutant mice. However, in mutant mice GDPs occur at a lower frequency comparted to controls. Furthermore, there is a delay in the development of synapses onto interneurons in mutant mice: at younger ages, the CT observed in interneurons is smaller relative to controls. Our data show that Arid1b haploinsufficiency in interneurons is sufficient to alter early patterned network activity necessary for proper hippocampal circuit development. Thus, the pathophysiological mechanisms of ASD likely occur long before the formation of mature circuits.

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Poster

354. Autism: Synaptic Mechanisms

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Topic: A.07. Developmental Disorders

Support: SFARI Pilot Award #724187
NIH Grant R01MH124870

Title: Cell-type specific transcriptomic signatures of neocortical circuit organization and their relevance to autism

Authors: *A. J. MOUSSA, J. C. WESTER;
Dept. of Neurosci., Ohio State Univ., Columbus, OH

Abstract: A prevailing challenge in neuroscience is understanding how diverse neuronal cell types select their synaptic partners to form circuits. In the neocortex, major subclasses of excitatory projection neurons and inhibitory interneurons are conserved across functionally distinct regions. There is evidence these subclasses form circuits that depend primarily on their identity; however, regional cues likely also influence their choice of synaptic partners. We mined the Allen Brain Institute's single-cell RNA-sequencing database of mouse cortical neurons to study the expression of cellular adhesion molecules (CAMs) necessary for synapse formation in two regions: the anterior lateral motor cortex (ALM) and the primary visual cortex (VISp). We used the Allen's metadata to parse cells by clusters representing major excitatory and inhibitory subclasses that are common to both ALM and VISp. We then performed two types of pairwise differential gene expression analysis: 1) between different neuronal subclasses within the same brain region (ALM or VISp), and 2) between the same neuronal subclass in ALM and VISp. We filtered our results for differentially expressed genes encoding CAMs and developed a novel bioinformatic approach to determine the sets uniquely enriched in each neuronal subclass in ALM, VISp, or both. This analysis provides an organized set of genes that may regulate circuit formation in a cell-type specific manner. Furthermore, it identifies candidate mechanisms for the formation of circuits that are conserved across functionally distinct cortical regions or that are region dependent. Finally, we used the SFARI Human Gene Module to identify CAMs from our analysis that are related to risk for autism spectrum disorder (ASD). From over 3,000 differentially expressed genes, we found 40 ASD-associated CAMs that are enriched in specific neuronal subclasses in both ALM and VISp. Our analysis provides clear molecular targets for future studies to understand neocortical circuit organization and abnormalities that underly autistic phenotypes.

Disclosures: **A.J. Moussa:** None. **J.C. Wester:** None.

Poster

354. Autism: Synaptic Mechanisms

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Topic: A.07. Developmental Disorders

Support: MSS Korea Grant S2611222

Title: Blood-brain barrier permeable neural activity modulators as therapeutic targets for the treatment of autism spectrum disorder

Authors: K. UM[1], M. CHOI[1], S. KWAK[1], H. PARK[1], H. KIM[1], A. HAM[1], D. LIM[1], S.-K. HWANG[2], ***K. C. AHN**[1];
[1]Astrogen R&D Ctr. for Intractable Brain Dis., Daegu-si, Korea, Republic of; [2]Pediatrics, Sch. of Medicine, Kyungpook Natl. Univ., Daegu-si, Korea, Republic of

Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by impaired social interaction and communication, repetitive, and rigid behaviors with higher incidence, and few effective treatments. Neuronal excitatory and inhibitory synaptic imbalance has been implicated in ASD. We herein identified a lead candidate, AST-001 as a novel treatment of ASD and just completed the phase 2 clinical trials advancing to the next round for the first time in Korea. Our studies uncovered acting mechanisms of AST-001 on the firing activity change of midbrain neurons, which was facilitated and recovered to the basal firing level during the proposed washout period. In alignment with spontaneous action potentials (APs), AST-001 reduced afterhyperpolarization (AHP) to help those neurons fire faster. In addition, AST-001 increased NMDA receptor excitatory postsynaptic currents (EPSC) evoked by electrical synaptic stimulation. In line with previous studies, decreased firing rate and small conductance of hyperpolarization-activated current (Ih) were also observed in the midbrain of valproic acid (VPA)-induced mouse model of ASD. Those alterations in neuronal activity were recovered to normal levels after 2 weeks of 500 mg/kg (per os) AST-001. Using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS), the pharmacokinetics and blood-brain barrier (BBB) permeability of AST-001 were validated in the present study. AST-001 levels reached significantly higher concentration than the basal level 30 minutes after oral administration and were sustained for a quite long time. The integrity of the in vitro BBB system was validated by the trans-endothelial electrical resistance (TEER) and Evans blue dye permeability test. In vivo microdialysis, AST-001 was orally administered or intraveously injected into mice, and their interstitial fluid (ISF) was collected for analysis through a microdialysis probe implanted into the brain region of interest. It was confirmed in both in vitro and in vivo BBB validation systems that AST-001 can be efficiently transported into the brain through the BBB and the drug can act on the brain changing neural activity. Finally, its therapeutic effects were evaluated in the VPA-induced ASD mouse model, AST-001 improved social behavior and anxiety-like behavior model using the three-chamber test and

elevated plus maze test, respectively. Taken together, the present preclinical studies have considerably supported our clinical studies that AST-001 may be a putative treatment for ASD.

Disclosures: K. Um: None. **M. Choi:** None. **S. Kwak:** None. **H. Park:** None. **H. Kim:** None. **A. Ham:** None. **D. Lim:** None. **S. Hwang:** None. **K.C. Ahn:** None.

Poster

354. Autism: Synaptic Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 354.23

Topic: A.07. Developmental Disorders

Support: Wallenberg Academy Fellowship
StratNeuro Bridging Grant
Karolinska Institute Principal Researcher
Vetenskaprådet Starting Grant

Title: Dysregulated synaptic transmission and plasticity in the striatum of the eIF4E mouse model of autism

Authors: *A. AALTONEN[1], C. CRISCUOLO[2], E. KLANN[3], A. BORGKVIST[1], E. SANTINI[1]; [1]Karolinska Inst., Karolinska Inst., Stockholm, Sweden; [2]The Nathan S. Kline Inst. for Psychiatric Res., New York, NY; [3]Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: A key symptom of autism spectrum disorders (ASDs) is the occurrence of repetitive behaviours, which is thought to originate from a dysregulated basal ganglia network. A prevailing hypothesis is that symptoms of ASD arise from disrupted synaptic function and structural plasticity caused by increased protein synthesis. Our previous work has shown that overexpressing eukaryotic initiation factor 4E (eIF4E) in mice results in increased rate of repetitive behaviours, cognitive inflexibility and social deficits, supporting the view that increased protein synthesis via eIF4E causes ASD-associated phenotypes. The aim of this project is to determine if the basal ganglia plays a role in the pathophysiology of ASD, by assessing striatal synaptic function in the eIF4E mouse model. We performed whole-cell electrophysiology to determine the synaptic strength, input strength and plasticity profile of D1- and D2-MSNs using recordings of miniature excitatory post-synaptic currents (mEPSCs), NMDA to AMPA ratio, paired pulse ratio and plasticity via high-frequency stimulation. eIF4E mice display increased mEPSC frequency in both D1- and D2-MSNs and a reduction in mEPSC amplitude in D2-MSNs. In addition, we found altered corticostriatal long-term potentiation in D2-MSNs. In conclusion, our preliminary data suggest increased global synaptic inputs in the eIF4E mice to both MSN types, as well as dysregulated corticostriatal synaptic plasticity particularly to D2-MSNs.

Disclosures: A. Aaltonen: None. **C. Criscuolo:** None. **E. Klann:** None. **A. Borgkvist:** None. **E. Santini:** None.

Poster

354. Autism: Synaptic Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 354.24

Topic: A.07. Developmental Disorders

Support: Wallenberg Academy Fellowship
StratNeuro Bridging Grant
Karolinksa Institute Principal Researcher
Vetenskaprådet Starting Grant
Karolinska Institute Doctoral Education Support (KID)

Title: Impaired dopamine release probability in the eIF4ETg mouse model of autism.

Authors: *J. CARBONELL, A. AALTONEN, A. BORGKVIST, E. SANTINI;
Dept. of Neurosci., Karolinska Inst., Stockholm, Sweden

Abstract: Autism Spectrum Disorders (ASDs) are a heterogeneous group of polygenic disorders characterized by various degrees of behavioral alterations, including repetitive behaviors, social impairment, and cognitive inflexibility. It has been suggested that these behaviors may result from disrupted basal ganglia network activity. Accordingly, studies in ASD mouse models and patients have shown altered basal ganglia connectivity and function. Our previous study showed that mice overexpressing the eukaryotic initiation factor 4E (eIF4E) display ASD-like behaviors accompanied by striatal synaptic impairments and increased brain protein synthesis. Given the importance of dopamine (DA) in basal ganglia function, this study aims to investigate whether striatal DA neurotransmission is dysregulated in the eIF4E Tg mice. To this end, we performed Fast-Scan Cyclic Voltammetry (FSCV) recordings in the acute striatal slices. We found a decreased DA release probability with no changes in firing activity or morphology of DA neurons of the eIF4E Tg mice. Since neuromodulation is critical for axonal DA release, we are currently investigating the effect of striatal glutamate, achetylcholine and GABA on altered DA neurotransmission of the eIF4E Tg mice. In summary, our results suggest impaired dopaminergic transmission of the eIF4E Tg mice, resulting from altered striatal neuromodulation.

Disclosures: J. Carbonell: None. **A. Aaltonen:** None. **A. Borgkvist:** None. **E. Santini:** None.

Poster

355. Developmental Disorders: Genetic Models II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 355.01

Topic: A.07. Developmental Disorders

Support: Gerogetown Graduate Student Research support funds

Title: Ephrin-a deletions produce limbic system circuit abnormalities in mouse models of developmental neurological spectrum disorders

Authors: A. HUGHES[1], P. CURCIO[2], A. PETSCHEK[2], *L. F. KROMER[1];
[1]Neurosci., Georgetown Univ. Med. Ctr., Washington, DC; [2]Biol., Georgetown Univ., WASHINGTON, DC

Abstract: Previous research has demonstrated neural circuit abnormalities and dendritic arborization changes in neurodevelopmental disorders, including autism, ADHD, and OCD. However, genetic and epigenetic factors underpinnings of these neurological changes remain elusive. To address a role for genetic factors in these disorders, we evaluated members of the EphA receptor/ephrin-A family of membrane bound intercellular signaling proteins, which are essential for neuronal migration, compartment formation, and axon guidance. Several studies have linked ephrin-A subclass deletions with phenotypic variations observed in mouse models of neurodevelopmental disorders, such as Autism and ADHD, which exhibit learning and memory disruptions. Yet, little is known regarding how these molecules regulate selective synapse formation on dendritic subzones of individual neurons. To address this question, we utilized the dentate gyrus of the hippocampus as a model system, as it has a well-characterized tri-laminar organization of afferent axonal projects to dentate granule cell apical dendrites. These well-defined laminae consist of an outer layer of input from the lateral entorhinal cortex (LECL) on distal dendrites, a middle afferent layer from the medial entorhinal cortex (MECL), and an inner lamina from CA3 neurons forming the associational/commissural layer (A/CL). This unique tri-laminar afferent organization was initially identified using TIMM silver stain, so this procedure was used to identify broad changes in laminar size and the interface integrity between lamina. Histological staining and data analysis were performed on mice with single or multi-gene deletions for ephrin-A2, -A3, and -A5. Statistical analysis was performed on morphometric measurements of afferent lamina obtained using Olympus CellSans imaging software. Microscopic evaluation of stained specimens revealed a consistent blurring of the boundaries between the three afferent input layers, which exhibited differences in the extent of interface disruptions between the mutant genotypes. Mice with multiple ephrin-A gene mutations exhibited the greatest boundary disruption with consistent invasion of axons from both the LECL and A/CL into the MECL. Our findings highlight the importance of Eph/ephrins in forming an intricate dendritic “molecular zip code” essential for the precise alignment of axonal terminals at specific dendritic subzones. We hypothesize that synaptic zone disruptions, like those observed in our study, could contribute to defining anatomical substrates underlying many functional abnormalities observed in human developmental behavioral spectrum disorders.

Disclosures: **A. Hughes:** None. **P. Curcio:** None. **A. Petschek:** None. **L.F. Kromer:** None.

Poster

355. Developmental Disorders: Genetic Models II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 355.02

Topic: A.07. Developmental Disorders

Support: CURE epilepsy award
NIH R01NS100893
American Epilepsy Society Postdoctoral Research Fellowship
Eunice Kennedy Shriver National Institute of Child Health and Human Development U54HD083092

Title: Glutamatergic and GABAergic neurons mediate distinct neurodevelopmental phenotypes of STXBP1 encephalopathy

Authors: *J. KIM, W. CHEN, E. S. CHAO, H. CHEN, A. RIVERA, M. XUE;
Baylor Col. of Med., Houston, TX

Abstract: Heterozygous pathogenic variants in syntaxin-binding protein 1 (STXBP1, also known as MUNC18-1) cause *STXBP1* encephalopathy and are among the most frequent causes of developmental and epileptic encephalopathies and intellectual disabilities. STXBP1 is an essential protein for presynaptic neurotransmitter release, and its haploinsufficiency impairs glutamatergic and GABAergic neurotransmission. However, the cellular origin of the broad spectrum of neurological phenotypes is poorly understood. Here we show that glutamatergic and GABAergic neurons-specific *Stxbp1* haploinsufficient mice exhibit different subsets of the cognitive and seizure phenotypes observed in the constitutive *Stxbp1* haploinsufficient mice. Developmental delay and most of the motor and psychiatric phenotypes are only recapitulated by GABAergic *Stxbp1* haploinsufficiency. Thus, glutamatergic and GABAergic neurons mediate distinct disease features with few overlaps. The contrasting roles of excitatory and inhibitory signaling in *STXBP1* encephalopathy identify GABAergic dysfunction as a main disease mechanism and reveal the possibility to selectively modulate disease phenotypes by targeting specific neurotransmitter systems.

Disclosures: J. Kim: None. **W. Chen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent holder. **E.S. Chao:** None. **H. Chen:** None. **A. Rivera:** None. **M. Xue:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Principal investigator for a drug study. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent holder, Stock option. F. Consulting Fees (e.g., advisory boards); Consultant, Advisory board.

Poster

355. Developmental Disorders: Genetic Models II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 355.03

Topic: A.07. Developmental Disorders

Support: NIH Grant P50HD103555
NIH Grant P30CA125123
Texas Children's Hospital

Title: Reactivating paternal Ube3a alleviates the disturbance of brain rhythms and sleep in juvenile and adult Angelman syndrome mouse models

Authors: *D. LEE[1,3,4], W. CHEN[1,3,4], H. KAKU[1,3,4], X. ZHUO[2], E. S. CHAO[1,3,4], A. SORIANO[5], A. KUNCHERIA[1], S. FLORES[1], J. KIM[1,3,4], F. RIGO[5], P. JAFAR-NEJAD[5], A. L. BEAUDET[2], M. S. CAUDILL[1], M. XUE[1,3,4,2];
[1]Neurosci., [2]Mol. and Human Genet., Baylor Col. of Med., Houston, TX; [3]The Cain Fndn. Labs., Houston, TX; [4]Jan and Dan Duncan Neurolog. Res. Inst., Texas Children's Hosp., Houston, TX; [5]Ionis Pharmaceuticals, Carlsbad, CA

Abstract: *UBE3A* encodes ubiquitin protein ligase E3A and is paternally imprinted in neurons because the *UBE3A* antisense transcript *(UBE3A-ATS)* represses paternal *UBE3A*. Maternal *UBE3A* deficiency causes Angelman syndrome, a severe neurodevelopmental disorder. A promising therapeutic approach to treating Angelman syndrome is to reactivate the intact paternal *UBE3A* by suppressing *UBE3A-ATS*. Prior studies show that many neurological phenotypes of maternal *Ube3a* knockout mice can only be rescued by reinstating *Ube3a* expression in early development, indicating a restricted therapeutic window for Angelman syndrome. Here we report that reducing *Ube3a-ATS* by antisense oligonucleotides in juvenile or adult maternal *Ube3a* knockout mice alleviates the abnormal electroencephalogram rhythms and sleep disturbance, two prominent clinical features of Angelman syndrome. The degree of phenotypic improvement correlates with the increase of Ube3a protein levels. These results indicate that the therapeutic window of genetic therapies for Angelman syndrome is broader than previously thought, and electroencephalogram power spectrum and sleep architecture should be used to evaluate the clinical efficacies of therapies.

Disclosures: **D. Lee:** None. **W. Chen:** None. **H. Kaku:** None. **X. Zhuo:** None. **E.S. Chao:** None. **A. Soriano:** A. Employment/Salary (full or part-time):; Ionis Pharmaceuticals. **A. Kuncheria:** None. **S. Flores:** None. **J. Kim:** None. **F. Rigo:** A. Employment/Salary (full or part-time):; Ionis Pharmaceuticals. **P. Jafar-nejad:** A. Employment/Salary (full or part-time):; Ionis Pharmaceuticals. **A.L. Beaudet:** A. Employment/Salary (full or part-time):; Luna Genetics. **M.S. Caudill:** None. **M. Xue:** None.

Poster

355. Developmental Disorders: Genetic Models II

Location: SDCC Halls B-H

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Topic: A.07. Developmental Disorders
NIH Grant R01NS100893
NIH Grant R01MH117089
NIH Grant F30MH118804
Eunice Kennedy Shriver National Institute of Child Health and Human Development Grant U54HD083092

Title: Adeno-associated virus-mediated gene replacement therapy in a mouse model of STXBP1-related developmental and epileptic encephalopathy

Authors: *W. CHEN[1,4,5], J. KIM[1,4,5], A. MICHAELS[1,4,5], A. RIVERA[1,4,5], C. LONGLEY[2,4,5], Z.-L. CAI[1,4,5], R. RESSLER[6], B. BONET[6], R. ELTERIEFI[6], J. OCTEAU[6], S. NGUYEN[1,4,5], Z. MOIN[1,4,5], S. ZOU[1,4,5], K. JIN[1,4,5], A. WANG[1,4,5], N. DHAR[1,4,5], P. PARK[1,4,5], J. VEGA[1,4,5], A. CHEN[1,4,5], H. CHEN[1,4,5], A. KNOLL[6], N. GOEDEN[6], N. FLYTZANIS[6], M. XUE[1,3,4,5];
[1]Neurosci., [2]Program in Developmental Biol., [3]Mol. and Human Genet., Baylor Col. of Med., Houston, TX; [4]The Cain Fndn. Labs., [5]Jan and Dan Duncan Neurolog. Res. Inst., Texas Children's Hosp., Houston, TX; [6]Capsida Biotherapeutics, Inc, Thousand Oaks, CA

Abstract: Developmental and epileptic encephalopathies (DEEs) are a group of devastating pediatric neurological disorders, manifesting with aggressive seizures and significant neurological comorbidities. De novo heterozygous pathogenic variants in *STXBP1* encoding syntaxin-binding protein 1 are one of the most frequent genetic causes of DEEs. The abnormal brain activity during early development is believed to contribute to the pathogenesis of *STXBP1* encephalopathy, presenting a great challenge for developing clinical treatments that can remain effective later in life. We previously demonstrated that restoring Stxbp1 protein level to the wildtype level in adult *Stxbp1* haploinsufficient mice was able to rescue most of the phenotypes including epileptic seizures, motor, cognitive, and psychiatric impairments. To develop a therapeutic approach with translational potential, we tested intravenous delivery of AAV expressing Stxbp1 in rescuing adult *Stxbp1* haploinsufficient mice. The vector biodistribution and mRNA/protein levels were quantified and correlated with the outcomes examined by health monitoring, video-electroencephalogram (EEG) recording and neurobehavioral tests. We found that gene replacement therapy in *Stxbp1* haploinsufficient mice could rescue different phenotypes in a dose-dependent manner. Thus, our results indicated that gene replacement is a promising gene therapy for *STXBP1*-related developmental and epileptic encephalopathy.

Disclosures: **W. Chen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent holder. **J. Kim:** None. **A. Michaels:** None. **A. Rivera:** None. **C. Longley:** None. **Z. Cai:** None. **R. Ressler:** A. Employment/Salary (full or part-time):; Capsida Biotherapeutics, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock options. **B. Bonet:** A. Employment/Salary (full or part-time):; Capsida Biotherapeutics, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock options. **R. Elteriefi:** A. Employment/Salary (full or part-time):; Capsida Biotherapeutics, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock options. **J. Octeau:** A. Employment/Salary

(full or part-time):; Capsida Biotherapeutics, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock options. **S. Nguyen:** None. **Z. Moin:** None. **S. Zou:** None. **K. Jin:** None. **A. Wang:** None. **N. Dhar:** None. **P. Park:** None. **J. Vega:** None. **A. Chen:** None. **H. Chen:** None. **A. Knoll:** A. Employment/Salary (full or part-time):; Capsida Biotherapeutics, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock options. **N. Goeden:** A. Employment/Salary (full or part-time):; Capsida Biotherapeutics, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent holder, Stock option. **N. Flytzanis:** A. Employment/Salary (full or part-time):; Capsida Biotherapeutics, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent holder, Stock option. **M. Xue:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Principal investigator for a drug study. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent holder, Stock option. F. Consulting Fees (e.g., advisory boards); Consultant, Advisory board.

Poster

355. Developmental Disorders: Genetic Models II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 355.05

Topic: A.07. Developmental Disorders

Support: Cain Foundation for Epilepsy
McNair Medical Institute at The Robert and Janice, McNair Foundation
NIH U01HG007709 (UDN)
Annie and Bob Graham
Burroughs Wellcome Fund
The Elkins Foundation
Wallace Endowment Award

Title: Rare PPFIA3 variants cause a syndromic neurodevelopmental disorder characterized by delayed development, intellectual disability, autism, and epilepsy

Authors: *M. S. PAUL[1], J. PFLIGER[1], V. LERMA[1], S. MICHENER[1], J. A. ROSENFELD[1], A. TRAN[1], L. MASSINGHAM[2], H. MEFFORD[3], R. BEKHEIRNIA[1], G. L. CARVILL[4], M. ZECH[5], M. WAGNER[5], H. ENGELS[6], K. CREMER[6], E. MANGOLD[6], R. A. LEWIS[1], C. A. BACINO[1], B. H. LEE[1], H.-T. CHAO[1];
[1]Baylor Col. of Med., Houston, TX; [2]Rhode Island Hosp. and Hasbro Children's Hosp., Providence, RI; [3]St. Jude Children's Res. Hosp., Memphis, TN; [4]Northwestern Univ. Feinberg

Sch. of Med., Chicago, IL; [5]Inst. of Human Genetics, Tech. Univ. Munich, Sch. of Med., Munich, Germany; [6]Inst. of Human Genetics, Univ. of Bonn, Univ. Hosp. Bonn, Bonn, Germany

Abstract: Protein Tyrosine Phosphatase Receptor Type F Polypeptide Interacting Protein Alpha-3 (PPFIA3) is a member of the LAR protein-tyrosine phosphatase-interacting protein (liprin) family involved in synaptic vesicle transport and presynaptic active zone assembly. The protein structure and function are well-conserved in invertebrates and vertebrates, but human diseases related to PPFIA3 dysfunction are not yet known. The gene *PPFIA3* has a pLI score of 1.0, indicating it does not tolerate haploinsufficiency and suggesting that *PPFIA3* loss-of-function variants may contribute to the pathogenesis of autosomal dominant disorders. Here, we report eight individuals with *de novo* variants in *PPFIA3* presenting with global developmental delay (6/8), intellectual disability (6/8), autism (3/8), epilepsy (3/8), and mortality at <1 year of age (2/8). These variants are not present in gnomAD. The CADD scores >24 and GERP scores >2 suggest that these variants are potentially deleterious. To determine the pathogenicity of *PPFIA3* variants *in vivo*, we generated transgenic fruit flies expressing human *PPFIA3* wild-type (WT) and missense variants with a C-terminal HA tag. Overexpression of human cDNA containing *PPFIA3* WT and variants in fruit flies was achieved using GAL4-UAS targeted gene expression. Expression of human PPFIA3 was confirmed by immunostaining and Western blotting in larval brain and adult flies, respectively. Act-GAL4 mediated ubiquitous expression of *PPFIA3* variants showed variable penetrance of pupal lethality, eclosion defects, and anatomical leg defects. Elav-GAL4 mediated neuronal expression of *PPFIA3* variants resulted in seizure-like phenotypes, motor defects, and bouton loss at the 3rd instar larval neuromuscular junction. Altogether, the variants located at the N-terminal coiled-coiled domain exhibited stronger phenotypes compared to those in the C-terminal region in the fly overexpression assays. In the loss-of-function fly assay, we show that the homozygous loss of fly Liprin-α leads to embryonic lethality. This lethality is partially rescued by the expression of human *PPFIA3* WT, suggesting human PPFIA3 function is partially conserved in the fly. However, the rare *PPFIA3* variants did not rescue lethality. Altogether, the findings observed with overexpression and lethality rescue assay of *PPFIA3* variants reveal the variants are dominant loss-of-function alleles leading to an autosomal dominant syndromic neurodevelopmental disorder.

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Poster

355. Developmental Disorders: Genetic Models II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 355.06

Topic: A.07. Developmental Disorders

Support: CCHMC Research Foundation Trustee Award
SFARI BTI Award

Title: Dopaminergic encoding of visual stimuli in the lateral nucleus accumbens: implications for cognitive dysfunction in neurofibromatosis type 1

Authors: *L. GONZALEZ[1], A. FISHER[2], K. RENEAU[1], E. COTELLA[2], J. ROBINSON[2]; [1]Neurosci. Grad. Program, Univ. of Cincinnati Col. of Med., Cincinnati, OH; [2]Cincinnati Children's Hospital-CCHMC, Cincinnati, OH

Abstract: Neurofibromatosis is an autosomal dominant disorder caused by haploinsufficiency of the *NF1* gene, whose protein product neurofibromin attenuates Ras-mitogen activated protein kinase (MAPK) signaling. Neurodevelopmental symptoms of NF1 include executive dysfunction, attentional deficits, speech and language delays, and visuospatial and visuoperceptual processing abnormalities. Previous work indicates that mesolimbic dopamine circuits may be involved in the pathophysiology of attentional and cognitive deficits in NF1. This mesolimbic dopamine system is an evolutionarily conserved set of brain circuits that plays a role in attention, appetitive behavior, and reward processing. In this circuitry, ascending dopaminergic projections from the ventral midbrain innervate targets throughout the limbic forebrain, such as the ventral striatum/nucleus accumbens (NAc). Dopaminergic signaling in the NAc has been widely studied for its role in behavioral reinforcement, reward prediction error encoding, motivational salience, and – to a lesser extent - responses to surprising or alerting sensory events. Previously, we used fiber photometry and the genetically encoded dopamine sensor dLight1 to explore dopamine responses to salient stimuli in NF1 model mice (Robinson et al., 2019). We found that mice modeling NF1 are hypersensitive to visual stimuli, which correlates with enhanced visually evoked dopamine release in the lateral NAc. In order to better understand the nature of this phenotype, we tested the ability of striatal dopamine release to encode the properties of salient sensory stimuli in wildtype mice. Here, we report that ventral striatum dopamine release encodes the physical properties of visual and auditory stimuli, rather than their emotional valence. In the case of visual stimuli, this encoding does not habituate with repeated exposure when the interstimulus interval is sufficiently long, is independent of circadian state or motor behavior, and likely involves multiple visual transduction pathways. Thus, we have described a novel ability of the mesolimbic dopamine system to encode sensory information in mice, which may play a role in their need to avoid detection by visual predators. Currently, we are examining how *Nf1* haploinsufficiency affects visual stimulus encoding by LNAc dopamine, as well as identifying neural circuits that mediate visual hypersensitivity phenotypes in NF1 model mice.

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Poster

355. Developmental Disorders: Genetic Models II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 355.07

Topic: A.07. Developmental Disorders

Support: Simons Foundation Autism Research Initiative (SFARI) Bridge to Independence Award
Cincinnati Children's Research Foundation
Gilbert Family Foundation Gene Therapy Initiative (GTI)

Title: Finding new strategies to treat behavioral deficits associated to neurofibromatosis type 1

Authors: *E. M. COTELLA[1], R. M. SALAZAR GONZALEZ[1], K. E. RENEAU[3,1], A. FISHER[1], N. G. RAUT[2], M. P. JANKOWSKI[2], J. E. ROBINSON[1];
[1]Div. of Exp. Hematology and Cancer Biol., [2]Dept. of Anesthesia, Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH; [3]Neurosci. Grad. Program, Univ. of Cincinnati, Cincinnati, OH

Abstract: Rasopathies are a family of genetic conditions characterized by aberrant amplification of the Ras/mitogen activated protein kinase (MAPK) signaling cascade. Neurofibromatosis type 1 (NF1) is an autosomal dominant Rasopathy caused by haploinsufiency of the NF1 gene, which codes for neurofibromin - a negative regulator of activated Ras. Symptoms of NF1 include increased risk for benign or malignant tumorigenesis, musculoskeletal abnormalities, hyper-pigmented macules, and cognitive deficits. Often, patients also present pain hypersensitivity. Cognitive symptoms of NF1 include impaired executive functioning, autistic features, speech and language delays, attention deficits, hyperactivity, and impulsivity. Based on work in rodent and fly models, the pathophysiology of behavioral and cognitive symptomatology may involve Ras-evoked excitation/inhibition (E/I) imbalance, as well as perturbations in neural circuits that release dopamine. Targeted gene therapies are a promising approach to treating these sequelae, as the expression of therapeutic proteins may be restricted to neural or glial populations of interest using unique gene regulatory elements or cell type-specific promoters/enhancer elements. Our lab has focused on identifying behavioral abnormalities in a mouse model of NF1 (Nf1+/- C57Bl/6J:129sv/J mouse) with the ultimate goal of designing viral vectors that can be administered systemically to reverse these effects. Using new adeno-associated viral vectors (AAVs) that can target populations of interest in the central and peripheral nervous system after intravenous injection, we screened AAV-encodable transgenes capable of therapeutically modulating Ras-MAPK signaling in NF1 haploinsufficient cells in vitro and in vivo, to correct behavioral and cellular phenotypes in NF1 model mice. These phenotype include alterations in cellular morphology in primary culture of embryonic hippocampal neurons and behavioral alterations such as fragmented grooming behavior, reduced psychomotor activity under bright light, and increased pain sensitivity assessed in the mechanical conflict-avoidance test. This work will provide important initial preclinical evidence for the utility of AAV-based gene therapies in the treatment of NF1 in non-oncological related symptoms.

Disclosures: **E.M. Cotella:** None. **R.M. Salazar Gonzalez:** None. **K.E. Reneau:** None. **A. Fisher:** None. **N.G. Raut:** None. **M.P. Jankowski:** None. **J.E. Robinson:** None.

Poster

355. Developmental Disorders: Genetic Models II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 355.08

Topic: A.07. Developmental Disorders

Support: CCHMC Center for Pediatric Genetics Pilot Project Award

Title: Novel behavioral and molecular phenotypes in a mouse model of Mapk1-dependent Rasopathy

Authors: *K. E. RENEAU[1], E. M. COTELLA[2], D. NARDINI[2], R. R. WACLAW[2], J. E. ROBINSON[2];
[1]Univ. of Cincinnati Col. of Med., Cincinnati, OH; [2]Exptl. Hematology and Cancer Biol., Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH

Abstract: MAPK1 (ERK2) is a key downstream signaling molecule in the Ras-mitogen activated protein kinase (MAPK) pathway. Recent work has identified gain-of-function (GOF) variants of *MAPK1* as a cause of neurodevelopmental disorder within the Rasopathy clinical spectrum that is similar to Noonan syndrome (Motta et al., 2020). To understand the impact of MAPK1 GOF mutations in the developing and postnatal brain, we generated an A172V knock-in mice (*Mapk1*$^{A172V/+}$) to mimic the human A174V Rasopathy mutation. *Mapk1*$^{A172V/+}$ *and* *Mapk1*$^{A172V/A172V}$ were viable to postnatal stages and showed increased expression of the MAPK target gene Etv5 and evidence of abnormal glial cells in the postnatal brain, which was similar to mouse models of neurofibromatosis type 1 (NF1) and PTPN11/Noonan syndrome. Cognitive and motor function was assessed using a battery of behavioral tests, and performance was compared to a widely used mouse model of NF1 (*Nf1*$^{+/-}$ mice on a C57BL/6J genetic background). In a novel object discrimination task, *Mapk1*$^{A172V/A172V}$ mice had impaired novel object discrimination compared to wild-type and *Mapk1*$^{A172V/+}$ mice. In contrast, C57BL/6J NF1 mice did not show impairments in novel object recognition. In an open field test, while there was no difference in overall locomotor activity, *Mapk1*$^{A172V/A172V}$ mice exhibited marked thigmotaxis, consistent with increased anxiety-like behavior compared to *Mapk1*$^{A172V/+}$ and wild-type mice. Using the sucrose splash test to measure stereotyped behavior via grooming, *Mapk1*$^{A172V/A172V}$ mice engaged in long bouts (longer than 15 seconds) of grooming more rapidly than *Mapk1*$^{A172V/+}$ or wild-type mice, despite no significant differences in overall grooming time or the number of long grooming bouts. NF1 model mice exhibited no difference in behavior in the sucrose splash test, and there was no significant difference between MAPK1 genotypes in the marble burying task. These data suggest that enhanced downstream MAPK signaling is sufficient to produce behavioral phenotypes in mice, which may mimic cognitive deficits seen in Noonan syndrome and/or other

Rasopathies. Future directions will seek to identify the neurobiological etiology of these phenotype, as well as explore candidate therapies targeting Ras-MAPK signaling.

Disclosures: K.E. Reneau: None. **E.M. Cotella:** None. **D. Nardini:** None. **R.R. Waclaw:** None. **J.E. Robinson:** None.

Poster

355. Developmental Disorders: Genetic Models II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 355.09

Topic: A.07. Developmental Disorders

Support: NIH Grant K01AG054729
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UNH Cole Neuroscience and Behavioral Faculty Research Awards
UNH Summer TA Research Fellowships

Title: Neuronal primary cilia regulate pyramidal cell positioning to the deep and superficial sublayers in the cerebral cortex

Authors: *J. YANG[1], L. QIU[1], X. CHEN[2];
[2]Dept. of Molecular, Cell. and Biomed. Sci., [1]Univ. of New Hampshire, Durham, NH

Abstract: It is well-recognized that primary cilia regulate embryonic neurodevelopment, but little is known about their roles during postnatal neurodevelopment. The hippocampal striatum pyramidal (SP) is subdivided into deep and superficial sublayers and gradually condensed into a compact lamina in the first postnatal two weeks, whereas simultaneously cortex volume greatly expands. It is elusive how pyramidal neurons position to two sublayers postnatally. Here, we show that the axonemes of primary cilia in the deep and superficial sublayers of hippocampal SP point in the opposite directions, while neuronal cilia in cortical sublayers display the same orientation. Neuronal primary cilia in the CA1 SP undergo marked changes in morphology and orientation from postnatal day 5 (P5) to P14, concurrent with cell positioning to two sublayers and with neural maturation. Surprisingly, the centrioles of late-born neurons migrate excessively to cluster at SP bottom before cilia protrusion and a reverse movement back to the main plate. Similarly, this “pull-back” movement of centriole/cilia is also identified on late-born cortical pyramidal neurons. Transgenic overexpression of Arl13b, a protein regulating ciliogenesis, not only elongates primary cilia and promotes earlier cilia protrusion, but also affects postnatal cilia orientation in hippocampal CA1 SP. We further reveal that ablation of neuronal cilia selectively in the mouse forebrain leads to megalencephaly and delayed condensation of SP lamina in the early postnatal stage. Together, this study provides the first evidence that primary cilia regulate

pyramidal neuronal positioning in the cerebral cortex and late-born pyramidal neurons undergo a reverse movement for final cell positioning.

Disclosures: J. Yang: None. **L. Qiu:** None. **X. Chen:** None.

Poster

355. Developmental Disorders: Genetic Models II

Location: SDCC Halls B-H

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Program #/Poster #: 355.10

Topic: A.07. Developmental Disorders

Support: MOST-108-2321-B001-026
AS-IA-109-L04
AS-TP-108- L08

Title: The interplay between CEP120 and protein Moonraker in the pathogenesis of Joubert syndrome

Authors: *C.-H. CHANG[1,2], T. K. TANG[2];
[1]UCSF, San Francisco, CA; [2]Inst. of Biomed. Sciences, Academia Sinica, Taipei, Taiwan

Abstract: Joubert syndrome (JS) is hereditary cerebellar ataxia in which cerebellar vermis hypoplasia is the main characteristic of all affected individuals. CEP120, a JS-associated protein, participates in centriole biogenesis and ciliogenesis. It was reported that knockout of *Cep120* in murine led to hydrocephalus and cerebellar hypoplasia. Here, using *in vivo* cerebellar electroporation, we identified a new role of CEP120 in regulating timely neuronal differentiation and the germinal zone exit of granule neuron progenitors (GNPs) during cerebellar development. Our *in vivo* assays showed that depletion of Cep120 perturbs GNP cell-cycle progression, resulting in a delay of cell-cycle exit. To dissect the potential molecular mechanism, we investigated the association between the CEP120 interactomes and the JS database and discovered another JS-associated protein, Moonraker (also known as KIAA0753/OFIP), as a CEP120-interacting protein. Surprisingly, we found that CEP120 recruits Protein Moonraker to centrioles and that loss of this interaction induces accumulation of GNPs in the germinal zone and impairs neuronal differentiation. Importantly, the replenishment of wild-type CEP120 rescues the above defects, whereas expression of JS-associated CEP120 mutants, which hinder Protein Moonraker recruitment, still result in ectopic cells in the germinal zone. Together, our data reveal a close interplay between CEP120 and Protein Moonraker for the germinal zone exit and timely neuronal differentiation of GNPs during cerebellar development, and mutations in CEP120 and Protein Moonraker may participate in the heterotopia and cerebellar hypoplasia observed in JS patients.

Disclosures: C. Chang: None. **T.K. Tang:** None.

Poster

355. Developmental Disorders: Genetic Models II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 355.11

Topic: A.07. Developmental Disorders

Support: NIH Grant RO1AR078663-01
Clinical and Translational Sciences Institute of Indiana

Title: Influence of Dyrk1a copy number on protein and transcript expression in the Ts65Dn mouse model of Down syndrome during early neurodevelopment

Authors: *L. E. HAWLEY[1], C. R. GOODLETT[2], R. J. ROPER[1];
[1]Biol., [2]Psychology, Indiana University-Purdue Univ. Indianapolis, Indianapolis, IN

Abstract: Down syndrome is caused by the triplication of human chromosome 21 (Hsa21) and includes hypocellular brain structures due to restricted cellular proliferation and precocious cell differentiation. The triplication of *DYRK1A,* found on Hsa21, has been implicated in the dysfunction of several associated molecular pathways. Historically, DS research has operated using a linear correlation between gene copy number and protein expression, assuming if two copies of a gene produce a normal amount of protein, three copies will produce a 1.5-fold overexpression. Inhibition of overexpressed DYRK1A is an attractive target for realigning neurodevelopmental trajectories in DS, and several preclinical studies are ongoing in humans and animal models. The Ts65Dn mouse model of DS contains a freely segregating segmental chromosome with ~104 genes homologous to those found on Hsa21, including *Dyrk1a*. Most experiments using the Ts65Dn model have been performed in adult males, preserving females for colony maintenance, creating a scarcity of data regarding other factors contributing to DS phenotypes during development. Quantification of DYRK1A at postnatal day (P) 15 found overexpression in the hippocampus, cerebral cortex, and cerebellum of male, but not female, Ts65Dn mice when compared to wild-type littermates. Preliminary investigation in our lab uncovered significant overexpression in both sexes at P6, suggesting DYRK1A is dynamically expressed during neurodevelopment and dependent on multiple factors including sex and age. We hypothesized reducing *Dyrk1a* copy number in Ts65Dn mice would significantly alter protein and mRNA expression compared to controls, and that expression profiles would be different between male and female mice. To test these hypotheses, Ts65Dn mice were bred to *Dyrk1a* +/- mice, functionally reducing *Dyrk1a* gene copy number while preserving the molecular environment created by the triplication of other genes on Hsa21. We quantified DYRK1A in the hippocampus, cerebral cortex, and cerebellum of male and female animals at multiple ages and quantified mRNA transcripts at P6 in matched animals. Our data support previous findings that *Dyrk1a* expression in the brain is influenced by mechanisms of developmental regulation beyond gene copy number, including genetic background, brain region, age, and sex. These results provide crucial temporal and spatial information for the design of DYRK1A inhibition therapies during development and support the further investigation of expression mechanisms in the brain that contribute to intellectual disability phenotypes present in individuals with DS.

Disclosures: **L.E. Hawley:** None. **C.R. Goodlett:** None. **R.J. Roper:** None.

Poster

355. Developmental Disorders: Genetic Models II

Location: SDCC Halls B-H

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Program #/Poster #: 355.12

Topic: A.07. Developmental Disorders

Support: NSERC Discovery Grant 2016-06035
ORSA Hope Fund

Title: Investigating the dose-dependent functional role of MeCP2 in the brain

Authors: *M. RASTEGAR;
Univ. of Manitoba, Univ. of Manitoba, Winnipeg, MB, Canada

Abstract: Methyl CpG-Binding Protein 2 (MeCP2) functions in a dose-dependent manner in the brain. MeCP2 is an important epigenetic factor that binds to different forms of methylated DNA to control a wide range of cellular function. Although MeCP2 is detected in different brain cell types, its expression level is the highest in neurons. Both MeCP2 gain- and loss-of-function lead to abnormal structural properties of neurons in the murine and/or human brain. Accordingly, altered MeCP2 expression or *MECP2* genetic mutations are associated with impaired neurodevelopment, mental disability, altered function of neurons, and compromised neuronal differentiation.

My lab has studied the regulation and function of MeCP2E1 and MeCP2E2 isoforms for over a decade. Accordingly, studies from us and others have indicated that MeCP2 homeostasis regulation in the brain involves a feedback regulatory loop, including MeCP2E1 and MeCP2E2 isoforms, the Brain-Derived Neurotrophic Factor (BDNF), and *miR132* that is a neuronal-specific microRNA. Our studies have included the analysis of newly synthesized transcript expression, steady-state RNA levels, increased MeCP2E1 and MeCP2E2 levels by lentiviral induction, as well as side-by-side analysis in post-mortem human brain of Rett Syndrome patients with that of animal models of Rett Syndrome with *Mecp2*-deficinecy [1, 2]. Recent data from my lab further suggest that the feedback regulatory loop of MeCP2E1/E2-BDNF-*miR132* could be differently regulated in human brain cells, versus what has been previously reported by others in the murine brain.

Our recent studies suggest that the functional regulatory role of MeCP2 isoforms on cell signaling pathways such as the mammalian target of rapamycin (mTOR), and protein translation initiation might be a dose-dependent regulatory role of MeCP2 isoforms [3]. Furthermore, our data suggests that MeCP2 isoforms themselves, may regulate the overall level of MeCP2 expression within the brain cells at different regulatory levels. Our recent research data would provide important insight towards the dose-dependent regulatory role of MeCP2 in the brain.

1. Olson CO, et al. MECP2 Mutation Interrupts Nucleolin-mTOR-P70S6K Signaling in Rett Syndrome Patients. Front Genet. 2018;9:635.

2. Pejhan S, et al. The MeCP2E1/E2-BDNF-miR132 Homeostasis Regulatory Network Is Region-Dependent in the Human Brain and Is Impaired in Rett Syndrome Patients. Front Cell Dev Biol. 2020;8:763.
3. Buist M, et al. Differential Sensitivity of the Protein Translation Initiation Machinery and mTOR Signaling to MECP2 Gain- and Loss-of-Function Involves MeCP2 Isoform-Specific Homeostasis in the Brain. Cells. 2022;11(9).

Disclosures: M. Rastegar: None.

Poster

355. Developmental Disorders: Genetic Models II

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 355.13

Topic: A.07. Developmental Disorders

Support: Nationwide Foundation Pediatric Innovation Fund

Title: Patient-derived cerebral organoids containing a SHANK3 nonsense mutation show deficits in synaptogenesis

Authors: D. JULIAN[1], G. GUO[2], A. BENCHOUA[3], T. BOURGERON[4], ***M. HESTER**[2,5]; [1]The Univ. of Arizona Col. of Med., Phoenix, Arizona, AZ; [2]Steve and Cindy Rasmussen Inst. for Genomic Med., Res. Inst. at Nationwide Children's Hosp., Columbus, OH; [3]CECS, I-STEM, Cedex, France; [4]Inst. Pasteur, Inst. Pasteur, Paris, France; [5]Dept. of Pediatrics and Neurosci., The Ohio State Univ., Columbus, OH

Abstract: Autism spectrum disorders (ASD) are complex neurodevelopmental disorders hallmarked by many behavioral and social deficits. While ASDs are genetically heterogeneous, defective synaptogenesis has emerged as a common pathological feature. Monogenic mutations in SHANK3, a critical scaffolding protein in post-synaptic neurons, account for a substantial amount (1-2%) of all ASD cases. While ASD has been extensively studied *in vivo* using animal models, the impact of SHANK3 mutations on human synaptogenesis is largely understudied. Cerebral organoids (COs) are a novel stem-cell based model that recapitulate features of early human corticogenesis. We have previously demonstrated COs functional activities can be assessed by a multi-electrode array (MEA) platform. To investigate synaptic properties of a truncated SHANK3 isoform, we generated COs from control iPSCs and iPSCs containing a SHANK3 nonsense mutation (Glu809X). To test for synaptic activity within both lines of COs, we prepared live organoid slices and performed immunohistochemistry for SYNAPSIN, a marker of excitatory synaptic contacts. We further assessed functional activities by measuring electrophysiological properties in both control and SHANK3 COs. Our SHANK3-deficient CO slice cultures showed a significant reduction in SYNAPSIN expression compared to controls. Treatment of the SHANK3 COs with Ataluren, a nonsense suppressor agent, significantly restored SYNAPSIN levels. Electrophysiological analyses using MEA showed reduced spiking

activity, increased inter-burst intervals, and a decline in burst rates in SHANK3 mutant COs compared to control organoids, all of which reflect a defect in synaptic transmission. Our results show for the first time that a loss of SHANK3 function leads to defective excitatory synaptic properties in COs. This study highlights both the important role for SHANK3 during human synaptogenesis in brain development and for the ability of COs to investigate clinically relevant mechanisms and therapeutic options for ASD.

Disclosures: D. Julian: None. **G. Guo:** None. **A. Benchoua:** None. **T. Bourgeron:** None. **M. Hester:** None.

Poster

355. Developmental Disorders: Genetic Models II

Location: SDCC Halls B-H

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Program #/Poster #: 355.14

Topic: A.07. Developmental Disorders

Support: NH&MRC Grant APP1163240 to RKS and JG
Australian Government Research Training Scholarship (International) to RB

Title: Depleted neural stem cell pool and their premature differentiation underpins the learning and memory problem in THOC2 patient variant inspired mouse model

Authors: *R. BHATTACHARJEE[1,3], L. JOLLY[2,3], M. WHITE[4], P. THOMAS[4,3], R. SHARMA[1,3], J. GECZ[1,3];
[1]Adelaide Med. Sch., [2]Sch. of Biomedicine, Univ. of Adelaide, Adelaide, Australia; [3]The Robinson Res. Institute, Univ. of Adelaide, Adelaide, Australia; [4]South Australian Genome Editing Facility, SAHMRI (South Australian Hlth. and Med. Res. Institute), Adelaide, Australia

Abstract: The X-linked *THOC2* gene encodes a subunit of the highly conserved TREX (Transcription-Export) complex that is involved in fundamental cellular processes like transcriptional regulation, stem cell maintenance, 3′ mRNA processing, mitotic progression, genome stability, and mRNA export in all eukaryotic cells. We have implicated >40 partial loss of function (pLOF) *THOC2* variants in neurodevelopmental disorders (NDD), with intellectual disability (ID) as the core phenotype. While many studies have investigated the effect of *THOC2* knockdown in various cell models, mostly cancer, the cellular and molecular basis of how the patient *THOC2* variants cause NDD/ID are elusive. We have approached answering these questions by generating a mouse model of *THOC2*. After multiple failed attempts with different missense variants (noting that IMPC also failed to generate conditional or knockout*Thoc2* mice), we successfully generated the first *Thoc2* Exon37-38 deleted mouse model by CRISPR-Cas9 gene editing, inspired by this variant we identified in a patient with ID, speech delay, hypotonia, and microcephaly. We confirmed the absence of gene editing-associated off-target effects by whole genome sequencing. Like the patient, the *Thoc2* Ex37-38 del male mice are smaller in size and weight (~15%) compared to their wild-type (WT) littermates. We confirmed deletion of

Thoc2 Ex37-38 sequence in the Ex37-38 del male mice mRNAs by RT-PCR and Sanger sequencing and that the Thoc2 protein in Ex37-38 del male mice brain, eye, and lungs is truncated and accumulates compared to the WT protein. Our behavioural testing using Morris water maze and Y-maze protocols (n=14 mice/genotype) revealed a significant deficit in spatial learning and working memory in the Ex37-38 del compared to the WT male mice. Further, the histological features of the E18.5 embryonic as well as adult mouse brains show significantly compressed cortical (ventricular and marginal zones of E18.5 mice brain) and corpus callosum architecture in Ex37-38 del male mice compared to the WT (n=7/genotype). Our investigations of *in vitro* primary cortical neuron and neural stem cell (NSC) cultures from E18.5 embryonic brains showed that the Ex37-38 del male mouse neurons have shorter primary axons and sub-optimal neural migration. Finally, the NSCs from Ex37-38 del male mice undergo premature differentiation and have significantly increased cell death. Together, our data show that *Thoc2* Ex37-38 del male mice recapitulate the patient phenotypes, providing a unique *in vivo* tool for undertaking in-depth cellular/molecular studies on *THOC2* variant-mediated brain pathology.

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Poster

355. Developmental Disorders: Genetic Models II

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Program #/Poster #: 355.15

Topic: A.07. Developmental Disorders

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The Nancy Lurie Marks Family Foundation
NIH NS093200
NIH HD096326
NIH MH115957

Title: Tissue and cell-type specific molecular and functional signatures of 16p11.2 reciprocal genomic disorder across mouse brain and human neuronal models

Authors: *D. J. C. TAI[1], P. RAZAZ[1], S. ERDIN[1], D. GAO[1], J. WANG[1], X. NUTTLE[1], C. E. DE ESCH[1], R. L. COLLINS[1], B. B. CURRALL[1], K. O'KEEFE[1], N. D. BURT[1], R. YADAV[1], L. WANG[1], K. MOHAJERI[1], T. ANEICHYK[1], A. RAGAVENDRAN[1], A. STORTCHEVOI[1], E. MORINI[1], W. MA[1], D. LUCENTE[2], A. HASTIE[3], R. J. KELLEHER[2], R. H. PERLIS[1], M. E. TALKOWSKI[1], J. F. GUSELLA[1];
[1]Ctr. for Genomic Med., Massachusetts Gen. Hospital/Harvard Med. Sch., Boston, MA; [2]Massachusetts Gen. Hosp., Boston, MA; [3]Bionanogenomics, San Diego, CA

Abstract: Recurrent copy number variants (CNVs) of 743 kilobases of chromosome 16p11.2 represent a well-defined reciprocal genomic disorder (RGD). The CNV segment is highly gene dense (31 protein-coding genes) and associated with autism spectrum disorder (ASD) as well as a range of variability in neuropsychiatric and anthropometric traits that may suggest differential tissue- and context-specific impact of multiple genes. We performed transcriptome profiling of 350 libraries from six tissues (cortex, cerebellum, striatum, liver, brown fat, white fat) in mouse models harboring CNV of the syntenic 7qF3 region, as well as cellular, transcriptional, and single-cell analyses in 54 isogenic neural stem cell, induced neurons, and cerebral organoid models of CRISPR-engineered 16p11.2 CNVs. Across tissues, differentially expressed genes (DEGs; FDR < 0.1) within the 16p11.2 segment were mostly uniform relative to their constitutive expression. Transcriptome-wide DEGs were largely tissue, cell-type, and dosage specific, though more effects were shared between deletion and duplication than expected by chance. The broadest effects were observed in the cerebellum (2163 DEGs), and the greatest enrichments were associated with synaptic pathways in mouse cerebellum tissues and human induced neuron cell lines. Energy and RNA metabolism were recurrently observed as shared processes disrupted by the 16p11.2 CNV, and co-expressed genes were enriched for highly constrained and ASD-associated gene sets. Intriguingly, despite the dosage-specific CNV effects on transcription, reciprocal 16p11.2 dosage changes resulted in consistent decrements in neurite function and electrophysiological features, while single-cell profiling of cerebral organoids showed reciprocal alterations to the proportions of excitatory and inhibitory GABAergic neurons. Both neuronal ratios and gene expression changes in our organoid analyses point most directly to calretinin GABAergic inhibitory neurons and the excitatory/inhibitory balance as a target of disruption in 16p11.2 carriers that may contribute to changes in neurodevelopmental and cognitive function. Collectively, our data indicate the genomic disorder involves disruption of multiple contributing biological processes, with relative impacts that are context-specific.

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Poster

355. Developmental Disorders: Genetic Models II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 355.16

Topic: A.07. Developmental Disorders

Title: Characterizing febrile seizure susceptibility in Pcdh19 mice

Authors: *A. C. BLASZKIEWICZ, F. E. FAGBEMI, J. M. RAKOTOMAMONJY, A. D. GUEMEZ-GAMBOA;
Neurosci., Northwestern University, Feinberg Sch. of Med., Chicago, IL

Abstract: PCDH19-related epilepsy, also known as PCDH19-CE, is one of the most common forms of early-onset epilepsy in females, affecting about 30,000 people in the U.S. alone. PCDH19-CE is an epileptic encephalopathy characterized by short and repeated seizure clusters. Patients present with predominantly focal seizures, which are often followed by developmental decline and intellectual disability. This disorder is caused by pathogenic variants in the PCDH19 gene, which codes for a protein known as protocadherin-19. PCDH19 is a member of the protocadherin (PCDH) subfamily, a group of cell-adhesion proteins which are important for the formation of neural circuits. PCDH19 consists of an extracellular domain that mediates calcium-dependent cell adhesion interactions and an intracellular domain that regulates intracellular signaling and trafficking. The PCDH19 gene is predominantly expressed in the central nervous system, particularly the limbic areas and cortex, and it has higher levels of expression during early development. This gene is located in the X chromosome; thus, PCDH19-CE is an X-linked disorder. However, hemizygous males are unaffected by PCDH19-CE, while heterozygous females experience severe symptoms. This unusual expression pattern is thought to be caused by a mechanism of cellular interference in which affected individuals have two different cell populations, caused by X-inactivation. This hypothesis is supported by symptomatic males presenting with mosaic pathogenic variants in PCDH19. Here, we established a mouse model to visualize the presence of two cell populations: WT (GFP-expressing) and PCDH19 KO by breeding wild-type PCDH19 males carrying XeGFP with heterozygous PCDH19 females. Previous studies investigating PCDH19 function in PCDH19 mouse models revealed that heterozygous females, homozygous null females, and hemizygous null males did not exhibit gross brain abnormalities or spontaneous seizures. Thus, we tested susceptibility to hyperthermia-induced seizures in our model, as patients with this disorder often experience uncontrollable seizures, initially triggered by fever. Then we aimed to determine if the presence of these two populations impacted the severity of febrile seizures. Results from this project provide insight into the pathophysiology of PCDH19-CE and contribute to the growing efforts to find novel therapeutic approaches for this devastating disorder.

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Poster

355. Developmental Disorders: Genetic Models II

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Program #/Poster #: 355.17

Topic: A.07. Developmental Disorders

Support: NIH Grant NS101596
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NIH Grant NS108874

Title: A Developmental and Epileptic Encephalopathy S4-S5 linker KCNQ2 variant leads to KCNQ2 mis-localization in the brain

Authors: *K. SPRINGER[1], B. HOU[1], N. VARGHESE[1], H. SOH[1], C. M. LUTZ[2], A. R. ZUBERI[2], A. TZINGOUNIS[1];
[1]Physiol. and Neurobio., Univ. of Connecticut, Storrs, CT; [2]Rare and Orphan Dis. Ctr., The Jackson Lab., Bar Harbor, ME

Abstract: *KCNQ2*, a gene that encodes a voltage-gated potassium channel subunit, has been the focus of studies related to neurodevelopmental disorders for decades. It is well known that KCNQ2 pathogenic variants could lead to disorders ranging in severity from Self-Limited (Familial) Neonatal Epilepsy to Developmental and Epileptic encephalopathy (DEE). Although many DEE variants have been identified in the voltage-sensor, pore-region, or C-terminus of the KCNQ2 channels, few have also been identified in the S4-S5 linker, a region critical for KCNQ2 electrocoupling. Here, we studied one such variant, $KCNQ2^{H228R}$, that has been identified in patients suffering from severe, early onset epilepsy. The $KCNQ2^{H228R}$ variant acts as a loss-of-function and dominant negative when expressed in heterologous cells. Using a $Kcnq2^{H228R}$ knockin mouse model developed by the Rare and Orphan Disease Center at the Jackson Lab (MMRRC stock #69623), we investigated the effects of the $KCNQ2^{H228R}$ variant on cellular physiology of neonatal and juvenile mice. We used $Kcnq2^{H228R/+}$ mice as the homozygous mice die perinatally, consistent with KCNQ2 loss of function in neurons. We compared the protein expression patterns between heterozygous mice and their wildtype littermates using immunohistochemistry (IHC) and Western blotting. IHC revealed that the variant appears to cause a dramatic shift in KCNQ2 localization in both the hippocampus and neocortex from fibers to the soma. This was an unexpected shift, as the S4-S5 linker has not been associated with KCNQ2 localization signals. This mis-localization was consistent across several developmental time points as well as different regions of the brain. Given this change, we also investigated the electrophysiological traits of neurons by recording in acute slices taken from $Kcnq2^{H228R/+}$ mice. Although mesoscale calcium imaging *ex vivo* revealed an increase in neuronal excitability in neonatal mice, there were no differences in the firing properties of hippocampal CA1 and L2/3 neocortical neurons between juvenile heterozygous mice and their wildtype littermates. Taken together, our data suggest that KCNQ2 loss of function variants could alter KCNQ2 protein localization independent of their location on KCNQ2 protein in neurons, suggesting that KCNQ2 subcellular mis-localization might be a KCNQ2 DEE endophenotype.

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Poster

355. Developmental Disorders: Genetic Models II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 355.18

Topic: A.07. Developmental Disorders

Support: NIH Grant R01HD104609
NIH Grant R01HD104609-01S1

Title: A subpopulation of glutamatergic projection neurons are altered in the neocortex of DDX3X syndrome model mice

Authors: *M. FLORES[1,2,3,4], M. GARCIA-FORN[1,2,3,4], A. VON MUEFFLING[1,2,3,4,5], P. OLA[1,2,3,4], S. DE RUBEIS[1,2,3,4];
[1]Psychiatry, [2]The Seaver Autism Ctr. for Res. and Treatment, [3]The Mindich Child Hlth. and Develop. Inst., [4]Friedman Brain Inst., Icahn Sch. of Med. at Mount Sinai, New York, NY; [5]Neurosci. and Behavior, Barnard Col., New York, NY

Abstract: DDX3X syndrome is a rare neurodevelopmental disorder that primarily affects females. It is caused by mutations in the X-linked gene *DDX3X*, yet its neurobiological mechanisms remain elusive. *Ddx3x* heterozygous female mice (*Ddx3x*$^{+/-}$) exhibit developmental and behavioral deficits closely resembling the clinical phenotypes of DDX3X syndrome. Further, *Ddx3x*$^{+/-}$ pups (postnatal day 3; P3) have an excess number of a glutamatergic cortical subpopulation that co-express molecular markers that lead to opposite cellular identities: CTIP2 for subcortical neurons and BRN1 for intracortical neurons (CTIP2+BRN1+). Given that little is known about this subpopulation and why it is altered by *Ddx3x* loss-of-function, we are investigating the development and circuitry of these neurons in *Ddx3x*$^{+/-}$ and control (*Ddx3x*$^{+/+}$) mice. We used immunohistochemical markers in P3 to 4-month-old mice to follow the co-expression timeline of CTIP2+BRN1+ neurons in the motor and primary somatosensory cortex of *Ddx3x*$^{+/-}$ and *Ddx3x*$^{+/+}$ mice. A window of neurogenesis for these neurons was identified by injecting bromodeoxyuridine (BrdU) into pregnant dams across different embryonic time points to mark proliferating cells in the developing embryo. The projection targets of these neurons were mapped by injecting a retrograde viral tracer that expresses GFP into cortical and subcortical areas. Our results show that CTIP2+BRN1+ neurons are present from P3 and are maintained at 4 months of age in *Ddx3x*$^{+/-}$ and *Ddx3x*$^{+/+}$ mouse cortices. At 4 months of age, there continues to be a surplus of these neurons in the primary motor cortex of *Ddx3x*$^{+/-}$ mice, in line with our previous observations at P3. CTIP2+BRN1+ neurons are decreased in premotor and primary somatosensory cortical areas of *Ddx3x*$^{+/-}$ mice. Our results also show that some CTIP2+BRN1+ neurons are generated at embryonic day 13.5 (E13.5), but the peak of their neurogenesis remains unknown. We also found that CTIP2+BRN1+ neurons reside within layer 5 of the neocortex and do not display corticothalamic or callosal projection identity. We expect the projections of these neurons to be altered in *Ddx3x*$^{+/-}$ mice. We propose that alterations in this subpopulation may underlie the developmental and behavioral impairments caused by DDX3X syndrome.

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Poster

355. Developmental Disorders: Genetic Models II

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Program #/Poster #: 355.19

Topic: A.07. Developmental Disorders

Support: Loulou Foundation [CDKL5-16-107-02]
UPenn Orphan Disease Center

Title: Functional, molecular, and neuropathological evaluation of two mouse models of CDKL5 deficiency disorder (CDD)

Authors: *D. L. CAMERON[1,2,3,4,5], A. ADHIKARI[2,6], F. K. BUCHANAN[1,2,3,4,5], N. COPPING[2,6], T. FENTON[2,6], I. VILLEGAS[1,2,3,4,5], J. A. HALMAI[1,2,3,4,5], J. L. SILVERMAN[2,6], K. FINK[1,2,3,4,5];
[1]Ctr. for Interventional Genet., [2]MIND Inst., [3]Stem Cell Program and Gene Therapy Ctr., [4]Inst. for Regenerative Cures, [5]Dept. of Neurol., [6]Dept. of Psychiatry and Behavioral Sci., Univ. of California Davis Hlth. Systems, Sacramento, CA

Abstract: CDKL5 deficiency disorder (CDD) is a rare X-linked neurodevelopmental disorder marked by epileptic encephalopathy and severe neurodevelopmental delay that is the result of loss-of-function mutations in the CDKL5 gene. CDD predominately affects females and the resulting mutations lead to a mosaic expression of Cdkl5 in the brain. Two mouse models of the disease, B6.129 FVB CDKL5 (exon 6 constitutive knockout) and CDKL5 R59X (patient mutation model) are commonly used in the field to study mechanisms underlying the disease progression, examine neuropathology and to evaluate novel therapeutic interventions. Our group has evaluated robust translational behavioral assays in both male and female wildtype and transgenic mice from both CDD models. An extensive motor, learning and memory, and neurophysiological battery was performed on both models describing functional endpoints in female mice. Hyperactivity and motor deficit phenotypes were validated in both lines. Following our tailored learning and memory and neurophysiology battery we were able to detect significant deficits in transgenic mice as compared to their wildtype littermates. To further replicate published protein levels, Cdkl5 substrates and gene expression Western blot and RT-qPCR was performed from discrete brain regions of both models. An immunohistochemical evaluation of the impact of the disease on perineuronal network formation (WFA), developmental memory processes (c-Fos) and vesicle facilitation of neurotransmission (SV2A) was performed in order to identify neuropathological endpoints of the disorder. Taken together these data will provide a series of reproducible quantitative and qualitative criteria to evaluate the efficacy of outcome of translational therapeutic intervention applied to the available animal models for CDKL5

Disclosures: **D.L. Cameron:** None. **A. Adhikari:** None. **F.K. Buchanan:** None. **N. Copping:** None. **T. Fenton:** None. **I. Villegas:** None. **J.A. Halmai:** None. **J.L. Silverman:** None. **K. Fink:** None.

Poster

355. Developmental Disorders: Genetic Models II

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Support: Fondation pour l'audition (FPA-RD-2020-8)
Association “autour de Williams”
Ministerio de Ciencia e Innovación (SAF2016-78508-R; AEI/MINEICO/FEDER, UE)

Title: Bk channels as a therapeutic target to treat rare neurodevelopmental disorders: preclinical evidence from a mouse model of williams-beuren syndrome

Authors: C. FERRAGUTO[1], M. PIQUEMAL[1], J.-F. QUIGNARD[2], J.-L. MOREL[1], B. BONTEMPI[1], E. LOUETTE[3], V. CAMPUZANO[4], **S. PIETROPAOLO**[1];
[1]INCIA, Bordeaux, France; [2]INSERM U1045, Ctr. de recherche cardiothoracique de Bordeaux, Bordeaux, France; [3]Assetsup, Paris, France; [4]Departament de biomedicina, university of Barcelona, Barcelona, Spain

Abstract: Williams-Beuren syndrome (WBS) is a rare hereditary neurodevelopmental disorder (NDD), characterized by several neurobehavioral and cardiovascular alterations. Despite the recent advances in engineering a pre-clinical animal model recapitulating the complete human chromosomic deletion (i.e., the CD transgenic mouse), the etiopathological processes of this complex syndrome are poorly understood and effective pharmacological treatments are still lacking. Because potassium (BK) channels play a crucial role in synaptic function and neuronal excitability, and are known to be altered in several NDDs, we tested the hypothesis that a reduction in BK channel expression and functionality underlies alterations in CD mice and that stimulating BK channels may therefore alleviate the pathological WBS-like phenotypes of CD mutants. We chose the FDA-approved BK channel agonist chlorzoxazone (CHLOR) typically prescribed for non-developmental muscular pathologies, but which has been recently proposed for treating neurodegenerative disorders in animal models. Adult (4-5 months-old) male and female CD mice and their WT littermates were used. BK expression and functionality were assessed at both neuronal and cardiovascular levels. For pharmacological studies, animals received either acute or chronic (for 10 days) intraperitoneal injections of CHLOR and were then screened for behavioral abnormalities resembling those observed in WBS patients. These included assessment of cognitive, social, sensory, motor and emotional abilities that are known to be altered in WBS. Furthermore, the effects of acute CHLOR on cerebral and cardiovascular alterations of CD mice were evaluated. We found that BK currents and the expression of their

constitutive and regulatory subunits were reduced in CD mice, both in neurons and lung vascular smooth muscle cells. Both acute and chronic CHLOR treatments were efficacious in rescuing the major neurobehavioral and cardiovascular abnormalities of CD mice. Our findings support the therapeutic value of CHLOR for the treatment of WBS and provide preclinical evidence for future clinical applications of CHLOR in WBS research. They also suggest that BK channelopathies may be a common ethiopathological mechanism involved in multiple neurodevelopmental rare syndromes.

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Poster

355. Developmental Disorders: Genetic Models II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 355.21

Topic: A.07. Developmental Disorders

Support: NIH Grant F32 HD105323
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NIH Grant GM130136
NIH Grant University of Rochester CTSA award UL1 TR002001

Title: Cell-specific transcriptional regulation of neurodevelopment by the Aristaless-related homeobox protein (ALR-1/ARX)

Authors: *C. A. DIAZ-BALZAC[1], M. I. LAZARO-PENA[2], D. S. PORTMAN[2];
[1]Dept. of Medicine- Div. of Endocrinology, Diabetes and Metabolism, [2]Dept. of Biomed. Genet., Univ. of Rochester, Rochester, NY

Abstract: Intellectual disabilities arise from disruption of normal brain function. ARX is a transcription factor known to regulate brain development and patterning, which when mutated causes an X-linked form of intellectual disability and other syndromes associated with neurological deficits. Moreover, several mutations have been identified in this gene, and there is a correlation between the class of mutation and the resulting neurological syndrome. This gene is conserved throughout evolution, and mutations in the *Caenorhabditis elegans* ortholog, *alr-1*, result in defects in neuronal development. We discovered that these defects can be rescued by

human ARX, creating an ideal model system to test the mechanistic underpinnings of ARX disease-causing mutations. Genetic analyses of *alr-1*/ARX mutant phenotypes support its role in regulating distinct gene regulatory networks cell/cell-group specifically, including axon termination, synaptogenesis, GABAergic neuronal differentiation, and male-specific neuronal differentiation. However, how this cell/cell group-specific regulation is achieved is still not known. Identifying how ARX achieves this specificity is crucial in order to understand the mechanism underlying the genotype/phenotype correlation observed in different ARX mutations. Current work aims at (1) identifying the regulatory sequences responsible for cell/cell group-specific expression of *alr-1*, (2) identifying its target genes, and (3) characterizing the effect of ARX disease-causing mutations in neurodevelopment.

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Poster

355. Developmental Disorders: Genetic Models II

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Topic: A.07. Developmental Disorders

Support: FOXG1 Foundation Australia PhD scholarship

Title: Antisense oligonucleotide therapy ameliorates FOXG1 syndrome in vivo

Authors: D. C. S. TAN[1], S. JUNG[1], Y. DENG[1], N. MOREY[1], G. CHAN[1], A. BONGERS[2], Y. KE[1], L. M. ITTNER[1], ***F. DELERUE**[1];
[1]Dementia Res. Ctr., Macquarie Univ., Sydney, Australia; [2]MWAC, Univ. of New South Wales, Sydney, Australia

Abstract: FOXG1 syndrome is a neuro-developmental disorder that affects the early development of the telencephalon, leading to severe cortical impairments. The disease is associated with mutations in the *FOXG1* gene, which encodes a transcription factor of the forkhead family. There is no cure or treatment for FOXG1 syndrome. Here, we report a novel mutation of the *FOXG1* gene, a single nucleotide deletion c.946del (p.Leu316Cysfs*10) resulting in the premature truncation of the FOXG1 protein. We generated and characterized the clinically-relevant Foxg1 c946del mouse model that recapitulates hallmarks of the human counterpart. Thorough genetic, molecular, cellular, physiological and behavioural analysis revealed that heterozygous mice display poorer grip strength and motor coordination. EEG recordings and chemically-induced seizures indicated an aberrant neuronal network underlying an increased seizure susceptibility. Gene expression profiling identified upregulation of oligodendrocyte and myelin related genes. Specifically, we showed that FOXG1 dysregulation is correlated with overexpression of proteolipid protein 1 (*Plp1*), a gene linked to white matter disorders. Postnatal administration of *Plp1*-targeting antisense oligonucleotides in Foxg1 c946del

mice improved motor-related deficits. These results positioned *Plp1* as an important new target for therapeutic strategies that benefit FOXG1 syndrome patients.

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Poster

355. Developmental Disorders: Genetic Models II

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Topic: A.07. Developmental Disorders

Support: DOD W81XWH-17-1-0238
CPRIT RP 140655
R01 NS115546
3R01MH116882-02S1

Title: Gator1 contributes to seizures: mechanisms and therapeutic rescue in Nprl2 models

Authors: *L. ANGELES-PEREZ[1], B. DENTEL[1], C. REN[1], V. JAKKAMSETTI[1], A. HOLLEY[2], D. CABALLERO[3], E. OH[2], J. GIBSON[2], J. M. PASCUAL[1], K. HUBER[2], B. TU[3], P. TSAI[1];
[1]Neurol., [2]Neurosci., [3]Biochem., Univ. of Texas Southwestern Med. Ctr., Dallas, TX

Abstract: One emerging cause of focal epilepsy is mutations in the gene nitrogen permease regulator-like 2 (*NPRL2*). NPRL2 is a part of the GAP activity towards the rags 1 (GATOR1) complex, which negatively regulates the mechanistic target of rapamycin complex 1 (mTORC1). To delineate the mechanisms underlying these epilepsies and further understand the role of NPRL2, we generated two conditional *Nprl2* mutant mouse models. Given that constitutive loss of *Nprl2* leads to embryonic lethality, we conditionally deleted *Nprl2* using a pan-neuronal and glial model ($Nestin^{cre}$) and an excitatory neuronal conditional model with deletion in the forebrain and hippocampus ($Emx1^{cre}$). Homozygous mutant *Nprl2* mice with the $Nestin^{cre}$ promoter showed early mortality around P8-12, weighed less than heterozygote and control littermates and demonstrated spontaneous seizures. The $Emx1^{cre}$ homozygous mutant mice for *Nprl2* showed mortality by P21 and weighed less than their littermates. In addition to spontaneous seizures, $Emx1^{cre}$ mutant mice displayed abnormal synaptic function characterized by increased excitatory and decreased inhibitory synaptic changes. To determine the specific contribution of excitatory neurons in the forebrain to seizure development and given their increased survival, we focused the rest of our studies on the $Emx1^{cre}$ mutants. Using neocortical tissue, we performed metabolomic studies, which revealed mutants had increased levels of the neurotransmitter, glycine. We next explored whether glycine contributed to the electrophysiological and survival phenotypes in these mice. Our studies show that blocking the co-agonist $glycine_B$ site at the NMDAR ameliorated the increased excitatory synaptic changes

present in the mutant mice. Additionally, probenecid treatments to inhibit glycine's actions at the NMDAR increased survival in *Emx1*^{cre} mutant mice. Altogether, we demonstrate *Nprl2* deletion in the brain results in spontaneous seizures, early mortality, synaptic dysfunction and metabolic disruptions in mice. Further, we show evidence of glycine actions at the NMDAR as a contributing mechanism underlying these phenotypes. These findings provide potential therapeutic targets for mTORC1-related epilepsies.

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Poster

355. Developmental Disorders: Genetic Models II

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Program #/Poster #: 355.24

Topic: A.07. Developmental Disorders

Support: NIH Grant R01HD104609
Fundación Alfonso Martín Escudero

Title: Impaired corticogenesis in a mouse model of DDX3X syndrome

Authors: *M. GARCIA-FORN[1,2,3,4], M. FLORES[1,2,3,4], P. OLA[1,2,3,4], A. VON MUEFFLING[1,2,3,4], M. HANNAN[1,2,3,4], K. NIBLO[1,2,3,4], S. DE RUBEIS[1,2,3,4];
[1]Seaver Autism Ctr. for Res. and Treatment, [2]Psychiatry, [3]The Mindich Child Hlth. and Develop. Inst., [4]Friedman Brain Inst., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: DDX3X syndrome accounts for 1-3% of unexplained intellectual disability (ID) in females, presenting also behavioral problems and motor impairments. Even though the genetic cause of the syndrome is known – mutations in the *DDX3X* gene –, the cellular and molecular mechanisms underlying it are still to be elucidated. DDX3X is an X-linked gene that regulates mRNA translation and has emerging functions in corticogenesis and synaptogenesis. We previously generated the first mouse model (*Ddx3x*$^{+/-}$) with construct and face validity for *DDX3X* loss-of-function mutations, which showed developmental and behavioral alterations accompanied by defective cortical lamination. Here, we aim to elucidate the cellular and molecular mechanisms driving DDX3X syndrome during development, particularly during corticogenesis. To this aim, we used our *Ddx3x*$^{+/-}$ mice to study the impact of *Ddx3x* haploinsufficiency on corticogenesis by assessing the presence of cortical progenitors using cell-specific markers at different time points of embryogenesis. We also evaluated the birthdate and migration of glutamatergic neurons using BrdU injections and *in utero* electroporation of a GFP plasmid. We found that *Ddx3x*$^{+/-}$ mice present with an increase in cortical progenitor cells during embryogenesis at the expense of postmitotic neurons compared to their *Ddx3x*$^{+/+}$ littermates. Overall, our data shed new light on the cellular mechanisms driving DDX3X syndrome.

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Poster

355. Developmental Disorders: Genetic Models II

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Program #/Poster #: 355.25

Topic: A.07. Developmental Disorders

Support: The National Cancer Institute (2U54CA132378)
The National Institute of General Medical Sciences (1SC1GM132035)

Title: Atm and Msh2 deficiency cause severe developmental defects in the visual system

Authors: *M. M. GHINIA-TEGLA[1], E. SIBLE[2], M. M. EMERSON[2], B. Q. VUONG[2];
[1]City Univ. of New York, New York, NY; [2]Biol., City Col. of New York, City Univ. of New York, New York, NY

Abstract: DNA damage response proteins play a crucial role in maintaining genomic integrity during genesis of the nervous system, when a pool of highly dividing cells generates a variety of neuronal and non-neuronal cell lineages. ATM (ataxia telangiectasia mutated) and MSH2 (MutS homologue 2) are two of the crucial DNA repair proteins, acting in two different pathways. Mutation of these genes are often associated with aberrant cell cycle and tumorigenesis. In the nervous system, ATM deficiency causes Ataxia telangiectasia (AT) in humans, which is characterized by progressive neurodegeneration manifested mainly as cerebellar ataxia, oculomotor dysfunction and oculocutaneous telangiectasias. Surprisingly, the ATM-deficient mouse models fail to recapitulate these neurological phenotypes. Loss of ATM and MSH2 causes a late embryonic lethality, suggesting an uncharacterized developmental defect in *Atm*$^{-/-}$ *Msh2*$^{-/-}$ embryos.
Gross anatomical analysis of the head of *Atm*$^{-/-}$*Msh2*$^{-/-}$ mouse embryos showed severe defects in eye development, such as anophthalmia, microphthalmia, or coloboma. We generated similar double mutants in the developing chick brain, by combining CRISPR/Cas9 gene editing and *in vivo* electroporation. The chick ATM and MSH2 double mutants showed a similar phenotype as the *Atm*$^{-/-}$*Msh2*$^{-/-}$ mouse embryo, suggesting a conserved mechanism of action of these genes during visual system development. To investigate the development of eye structures, we examined the retinal pigment epithelium (RPE), neuronal retina as well as components of the neuro-vascular unit. Both the mouse and the chick ATM and MSH2 double mutants showed defects in eye structure patterning, such as enlarged optic stalk, incomplete formation of the RPE and different degrees of delay in the generation of the neuronal retina. Importantly, the ATM and MSH2 double mutants show vasculature leakage around the developing eye, as well as in other regions of the embryonic brain, which is reminiscent of telangiectasia, a hallmark of AT patients. We hypothesize that the combined absence of ATM and MSH2 triggers the accumulation of genomic DNA damage in vulnerable cell populations. In parallel, we will investigate the role of

reactive oxygen species (ROS), known to be accumulated during mitosis and to activate ATM and MSH2. The mechanisms by which ATM and MSH2 regulate vascularization and visual system development in mice and chicken remain to be determined. Upon completion, this work will advance our understanding of the molecular mechanisms that determine AT features such as visual telangiectasia as well as the vulnerability of the visual system in these cases of faulty DNA repair.

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Poster

355. Developmental Disorders: Genetic Models II

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Program #/Poster #: 355.26

Topic: A.07. Developmental Disorders

Title: In vivo tracking of KCC2b expression during early brain development

Authors: *E. GAHTAN[1], E. F. JONES[2], G. BUTLER[1], L. JERNIGAN[1], M. S. MENDEZ[1], J. STEELE[3];
[1]Cal Poly Humboldt, Arcata, CA; [2]Univ. of Alabama at Birmingham, Birmingham, AL; [3]Takeda Pharmaceut. Co., San Diego, CA

Abstract: The neuronal chloride (Cl-) exporter, KCC2, is an important regulator of neuron excitability and development. KCC2 expression undergoes a stereotypical pattern of delayed upregulation as neurons are born and mature. KCC2 upregulation favors neural inhibition by establishing a negative Cl- gradient, ensuring GABA-induced Cl- currents are inward and inhibitory. We developed a zebrafish fluorescent reporter line, KCC2b:mCitrine, to track KCC2 expression in vivo during early brain development. KCC2b:mCitrine expression was first detected at 16 hours post fertilization in the earliest differentiating neurons, and labeled most central and peripheral neurons and axonal and dendritic processes as they developed across 6 days. At 20 hours expression was greatest in the soma-dense basal neuroepithelium but largely absent in apical and mantle zones where differentiation and migration primarily occurs, and time lapse imaging at this stage supports a post-migration upregulation of KCC2. Central dopamine neurons have been shown to have low KCC2 expression in other species and we found preliminary evidence for this in larval zebrafish. KCC2b:mCitrine fluorescence was stable over minutes in most neurons, but brightness transients observed in single cells fit our expectation for real-time tracking of KCC2 gene upregulation in new neurons. To further assess whether fluorescence brightness tracks KCC2 expression, zebrafish embryos were exposed to BPA, which is known to suppress KCC2 expression. BPA decreased whole-brain mCitrine fluorescence after 6 days of exposure but not after 2 or 4 days of exposure, suggesting fluorescence may be an accurate but delayed indicator of gene regulation. KCC2b:mCitrine

zebrafish present a new way to visualize KCC2b's complex dynamics during brain development, and potentially to screen compounds aimed at modulating KCC2 expression.

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Poster

355. Developmental Disorders: Genetic Models II

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Program #/Poster #: 355.27

Topic: A.07. Developmental Disorders

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1.3.5 project for disciplines of excellence of West China Hospital, ZYJC18011

Title: Impaired neuritogenesis during neuronal maturation in models of NIBP/TRAPPC9 syndrome

Authors: *B. BODNAR[1], M. HU[2], Y. ZHANG[3], F. XIE[3,4], F. LI[1], S. LI[2], J. ZHAO[2], R. ZHAO[1], N. GEDUPOORI[1], Y. MO[1], X. LI[2], W. MENG[2], X. YANG[1], H. WANG[1], M. F. BARBE[1], S. SRINIVASAN[5], J. R. BETHEA[6], H. XU[2], W. HU[1];
[1]Ctr. for Metabolic Dis. Res., Lewis Katz Sch. of Med. at Temple Univ., Philadelphia, PA; [2]Lab. of Stem Cell Biology, State Key Lab. of Biotherapy, West China Sch. of Medicine, West China Hosp. of Sichuan Univ., Chengdu, China; [3]Ctr. for Stem Cell Res. and Application, Inst. of Blood Transfusion, Chinese Acad. of Med. Sci. & Peking Union Med. Col., Chengdu, China; [4]Clin. Lab., Xi'an No. 3 Hosp., Xi'an, China; [5]Div. of Digestive Dis., Emory Univ. Sch. of Med., Atlanta, GA; [6]Dept. of Biol., Drexel Univ., Philadelphia, PA

Abstract: NIBP (NIK-and-IKK2-binding protein; also known as TRAPPC9) is an important mediator of both NFκB signaling and protein transport particle (TRAPP) trafficking. Increasing clinical evidence shows that *NIBP/TRAPPC9* mutations have been linked to a novel autosomal recessive intellectual disability syndrome, NIBP Syndrome, characterized by various symptoms including intellectual disability, developmental delays, postnatal microcephaly, dysmorphic facial features, and obesity. Clinical evidence has confirmed that patients with this disorder have significantly decreased NIBP mRNA and protein expression, suggesting that NIBP deficiency is directly responsible for its pathogenesis, although the pathways involved remain unclear. To better understand the cellular/molecular mechanisms underlying NIBP Syndrome, we developed two separate animal models to phenotype NIBP deficiency. Zebrafish models were generated using morpholino knockdown and CRISPR/Cas gene editing and mouse models were generated

using traditional gene targeting of floxed exon 2-5. NIBP-deficient zebrafish and mice presented with microcephaly marked by decreased volume of white and grey matter. Additionally, NIBP knockout mice displayed deficits in learning and memory. Immunofluorescent analysis showed defective neuritogenesis in NIBP-deficient mice and zebrafish, characterized by impaired elongation and branching of dendrites and axons during neuronal maturation. Proteomics highlighted decreased expression of several other TRAPPII related proteins, suggesting impaired stability of TRAPPII complexes. We confirmed that NIBP deficiency impaired TRAPPII trafficking in actin filaments and microtubules of neurites and growth cones. Altogether, these findings suggest that NIBP deficiency may lead to impaired TRAPPII trafficking resulting in defective axon and dendrite elongation/branching. This provides novel genetic/molecular evidence to help characterize the mechanisms underlying a novel intellectual disability. These findings highlight the importance of protein trafficking during neuronal maturation and offers a potential therapeutic target for neurodevelopmental disorders.

Disclosures: B. Bodnar: None. **M. Hu:** None. **Y. Zhang:** None. **F. Xie:** None. **F. Li:** None. **S. Li:** None. **J. Zhao:** None. **R. Zhao:** None. **N. Gedupoori:** None. **Y. Mo:** None. **X. Li:** None. **W. Meng:** None. **X. Yang:** None. **H. Wang:** None. **M.F. Barbe:** None. **S. Srinivasan:** None. **J.R. Bethea:** None. **H. Xu:** None. **W. Hu:** None.

Poster

355. Developmental Disorders: Genetic Models II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 355.28

Topic: A.07. Developmental Disorders

Support: NIH Grant 1F31DA053796-01A1

Title: Cell-type Specific Alternatively-Spliced Fgf13 Regulates Cortical Development

Authors: *S. LIN, A. GADE, H. WANG, A. GALANTE, I. DISTEFANO, A. RAJADHYAKSHA, G. S. PITT;
Cornell University: Weill Cornell Med. Col., New York, NY

Abstract: Fibroblast growth factor homologous factor 13 (FGF13) is a non-canonical member of the fibroblast growth factor (FGF) superfamily that is not a secreted growth factor but instead functions intracellularly as a voltage-gated sodium channel auxiliary protein. C**linical studies revealed that patients with disruptions or mutations in the X-linked *FGF13* gene have early onset cognitive impairment and febrile seizures. The X-linked *Fgf13* produces two major alternatively-spliced isoforms in the cerebral cortex, one which is predominantly expressed in excitatory and the other in inhibitory neurons. Proteomic screens and transcript sequencing studies predict different roles for the two splice variants.** To study cell-type specific FGF13 functions in the cerebral cortex, I generated mice lacking FGF13 throughout *Emx1*-expressing excitatory neurons or *Gad2*-expressing GABAergic interneurons. *Gad2*-mutant

mice suffer epileptic seizures, consistent with the human clinical condition, and hemizygous knockout mice die perinatally. Mice with *Fgf13* deficiency in glutamatergic cells survive through adulthood. To further define the functional roles of FGF13, we recorded sodium currents in *Emx1*-expressing or *Gad2*-expressing knockout neurons. My findings reveal cell type-specific roles of *Fgf13* in cortical development, likely driven by distinct splice variants. **These data show that differential expression of *Fgf13* via alternative splicing generates distinct proteins with different neuronal functions.**

Disclosures: S. Lin: None. **A. Gade:** None. **H. Wang:** None. **A. Galante:** None. **I. Distefano:** None. **A. Rajadhyaksha:** None. **G.S. Pitt:** None.

Poster

355. Developmental Disorders: Genetic Models II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 355.29

Topic: A.07. Developmental Disorders

Support: 1F30HD108986-01

Title: Investigation of Fmnl2 in Cerebellar Development

Authors: *J. TRAN[1], S. L. ACKERMAN[2];
[1]UCSD, La Jolla, CA; [2]Univ. of California San Diego, La Jolla, CA

Abstract: The coordinated regulation of the dynamic cytoskeletal network is essential for proper brain development. This plays an integral role in cellular movement and orientation during proliferation, as well as growth cone protrusion during axonal guidance. Primary cilia, which are made up of actin and microtubules, serve as a hub for various key developmental signaling cascades. Dysfunction in actin and ciliary proteins lead to rare genetic disorders affecting many organs, including the brain. Congenital ataxias generally result from dysfunction and malformation of the cerebellum, particularly the vermis. These disorders may be caused by the lack of proper developmental signaling cascades that dictate the proliferation and migration of neurons.
Our lab identified the *nmf418* mutation in a chemical mutagenesis screen for neurological phenotypes in mice. *Nmf418*$^{-/-}$ mice are ataxic, and most die at postnatal day 0. They have vermis malformation abnormal foliation, and elongated shape along the anterior-posterior axis of the cerebellum. These mice also have reduced axonal tract fibers in the corpus callosum and anterior commissure, as well as a lack of superior cerebellar peduncle axonal tract decussation.
Strikingly, many of these phenotypes are reminiscent of symptoms seen in humans with dysfunctional cilium. By positional cloning, we identified a mutation in *Fmnl2*, a member of the formin protein family which leads to a dramatic reduction in FMNL2 protein.
FMNL2 is an autoinhibited cytoskeletal effector that has been previously shown to drive actin polymerization at filopodia and lamellipodia tips of cultured cells. Although other formins have

been shown to bind and regulate microtubules and actin, as well as influence cilia formation, the function of FMNL2 in these processes during brain development is unknown. Our next step is to determine the function of FMNL2 in cerebellar granule cell actin polymerization, particularly during growth cone formation. We will also investigate the loss of FMNL2 and its impact on ciliary function and developmental cascades. These studies will also shed light on the mechanisms underlying the role of cilia and actin in human cerebellar malformations.

Disclosures: J. Tran: None. **S.L. Ackerman:** None.

Poster

356. Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 356.01

Topic: A.07. Developmental Disorders

Support: MOST 110-2410-H-227 -006

Title: Changes of Auditory and Visual Event Related Potential in Unilateral Hearing Loss School Age Pupils in Mandarin Learning Environment

Authors: Y. KAO[1], H. WU[2], H. FOO[4], C. TANG[3], C. CHANG[5], M. WU[6], Y. LO[7], ***S.-M. WENG**[4];
[1]Dept. Of Speech Language Pathology And Audiol., Natl. Taipei Univ. Of Nursing And Hlth. Sci., Taipei, Taiwan; [2]Dept. of Otolaryngology, [3]Dept. of Ophthalmology, Shin Kong Wu-Ho-Su Mem. Hosp., Taipei, Taiwan; [4]Dept. Of Speech Language Pathology And Audiol., Natl. Taipei Univ. of Nursing And Hlth. Sci., Taipei, Taiwan; [5]Artise Biomed. Co., Ltd, Hsinchu, Taiwan; [6]Donghu Elementary Sch., Taipei, Taiwan; [7]The Ph.D. Program for Neural Regenerative Medicine, Col. of Med. Sci. and Technol., Taipei Med. Univ., Taipei, Taiwan

Abstract: Our study investigated the differences in speech performance and neurophysiological response in groups of school-aged children with unilateral hearing loss (UHL) or typically developed (TD) characteristics. Total 16 primary school-aged children were recruited in our study (UHL=9/TD=7, diagnosed in Shin Kong Wu-Ho-Su Memorial Hospital). Word comprehension is tested by the Peabody Picture Vocabulary Test-Revised (PPVT-R), and the PPVT-R PR value is proportional to the auditory memory score (by The Children's Oral Comprehension Test) in both groups. Later we assessed the latency and amplitude of auditory and visual ERPs and found that the latency of auditory ERP N200 in the UHL group is prolonged compared with which in the TD group. The amplitudes of auditory and visual ERP P300 were both lower in the UHL group showing the implication of attenuated auditory and visual processing. Although UHL pupils have one-side normal hearing, based on our results, long-term one-side hearing deprivation might be the origin of aberrant reorganization of brain areas for auditory or even visual perceptions attributed to speech delay and learning difficulties.

Disclosures: **Y. Kao:** None. **H. Wu:** None. **H. Foo:** None. **C. Tang:** None. **C. Chang:** None. **M. Wu:** None. **Y. Lo:** None. **S. Weng:** None.

Poster

356. Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 356.02

Topic: E.05. Brain-Machine Interface

Title: Acute effects of aerobic exercise on cortical excitability in young adults with attention deficit hyperactivity disorder

Authors: *Y.-H. WANG[1], C.-R. HONG[3], L.-G. YANG[4], Y.-C. LIOU[2], H.-I. KUO[2];
[1]Sch. and Grad. Inst. of Physical Therapy Col. of Med. Natl. Taiwan Univ., Sch. and Grad. Inst. of Physical Therapy Col. of Med. Natl. Taiwan Univ., taipei, Taiwan; [2]Sch. and Grad. Inst. of Physical Therapy Col. of Med. Natl. Taiwan Univ., Taipei, Taiwan; [3]Natl. Pingtung Univ., Pingtung, Taiwan; [4]Tri-Service Gen. Hosp. Beitou Br., Taipei, Taiwan

Abstract: Attention deficit hyperactivity disorder (ADHD) is one of the most common neurodevelopmental disorders worldwide. Evidence has shown that aerobic exercise might have positive impact on relieving symptoms in subjects with ADHD. However, the underlying mechanism is still missing. The study aims to explore the effects of acute aerobic exercise on brain physiology in subjects with ADHD. The study was conducted in a cross-over design. Six ADHD subjects and 6 matched healthy controls were assessed by transcranial magnetic stimulation (TMS) before and after exercise and control (rest) intervention. The results found that acute aerobic exercise significantly decreased cortical excitability (enhanced intracortical inhibition, and decreased intracortical facilitation) in subjects with ADHD. In contrast, cortical excitability enhancement was found in healthy controls after acute aerobic exercise. The study gave preliminary evidence that acute exercise can modulate cortical excitability in subjects with ADHD which might explain the treatment effects of exercise intervention.

Disclosures: **Y. Wang:** None. **C. Hong:** None. **L. Yang:** None. **Y. Liou:** None. **H. Kuo:** None.

Poster

356. Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 356.03

Topic: H.12. Aging and Development

Title: Preliminary Findings for Early Child Development on Cognitive, Social, and Emotional Functions among the COVID generationPreliminary Findings for Early Child Development on Cognitive, Social, and Emotional Functions among the COVID generation

Authors: J. LIM[1], H.-Y. CHOI[2], G. KANG[3], *S. EOM[4];
[1]LumanLab, LumanLab, Seoul, Korea, Republic of; [2]Yonsei university, Seoul, Korea, Republic of; [3]Lumanlab, Lumanlab, Seoul, Korea, Republic of; [4]Yonsei Univ. Col. of Med., Yonsei Univ. Col. of Med., Seoul, Korea, Republic of

Abstract: Since early child development is affected by environmental factors, the impact of the COVID-19 pandemic on children's development should be examined in a timely manner. Decreased physical activities and social interactions, due to closures of childcare sites and social distancing policies, may have caused deficits in cognitive and social stimulation that children would have normally acquired from their environment, resulting in delayed development. As a preliminary study for planning adequate interventions for children going through the pandemic, the present study aimed to delineate the developmental profiles of the COVID generation. The present study identified 147 infants without known developmental disabilities born between late 2018 and early 2021 in South Korea. Their developmental profiles in Cognitive, Physical, Social functions, behavioral problems, as well as their caregivers' depression, were assessed. The results showed that the rates of children at high risk of developmental delays in Communication and Physical functions were higher than standardized base rates by 11.15% and 13.7%, respectively. The high-risk rates for Social function (22.2%) were notably higher than base rates, suggesting a significant delay in social development. 10% of the children exhibited more behavioral problems compared to children in the normal range, showing 8% more Internalization and Externalization each. When the extent of COVID-related regulations is accounted for, children residing in areas with more strict regulations showed higher rates of Hyperactivity (p<.001) and more delays in daily living skills (p<.05). Lastly, 22% of the caregivers reported significantly higher rates of depression than average. There was also a trend for a positive correlation between time passed during the pandemic and children's developmental delays, behavioral problems, and caregivers' depression. It is noteworthy that these preliminary findings revealed the negative effects of the pandemic on child development even with no nationwide lock-down. Further examination of developmental state for more children during-pandemic and its long-term effects is needed. Also, the application of early developmental and psychological interventions for children and parenting education programs should be considered.

Disclosures: **J. Lim:** None. **H. Choi:** None. **G. Kang:** None. **S. Eom:** None.

Poster

356. Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 356.04

Topic: A.07. Developmental Disorders

Title: ADHD-associated alterations in network topography and DNA methylation in discordant ADHD twins

Authors: *H. ABUAD[1], R. HERMOSILLO[1,2], M. MOONEY[4,5], S. KOIRALA[1], F. ESTRADA SANCHEZ[6], E. EARL[7], A. RANDOLPH[1,2], E. FECZKO[1,2], D. FAIR[1,2,3], J. NIGG[8,9,10];
[1]Masonic Inst. for the Developing Brain, [2]Dept. of Pediatrics, [3]Inst. of Child Develop., Univ. of Minnesota, Minneapolis, MN; [4]Dept. of Med. Informatics and Clin. Epidemiology, [5]Knight Cancer Inst., Oregon Hlth. & Sci. Univ., Portland, OR; [6]Portland State Univ., Portland, OR; [7]Natl. Inst. of Mental Hlth., Bethesda, MD; [8]Dept. of Psychiatry, [9]Ctr. for ADHD Res., [10]Behavioral Neurosci. Grad. Program, Oregon Hlth. & Sci. Univ. Sch. of Med., Portland, OR

Abstract: The observed variety of symptoms of attention-deficit/hyperactivity disorder (ADHD) suggests a multiplicity of sources for the disorder, though environmental factors can affect the degree of gene expression. Using a cohort of monozygotic twins (n=28) with discordant ADHD diagnoses, we quantified the topography of neural networks using a template matching procedure with resting-state functional connectivity MRI and demonstrated that, despite different diagnoses, corresponding twins had a similar topography (measured using normalized mutual information) to each other compared to mismatched twins pairs ($t(13.4)= -11.4$, $p= 3.00x10^{-6}$). We also found the size of the frontoparietal (FP) and the visual networks surface area to be significantly decreased in the ADHD twin ($t(12)$, FP: $p= 0.023$; visual: $p= 0.007$). This provides evidence of a strong genetic component to topography, but also indicates epigenetic modifications contribute to symptoms. We then used network topographies from typically-developing children from two matched cohorts (n=2625; n=2663) from the Adolescent Brain Cognitive Development (ABCD) study to examine the relationship between attentional scores from the Child Behavior Checklist and network surface area. Linear models using demographic covariates showed a reliable correlation between the FP network surface area and attention scores (ABCD group1 $r(2582)= 0.252$, $p= 0.021$, Benjamini-Hochberg (BH) correction; ABCD group2 $r(2620)= 0.2296$, $p= 0.001$, BH correction). However we found no correlation between the difference in FP network surface area and in the ADHD rating scale for our discordant twins (hyperactivity $r(13)= -0.1803$, $p= 0.537$; inattentiveness $r(13)= 0.1467$, $p= 0.617$). To investigate how differential DNA methylation in those with ADHD contributes to symptoms and possibly network topography, we examined DNA methylation in the blood and saliva of the discordant twins. Only the sensorimotor network significantly correlated with DNA methylation at cg06218533 within SETBP1 ($r(13)= -0.789$, $p= 0.014$, BH correction), a gene previously shown to be associated with intellectual disability. These findings show gene expression can modify network cortical surface area, however how network topography differences relate to behavioral symptoms merits further exploration.

Disclosures: H. Abuad: None. **R. Hermosillo:** None. **M. Mooney:** None. **S. Koirala:** None. **F. Estrada Sanchez:** None. **E. Earl:** None. **A. Randolph:** None. **E. Feczko:** None. **D. Fair:** None. **J. Nigg:** None.

Poster

356. Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 356.05

Topic: A.02. Postnatal Neurogenesis

Title: Comparison of longitudinal improvement between early and late helmet therapy in brachycephaly

Authors: *H. KIM[1], C. LEE[2];
[1]Soonchunhyang Univ. Bucheon Hosp., Gyeonggi-do, Korea, Republic of; [2]Bundang Yonsei Intrnl. Med. Clin., Gyeonggi-do, Korea, Republic of

Abstract: Introduction: Neurocranium growth occurs fastest in the first year after birth. Cranial molding helmet therapy is a non-invasive treatment for positional brachycephaly. There have been efforts to find when to start the therapy. However, previous studies compared the parameters before and after the treatment, and studies dealing with longitudinal change during helmet therapy are scarce. We aimed to investigate whether the monthly improvement of early helmet therapy is better than late therapy.
Methods: We divided patients into two groups, the early helmet therapy group(EG) in which patients started therapy before 5 months old, and the late helmet therapy group(LG) in which patients started therapy after 5 months old. All patients received custom-made helmet therapy. The cephalic index(CI) was calculated by dividing the cephalic width by the cephalic length obtained by the scanner. Scan data were collected before the treatment and every month after starting the treatment for 6 months. We compared the slopes of changes in CI between two groups to investigate longitudinal change.
Results: The baseline characteristics of the study population are shown in Table 1. The decline rate of CI was -1.18 per month in EG and -0.78 per month in LG (p=0.01), showing that the monthly decline rate of CI was steeper in EG than in LG (Figure 1).
Conclusions: The earlier the patient receives helmet therapy, the faster monthly improvement of head shape will be achieved in brachycephaly.

Table 1. Baseline characteristics of subjects

	LG (N=26)	EG (N=10)	total (N=36)	p-value
Male/Female	16/10	6/4	22/14	0.95
GA (weeks)	35.5 ± 3.4	38.4 ± 2.6	36.3 ± 3.4	0.1
Therapy starting age (months)	6.1 ± 1.0	4.6 ± 0.3	5.7 ± 1.1	0.004
Preterm/Full term	14/12	2/8	16/20	0.2
NSVD/C-SEC	2/24	4/6	6/30	0.17
Twin	8 (31%)	0 (0%)	8 (22%)	0.16
Torticollis	4 (15%)	6 (60%)	10 (28%)	0.058
CI	92.3 ± 4.4	96.0 ± 7.4	93.4 ± 5.4	0.2

LG: Late helmet therapy group

EG: Early helmet therapy group

GA: Gestational age

NSVD: Normal Spontaneous Vaginal Delivery

C-SEC: Cesarean section

CI: Cephalic index at starting helmet therapy

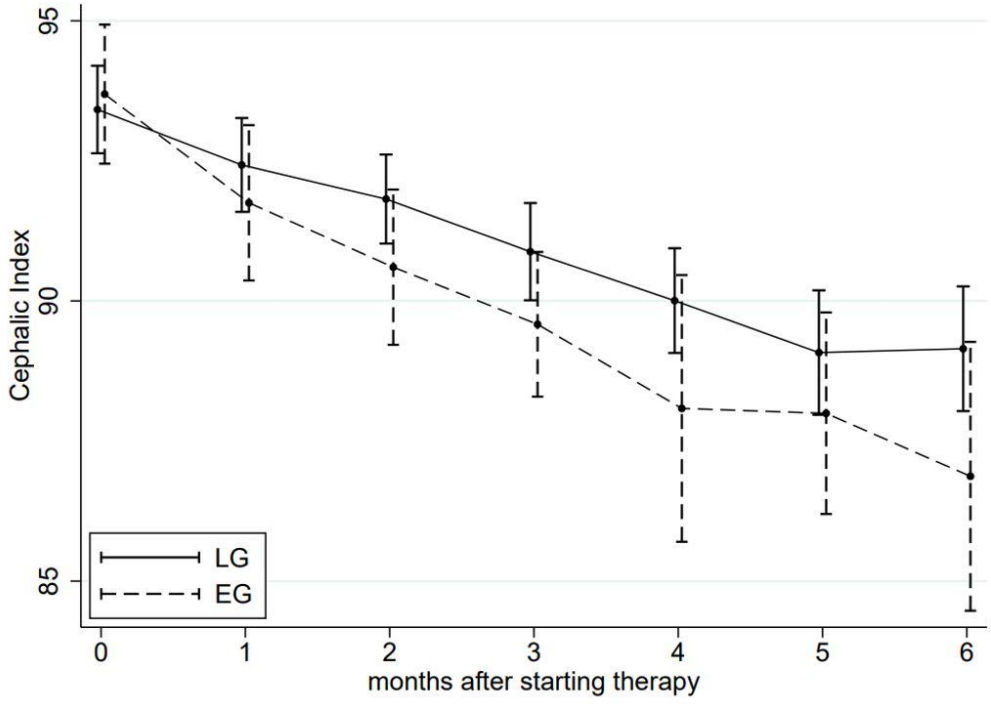

Figure 1. Longitudinal change of cephalic index

LG: Late helmet therapy group

EG: Early helmet therapy group

Disclosures: H. Kim: None. **C. Lee:** None.

Poster

356. Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 356.06

Topic: A.07. Developmental Disorders

Title: Acetaminophen use induces hyperactivity in male mice

Authors: *J. LEE, O. CIVELLI, A. ALACHKAR;
Pharmacol. and Pharmaceut. Sci., Univ. of California Irvine, Irvine, CA

Abstract: Acetaminophen use induces hyperactivity in male mice Justine Lee, Olivier Civelli, Amal AlachkarDepartments of Pharmacology and Pharmaceutical SciencesSchool of Pharmacy369 Med Surge IIUniversity of California, IrvineIrvine, CA 92697-4625A growing body of evidence in recent years has linked prenatal acetaminophen APAP use with adverse neurodevelopmental outcomes in children. Correlations between early life APAP exposure and attention-deficit hyperactivity disorder (ADHD) have been particularly compelling. Human epidemiological findings, as well as results from animal studies, suggest there is a causative link between prenatal APAP use and ADHD in children though the underlying mechanisms relating prenatal APAP exposure and ADHD phenotypes in offspring have yet to be identified. Results from our recent study show that long-term exposure to high doses of APAP in adult male mice was correlated with increases in locomotor activity and exhibited an escalating trend over time. Specifically, total distance traveled, ambulatory time, and ambulatory counts were consistently higher in APAP-treated mice. Moreover, differences between the two groups grew increasingly larger over time and APAP-treated animals often exhibited greater than twice the level of activity of their vehicle-treated counterparts. Our results show that not only are animals treated with APAP more hyperactive, they become even more active following chronic APAP exposure. Our findings suggest a causal agent of hyperactive phenotypes may be present following APAP administration which may shed light on potential pathophysiological mechanisms linking APAP use during pregnancy and ADHD-like behaviors in offspring.

Disclosures: **J. Lee:** None. **O. Civelli:** None. **A. Alachkar:** None.

Poster

356. Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 356.07

Topic: A.07. Developmental Disorders

Support: JSPS KAKENHI 20K03478
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JSPS KAKENHI 21K13760
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Japan Health Research Promotion Bureau Research Fund for Young Investigators JH2022-Y-03
Intramural Research Grant for Neurological and Psychiatric Disorders of NCNP 2-7

Title: Does feedback of a happy face improve response inhibition in adults with attention-deficit/hyperactivity disorder?

Authors: *S. HAYASHI, Y. EGASHIRA, S. UONO, M. TAKADA, M. UKEZONO, T. OKADA;
Dept. of Developmental Disorders, Natl. Inst. of Mental Hlth., Natl. Ctr. of Neurol. and Psychiatry (NCNP), Kodaira, Japan

Abstract: Individuals with attention-deficit/hyperactivity disorder (ADHD) show a deficit in response inhibition. Previous studies have demonstrated that feedback using a happy face improves response inhibition in children with ADHD; however, the findings were limited to children. This study investigated whether feedback using a happy face improved the response inhibition in adults with ADHD. The feedback effects were determined with and without low-probabilistic uncertainty, considering difficulties in eliminating uncertainty in social feedback. Forty-three adults with ADHD and 43 typically developed controls (TD) performed a social incentive go/no-go task under three reward conditions (no-reward/certain-reward/uncertain-reward). The participants observed a mosaic picture as meaningless feedback in the no-reward condition. They observed a happy face for successful inhibition in the certain-reward condition. They observed a happy face (87.5%) or a neutral face (12.5%) for successful inhibition in the uncertain-reward condition. In both reward conditions, they observed a neutral face for unsuccessful inhibition. The false alarm rate (FA), hit rate (HIT), and correct response times (RTs) during the task were calculated. We confirmed attenuation of the FA in the certain- and uncertain-reward conditions only in TD. For the participants with ADHD, there was no significant improvement in the accuracy of inhibition by the feedback. Furthermore, the FA in the participants with ADHD was lower than that of TD in the certain- and uncertain-reward conditions. There were no group differences in HIT and RTs. We additionally investigated a correlation between ADHD symptoms and the effects of the feedback on the FA in the participants with ADHD. The analysis indicated that the high score of inattention was associated with high FA in the uncertain-reward condition compared to no-reward. Our findings support the attenuated effects of the feedback of a happy face on response inhibition in adults with ADHD. The attenuation of the feedback effects may cause lower accuracy of response inhibition in adults with ADHD compared with TD in situations where they receive positive social feedback from others.

Disclosures: S. Hayashi: None. **Y. Egashira:** None. **S. Uono:** None. **M. Takada:** None. **M. Ukezono:** None. **T. Okada:** None.

Poster

356. Neurodevelopmental Disorders

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Program #/Poster #: 356.08

Topic: A.07. Developmental Disorders

Support: NIH Grant R01NS080844
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Brain Wellness Constellation (BWC) Neuroscience Summer Research Education Program, University of Mississippi
Newborn Medicine Funds from the Department of Pediatrics, University of Mississippi Medical Center

Title: Machine learning-based pattern analysis of chronic inflammation involvement in sleep disorders and attention-deficit/hyperactivity disorder (ADHD)-like behaviors in adolescent rats exposed to perinatal inflammation

Authors: *L.-W. FAN[1], S. LU[2], J. W. LEE[1], J. C. CROSBY[1], J. P. SHAFFERY[3], L.-T. TIEN[6], M. A. TUCCI[4], H. WANG[7], Z. CHEN[8], M. PATEL[5], P. SANAPUREDDY[9], N. B. OJEDA[1]; [1]Dept. of Pediatrics, Div. of Newborn Med., [2]Dept. of Neurol., [3]Dept. of Psychiatry and Human Behavior, Animal Behavior Core, [4]Dept. of Anesthesiol., [5]Dept. of Pediatrics, Div. of Pediatric Pulmonary, Univ. of Mississippi Med. Ctr., Jackson, MS; [6]Sch. of Med., Fu Jen Catholic Univ., New Taipei City, Taiwan; [7]Dept. of Industrial and Systems Engin., [8]Dept. of Computer Sci. and Engin., Mississippi State Univ., Mississippi State, MS; [9]Dept. of Med., G.V. (Sonny) Montgomery Veterans Affairs Med. Ctr., Jackson, MS

Abstract: Perinatal exposure to inflammation may play an important role in the association between sleep disturbances and neurodevelopmental disorders such as attention-deficit/hyperactivity disorder (ADHD) development. The objective of this study was to examine whether machine learning-based pattern analysis identified sleep patterns associated with ADHD in juvenile rats exposed to perinatal inflammation and sleep disruptions. Intraperitoneal injections of LPS (2 mg/kg) or saline were administered on postnatal day 5 (P5) to Sprague-Dawley male rat pups, followed by behavioral testing at P35, implantation of sleep recording electrode on P39, and exposure to sleep disruptions on P47. Baseline sleep, sleep disruption, and recovery sleep were recorded on P46, P47 and P48, respectively, for 24 hours. Four groups (n=5) were included in this study: Saline-Baseline, Saline-Recovery, LPS-Baseline, and LPS-Recovery. Histological and molecular assessments for brain inflammation and neuronal damage were performed at the end of experiments at P49. To ensure scientific rigor the molecular assays and histological assessments were evaluated by triplicate, and the sample sizes were calculated to reach a statistical power of at least 0.85 for a $p<0.05$. All animals were from the same strain and same vendor. Data were analyzed by two-way ANOVA followed by the Student-Newman-Keuls test. Our results showed that neonatal LPS treatment induced ADHD-like behaviors, including hyperactivity and inattention on P35. Neonatal LPS treatment interfered with REM sleep and

sleep homeostatic responses (recovery sleep) to sleep disturbances in adolescent rats (P49). Six unsupervised machine learning models were applied to analyze the feature interaction patterns among the collected high-dimensional sleep data. Our approaches identified relative theta and spindle power as features significantly associated with ADHD and perinatal inflammation in this experimental model of sleep disruption. Additionally, LPS exposure induced brain changes at P49 depicted by chronic microglia (Iba1+) activation and loss of TH+ neurons in the locus coeruleus, and chronic astrocyte (GFAP+) activation and loss of immature neurons (DCX+) in the hippocampal dentate gyrus. These results suggest that machine learning-based analysis is a strong tool to identify neurodevelopmental disorders utilizing sleep data in subjects exposed to perinatal inflammation and sleep disruptions. In addition, these results could help in developing new treatments for sleep disorders associated with ADHD.

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Poster

356. Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 356.09

Topic: A.07. Developmental Disorders

Support: NIH/NIMH MH105625

Title: Neural mechanisms underlying dual models of cognitive control in children with ADHD

Authors: *Z. GAO, K. DUBERG, V. MENON, W. CAI;
Psychiatry and Behavioral Sci., Stanford Univ., Palo Alto, CA

Abstract: Attention-deficit hyperactivity disorder (ADHD), characterized by significant deficits in cognitive control, is one of the most common neurodevelopmental disorders. Recent dual control model suggests that cognitive control operates via two distinct modes: proactive and reactive. However, the brain mechanisms underlying proactive and reactive control and their relation to individual differences in behavioral symptoms associated with childhood ADHD remain unknown. To address this issue, 26 ADHD and 27 typical developing children (TD) (mean age = 10.7 years, 31 males) were recruited to perform a stop-signal task (SST) and a cued SST in the MRI scanner. In the SST, participants made a choice based on the direction of a green arrow (Go signal). Occasionally (33%), the green arrow quickly turned to red (Stop signal) and participants need to withhold responses. In the cued SST, each trial started with a white or green cue, indicating the probabilities of stop signal being 33% (Uncertain Go) or 0% (Certain Go), respectively. Participants' behaviors were highly stable as the stop signal reaction times were correlated between tasks ($r = 0.90$, $p < 0.001$). Reactive and proactive control were probed by the contrast of Successful Stop versus Go in the SST and the contrast of Uncertain versus Certain Go

in the cued SST, respectively. Reactive control elicits greater activation in bilateral insula, pre-supplementary motor area (pre-SMA) and ventral temporal areas in TD children ($t > 3.1$). A similar, less distributed, activation pattern was found in ADHD children ($t > 3.1$). Proactive control elicits activation in bilateral insular and pres-SMA ($t > 2.6$, uncorrected). Regions of interest (ROIs) analysis found that TD children had greater activation in right superior marginal gyrus and caudate than ADHD for proactive control ($p = 0.018$ and $p = 0.049$, uncorrected). Multivariate classification analysis found that the activation map of proactive control can successfully differentiate children with ADHD from TD children (CV Accuracy = 75%, $p=0.004$). Moreover, we correlated brain activation and clinical symptoms estimated by the SWAN rating scale and found that activation in paracingulate gyrus and precuneus during proactive control was negatively associated with hyperactivity symptoms ($p=0.003$, $p=0.002$), and activation in the right insular and bilateral occipital cortex during reactive control were positively associated inattention symptoms ($p=0.005$, $p<0.001$). Together, our findings reveal aberrant neural mechanism underlying dual model of cognitive control and provide new insights into the aberrant brain functioning associated with clinical symptoms in childhood ADHD.

Disclosures: **Z. Gao:** None. **K. Duberg:** None. **V. Menon:** None. **W. Cai:** None.

Poster

356. Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 356.10

Topic: A.07. Developmental Disorders

Support: FAPERGS
CNPq
CAPES

Title: Caffeine counteracts behavioral phenotypes, altered brain oscillations pattern, and dopaminergic changes in a sex-dependent manner in the rat model of Attention Deficit and Hyperactivity Disorder

Authors: *L. O. PORCIUNCULA[1], F. NUNES[2], M. S. LARA[2], D. M. MARQUES[2], A. S. ALMEIDA[2], M. CALCAGNOTO[2];
[1]Biochem., [2]Univ. Federal do Rio Grande do Sul, Porto Alegre, Brazil

Abstract: Attention Deficit and Hyperactivity Disorder (ADHD) is one of the most common neurodevelopmental disorders, characterized by inattention, hyperactivity and impulsivity. This symptomatology differs between boys and girls, since girls present predominantly the inattentive subtype, and boys, the hyperactive and impulsive combined subtype. Caffeine is the most consumed psychostimulant worldwide with benefits in preventing attention, spatial and recognition memory impairment in the ADHD model. However, few studies have evaluated sexual dimorphism in the effects of caffeine in the ADHD model. In this study, the effects of

caffeine were investigated in a learning and decision making task (Dig task), hyperactivity and brain oscillations in adolescent rats from both sexes of the ADHD rat model. Male and female SHR (Spontaneously hypertensive rats, ADHD model) and Wistar Kyoto (control strain) received either caffeine (0.3 g/L, drinking water) or water from 15 post-natal day (PND) to 55-59 PND. Dig task involves two phases of odor discrimination and both strains started to be trained at PND 26. Superficial electrodes were implanted in the frontal cortex (FC) and locomotor activity along with electroencephalogram (EEG) recordings started at PND 55. Dopamine receptors D4 (DRD4) and the transporter (DAT) levels were evaluated in the FC. Female SHR required more trials to complete learning phase, while both sexes of the SHR required more trials and had a lower percentage of right choices in the novel learning phase. Caffeine was able to improve the performance in the learning phase for female SHR, and the novel learning phase for male SHR. The hyperlocomotion was attenuated by caffeine. DAT was decreased in the SHR from both sexes, and caffeine recovered DAT only in male SHR. Brain oscillations were mostly altered in female SHR, with increases in delta and theta/beta power during locomotor activity, which were prevented by caffeine treatment. Therefore, the worsened performance in a task that requires FC functioning displayed by female SHR involves alterations in brain oscillations pattern. Besides, caffeine extends its benefits by attenuating behavioral phenotypes related to FC dysfunctions in ADHD by modulating the power of brain oscillations associated with less wakefulness. Finally, these findings shed light on the importance of sex as biological variable in ADHD, and also when making treatment decisions in neuropsychiatric disorders.

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Poster

356. Neurodevelopmental Disorders

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Program #/Poster #: 356.11

Topic: A.07. Developmental Disorders

Support: R21 1NS119999
F30MH122100
R01 CA74177

Title: Corticostrial circuitry plays key role in modulating ADHD phenotype in translational model of Neurofibromatosis Type 1

Authors: *J. LUKKES, T. GALBARI, M. SULLIVAN, H. BOERNER, H. DROZD, D. CLAPP;
Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Neurofibromatosis type 1 (NF1) is an autosomal dominant disease with a mutation in one copy of the NF1 gene, with a prevalence of approximately 1 in 3000 individuals, and no

curative treatment. NF1 patients are commonly diagnosed with ADHD, with estimates of 60 - 90% having attention difficulties in school. There is limited preclinical data investigating ADHD in NF1, particularly in females. Our recent data demonstrate that male mice haploinsufficient for the neurofibromin gene (*Nf1*$^{+/-}$) exhibit hyperactivity in an open field, increased risky behavior in a cliff avoidance test, and increased impulsivity in a delay discounting task compared to wild-type males. These deficits were all attenuated with systemic treatment with a commonly prescribed, non-stimulant ADHD drug, guanfacine. Preclinical studies using murine experimental systems of ADHD have shown that lesions of the prefrontal cortex (PFC) or nucleus accumbens (NAc) increase impulsivity in a delay discounting task (DDT). The aim of the current study was to determine sufficiency of *Nf1* knock-down in cortical-striatal circuitry to cause executive dysfunction during clinically-relevant behavioral tasks through the use of *Nf1*$^{flox/flox}$ male and female mice. *Nf1*$^{flox/flox}$ male and female adult mice were injected bilaterally with either control virus (AAV5-CMV-GFP) or the Cre virus (AAV5-CMV-Cre-GFP) into the PFC, NAc, or ventral tegmental area (VTA). We found that selective deletion of the neurofibromin gene (*Nf1*) in the NAc increased hyperactivity to a novel open field and increased risky behavior in a cliff avoidance reaction test (CAR) in males but not females. However, both sexes of *Nf1*$^{flox/flox}$ mice injected with AAV5-CMV-Cre-GFP into the NAc exhibited deficits in behavioral inhibition measured by increased frequency of small reward choice in DDT. In contrast, selective deletion of *Nf1* in the PFC or VTA of males only increased impulsive behavior during the DDT in males but not in females. No effects of treatment nor sex were observed following selective deletion of Nf1 in the PFC or VTA on distance travelled in a novel open field. We also found that injection of Cre virus into the VTA of *Nf1*$^{flox/flox}$ female, but not male mice increased risk-taking and impulsive behavior in the CAR test. These data suggest that selective deletion of *Nf1* has region- and sex-specific effects on hyperactivity and impulsivity. Furthermore, our data show that the NAc plays an integral role in modulating the observed deficits in behavioral inhibition of Nf1 animals. Overall, these studies will help elucidate underlying molecular and neural mechanisms driving impulsivity.

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Poster

356. Neurodevelopmental Disorders

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Program #/Poster #: 356.12

Topic: A.07. Developmental Disorders

Support: NIH NICHD R15HD087937
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Title: Orthographic depth may influence the degree of severity of maze learning performance in children at risk for reading disorder

Authors: *L. A. GABEL[1], A. BATTISON[4], D. T. TRUONG[7], E. R. LINDSTROM[8], K. VOSS[2], Y.-C. YU[3], K. SHYNTASSOV[3], S. RIEBESELL[2], N. TOUMANIOS[2], C. NIELSEN-PHEIFFER[8], S. PANIAGUA[5], J. R. GRUEN[6];
[1]Dept. of Psychology & Program in Neurosci., [2]Program in Neurosci., [3]Dept. of Electrical & Computer Engin., Lafayette Col., Easton, PA; [4]Dept. of Chem., [5]Dept. of Genet., [6]Dept. of Pediatrics & Genet., Yale Univ., New Haven, CT; [7]Dept. of Pediatrics, Yale Sch. of Med., New Haven, CT; [8]Dept. of Educ. and Human Services, Lehigh Univ., Bethlehem, PA

Abstract: Reading disabilities (RDs), which affect between 5-17% of the population worldwide, are the most prevalent form of learning disabilities and are associated with underactivation of a universal reading network in children. However, recent research suggests there are differences in learning rates on cognitive predictors of reading performance, as well as differences in activation patterns within the reading neural network, based on orthographic depth in children with RD. Recently, we showed that native-English-speaking children with RD exhibit impaired performance on a maze learning task that taps into the same neural networks that are activated during the reading. In addition, we demonstrated that genetic risk for RD strengthens the relationship between reading impairment and maze learning performance. However, it is unclear whether the results from these studies can be broadly applied to children from other language orthographies. In this study we examined whether low reading skill was associated with poor maze learning performance in native English-speaking and native German-speaking children, and the influence of genetic risk for RD on cognition and behavior. In addition, we investigated the link between genetic risk and performance on this task in an orthographically diverse sample of children attending an English-speaking international school in Germany. The results from our data suggest that children with low reading skill, or with a genetic risk for reading impairment, exhibit impaired performance on the maze learning task, regardless of orthographic depth. However, these data also suggest that orthographic depth influences the degree of impairment on this task. The maze learning task taps into various cognitive processes and neural networks that underlie reading, but is not influenced by potential differences in reading experience due to lack of text or oral reporting. As a fully automated tool, it does not require specialized training to administer, and current results suggest it may be a practicable screening tool for early identification of reading impairment across orthographies.

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Poster

356. Neurodevelopmental Disorders

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Program #/Poster #: 356.13

Topic: A.07. Developmental Disorders

Support: 5R01MH114888-04

Title: Elucidating the Roles of Brain Endothelial Cells in Early Onset Schizophrenia

Authors: *I. STANKOVIC, M. NOTARAS, P. WOLUJEWICZ, D. COLAK;
Cornell Univ. / Weill Cornell Medic Neurosci. Program, New York, NY

Abstract: Schizophrenia (Scz) is a heterogeneous neurodevelopmental disorder characterized by the manifestation of psychiatric symptoms in early adulthood. While many research avenues have explored the origins of Scz during brain development, the contribution of endothelial/vascular dysfunction to the disease remains largely elusive. To model the neuropathology of Scz and map cell-specific signatures during early critical periods of brain development, we utilized patient-derived induced pluripotent stem cells (iPSCs) to generate 3D cerebral organoids. Single-cell sequencing revealed that while Scz organoids were similar in their macromolecular diversity to organoids generated from healthy controls (Ctrl), they showcased a higher percentage of endothelial cells. Differential gene expression analysis between endothelial cells of Scz and Ctrl organoids showed significant enrichment ($p < 0.05$) in genes involved in vessel formation, pericyte formation, and vasculature regulation. As endothelial cells are known to comprise the blood-brain barrier (BBB), this prompted us to explore the role of the BBB dysfunction in Scz. Data from 25 different donors showed that Scz organoids had longer and more abundant CD31+ positive microvascular vessels, as compared to Ctrls organoids. We further characterized the observed enrichment in endothelial cells functionally by generating 2D brain microvascular endothelial cells from Ctrl/Scz iPSCs. Functionally, compared to Ctrl cells, Scz brain microvascular endothelial cells showed higher permeability and increased proinflammatory response upon stimulation with TNF-α, a protein heavily implicated in the onset of schizophrenia. Collectively, our work suggests that braain microvascular cells might play an important role in the early onset of Scz, by affecting the developing BBB's permeability and proinflammatory response.

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Poster

356. Neurodevelopmental Disorders

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Topic: A.07. Developmental Disorders

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2-7

Title: Time perception deficits in individuals with comorbidity of attention-deficit/hyperactivity disorder and autism spectrum disorder

Authors: *Y. EGASHIRA[1], S. HAYASHI[1], S. UONO[1], M. TAKADA[1,2], M. UKEZONO[1], T. OKADA[1];
[1]Dept. of Developmental Disorders, Natl. Inst. of Mental Health, Natl. Ctr. of Neurol. and Psychiatry, Kodaira, Japan; [2]Grad. Sch. of Med. and Pharmaceut. Sci., Chiba Univ., Chiba, Japan

Abstract: Both individuals with attention-deficit/hyperactivity disorder (ADHD) and autism spectrum disorder (ASD) which frequently coexist have deficits in time perception, yet demonstrate different patterns. While individuals with ADHD have low accuracy of time reproduction duration and a high threshold of duration discrimination, individuals with ASD exhibit low accuracy of time reproduction duration as well, but a low threshold of duration discrimination. However, characteristics of the deficits in time perception for those individuals with comorbidity of ADHD and ASD are still unclear. Thus, we investigated the deficits in these individuals by using multiple time perception tasks which required different cognitive neural basis.Twenty-six individuals with ADHD (ADHD, age 21-51, 8 males), 23 comorbidities of ADHD and ASD (COM, age 18-51, 10 males), and 74 neurotypical individuals (NT, age 16-55, 18 males) participated. The following 3 tasks were performed: the time reproduction (TR), the duration discrimination (DD), and the tapping. In the TR, the participants sustained a button pressing for the same duration as the preceding stimulus (5 seconds). In the DD, the participants detected a target tone among three tones, consisting of two pure tones(1200ms) as standard and one target tone (400, 700, 800, 900, 1000, or 1100ms). In the tapping, the participants pressed the button to 50ms tones with 450ms intervals for 15 seconds, after that, kept the button pressing for 15 seconds without tones.In the TR, the mean reproduction durations were as follows; ADHD: 4751(SD: 310) ms, COM: 4661(SD: 392) ms, NT: 4773(SD: 234) ms. The mean reproduction duration of COM was significantly shorter than that of NT. The SD of reproduction durations was as follows; ADHD: 379(SD: 245) ms, COM: 455(SD: 302) ms, NT: 279(SD: 174) ms. The SDs of reproduction durations of ADHD and COM were significantly larger than that of NT. The discrimination rate of the DD and the button pressing intervals, or SD of the tapping had no significant differences between groups.Each task relates to different brain activities - the supplementary motor area (SMA) and the prefrontal area for TR; the cerebellar-basal ganglia network for DD; the cerebellum and SMA for the tapping. The shorter mean reproduction duration of the COM suggests that they have difficulty recognizing the reference duration. These results showed that time perception deficits of the comorbid group may be more severe than those of ADHD. In addition, both ADHD and COM had difficulty with time perception that required SMA and prefrontal cortex activity.

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Poster

356. Neurodevelopmental Disorders

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 356.15

Topic: A.07. Developmental Disorders

Title: Novel computationally designed peptide negative allosteric modulator of mGluR5 regulates impulsivity in delay discounting paradigm in rats indicating its potential for correction of ADHD

Authors: *A. MALYSHEV, N. MITKIN, A. ZLOBIN, I. SUKHANOVA, V. GEDZUN, I. DORONIN, G. BABKIN, V. PAVSHINTSEV;
Lactocore, Inc., Newton, MA

Abstract: Background. The group I metabotropic glutamate receptor 5 (mGluR5) are implicated in the pathology of various mental disorders, including attention deficit hyperactivity disorder (ADHD) and addiction. Inhibition of mGluR5 activity with pharmacological antagonists results in reduced impulsive behavior, which is considered as a beneficial strategy for correcting both ADHD and addiction. Our aim was to design a novel mGluR5 negative allosteric modulator of peptide nature and to test its ability to regulate impulsivity in choice behavior of rats in the delay discounting task.
Methods. We designed a list of peptides with high calculated affinity for the binding site of negative allosteric modulators of the mGluR5 receptor by applying the proprietary Reptide algorithm (https://doi.org/10.3389/fnins.2021.705590). The peptides were tested in vitro to decrease the activity of mGluR5 using calcium-flux imaging in CHO cell line expressing human mGluR5. We measured [Ca^{2+}] responses of CHO-mGluR5 cells to 1 mM sodium glutamate (GluNa) in the presence of designed peptides or control MPEP antagonist in different concentrations. The best candidate was then tested in vivo in the delay discounting paradigm. LCGM-10 peptide was administered for seven days intranasally in a dose of 1 and 10 mg/kg to Wistar rats, the testing was performed the next day after the last treatment.
Results. Of all the peptides tested, LCGM-10 pretreatment resulted in abolished GluNa-induced activation comparable to the effect of MPEP in CHO-mGluR5 cells. In the delay-discounting test we observed a significant increase in the proportion of choices of the Large/Delayed lever after chronic 10 mg/kg LCGM-10 administration. Rats treated with the peptide preferred large but delayed reward, which suggests decreased reward choice impulsivity.
Conclusion. Reduction of the GluNa-induced cytoplasmic [Ca^{2+}] levels in vitro by LCGM-10 is typical for mGluR5 receptor antagonists. LCGM-10 decreased reward choice impulsivity after chronic administration, which indicates its potential for the treatment of ADHD and addiction.

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Poster

356. Neurodevelopmental Disorders

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Topic: A.07. Developmental Disorders

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Title: Comparison of global and local brain dynamics between pure ADHD and autistic ADHD

Authors: *T. WATANABE;
WPI-IRCN, Univ. of Tokyo, Tokyo, Japan

Abstract: Autism spectrum disorder (ASD) and attention-deficit/hyperactivity disorder (ADHD) show seemingly opposite symptoms and were once considered mutually exclusive. Clinically, however, they are known to often co-occur in the same individuals, and the current diagnosis system—DSM-5—allows such co-existence of the two prevalent neurodevelopmental conditions. Despite such changes in clinical classification, neurobiological mechanisms that enable such apparently contradictory conditions to co-exist in the same individuals remain unclear. Here we partly resolve this conundrum by demonstrating that ADHD co-occurring with ASD children is neurobiologically different from pure ADHD. First, by applying energy landscape analysis to resting-state function MRI data, we compared global brain state dynamics between pure ADHD (N = 30), ASD+ADHD (N = 33), pure ASD (N = 30) and typically developing (TD, N = 67) children. The data-driven analysis identified multiple state transition pathways that were seen in pure ADHD but not in ASD+ADHD, ASD or TD cohort. In fact, an atypical increase in such pure-ADHD-specific state transition frequency was significantly correlated with the severity of ADHD symptoms. In contrast, one state transition pathway was highly enhanced in the ASD+ADHD children but rarely seen in ASD and TD groups, and such enhancement seen in the ASD+ADHD children was correlated with less cognitive rigidity. Moreover, through investigation of intrinsic neural timescale, we revealed that such atypically flexible brain state dynamics in the pure ADHD and ASD+ADHD groups are attributable to the atypically fast neural fluctuation of different brain areas. The neural flexibility in the pure ADHD children was closely correlated with a shorter neural timescale in the left inferior parietal sulcus, whereas that in the ASD+ADHD children was linked to an overly short neural timescale in the left prefrontal pole. These results demonstrate that ADHD-like behaviours often seen in ASD children are driven by different neural mechanisms from those underpinning pure ADHD symptoms. These findings are expected to accelerate our biological understanding of relationships between multiple neuropsychiatric conditions.

Disclosures: T. Watanabe: None.

Poster

357. Animal Models of Autism: Developmental

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 357.01

Topic: A.07. Developmental Disorders

Support: Institute of Brain and Cognitive Sciences, University of Connecticut, Storrs CT 06269, USA

Title: The role of FOXP1 in cerebellar development and function

Authors: *R. CHASSE[1], R. FITCH[1], J. LI[2];
[1]Univ. of Connecticut, Univ. of Connecticut, Storrs, CT; [2]Genet. and Genome Sci., UConn Hlth., Farmington, CT

Abstract: FOXP1 has been implicated in disorders associated with features of autism (ASD), including Foxp1 Syndrome. Gene expression has been recently identified in the cerebellum, a region known to express FOXP2 gene proteins (although not FOXP1). Unpublished work from our lab has shown that absence of FOXP1 during cerebellar development in mice leads to a decrease in vermis size and an increased size of hemispheres, whereas absence of FOXP2 leads to an increase in vermis size and a decrease in hemisphere size. Although FOXP2's role in cerebellar development and function has been well characterized, the role of FOXP1 still remains poorly understood. In the current study, we sought to elucidate the behavioral implications of a conditional knockout of FOXP1 genes in the cerebellum, through the use of transgenic mouse models and a behavioral battery testing multiple facets of behavior associated with cerebellar dysfunction, as well as ASD. Mice with this knockout (n=31), along with wildtype littermate controls (n=34), were assessed on a behavioral battery including assessments of motor control in generalized as well as orafacial tasks, motor learning, general coordination, locomotion, anxiety, auditory processing, and vocalization. On motor coordination tasks, KO mice were found to have significantly weaker grip strength as well as a longer latency to escape from a water maze onto a hidden platform. On motor learning tasks, KO mice were able to spend significantly longer on an accelerating rotarod than WT littermates. When analyzing vocalizations during various days of early postnatal development, KO and WT mice showed similar developmental vocalization patterns (peak postnatal (P)7), however KOs showed a significant reduction in the number of peak vocalizations relative to four other timepoints in development. During adulthood, vocalizations were again assessed, revealing that KOs again vocalized less compared to WTs. There were no adult differences observed in orafacial movement, anxiety, nor working and spatial memory. The current study provides key insights to the role of FOXP1 in cerebellar function and associated behaviors, with implications for improved understanding of several neurodevelopmental disabilities. We also found intriguing evidence of sex differences which may inform sex differences in prevalence of communicative disorders. This work was supported by a Science of Learning and Art of Communication (SLAC) training grant from NS; the University of Connecticut Institute of Brain and Behavioral Sciences (IBACS); and the Murine Behavioral Neurogenetics Facility (MBNF).

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Poster

357. Animal Models of Autism: Developmental

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Program #/Poster #: 357.02

Topic: A.07. Developmental Disorders

Support: Autism Speaks
Nancy Lurie Marks Family Foundation
Advancing Women in Science and Medicine

Title: Altered parvalbumin interneuron development and dysfunction in maternal anti-Caspr2 antibody induced ASD

Authors: *C. BAGNALL-MOREAU, B. SPIELMAN, D. CAMPBELL, C. CRUZ, P. HUERTA, L. BRIMBERG;
Inst. of Mol. Med., Feinstein Inst. for Med. Res., Manhasset, NY

Abstract: Autism Spectrum Disorder (ASD) is a heterogenous group of neurodevelopmental disorders that is characterized by impairments in social interactions, communication and the presence of stereotypic behaviors. ASD affects 1 in every 44 children in the U.S. and is four times more prevalent in boys than in girls. Both genetic and environmental factors converge on deficits in the GABAergic system, suggesting that inhibitory interneurons might be particularly susceptible and contribute to ASD pathophysiology. Several studies, including our own, have demonstrated that 10-20 % of mothers of a child with ASD harbor brain-reactive antibodies (IgG). One target of these antibodies is Caspr2, a protein involved in neural development and synaptic transmission, and present in up to 40% of mothers with anti-brain antibodies and an ASD child. We have developed a model in which female mice are immunized with Caspr2 and harbor endogenous polyclonal anti-Caspr2 IgG throughout gestation. Male, but not female offspring, display ASD-like behaviors.Single nucleus (sn) RNA-seq of adult mice exposed in utero to anti-Caspr2 or Control IgG revealed that GABAergic cells are the most affected neuron subtype in the hippocampus, as indicated by the high burden of differentially expressed genes (DEG) in these cells. Gene ontology analysis revealed that DEGs were related to GO categories as brain development and synaptic transmission. Immunohistochemistry revealed changes in subtype of GABAergic parvalbumin interneurons (PV) but not in the total of the GABA interneuron population, suggesting that exposure in utero to anti-Caspr2 IgG affect PV interneurons selectively. Perineuronal nets (PNN) are specialized extracellular matrix components that specifically surround PV interneurons and are implicated in the regulation of their function. The association of PNN with PV cells were visualized using immunohistochemistry and staining with the marker Wisteria Floribunda Agglutinin. While mice exposed in utero to Control IgG showed positive correlation in the hippocampus between PV and PNN expression as measured by intensity, such correlation was lost in mice exposed in utero to anti-Caspr2 IgG. Furthermore, these mice also exhibited a reduction of vGLUT2+ excitatory synaptic puncta density on hippocampal CA1 PV interneurons in accordance with our sn-RNA

seq data. Since alterations to PNNs have been shown to influence PV interneuron activity, and dysregulation of these cells is a proposed mechanism underlying ASD; ongoing studies are focused on the trajectory of PV interneuron development and the effect of exposure in utero to anti-Caspr2 IgG on the intrinsic physiology of PV interneurons.

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Poster

357. Animal Models of Autism: Developmental

Location: SDCC Halls B-H

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Program #/Poster #: 357.03

Topic: A.07. Developmental Disorders

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MOST 108-2320-B-002-006-MY3
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MOST 103-2314-B-002-055-MY3
MOST 103-2321-B-002-021

Title: The hippocampal phenotype of Dlgap2 mutant mice, a genetic model of autism spectrum disorder

Authors: M.-Y. HSIEH[1], H.-C. CHANG[1], L.-H. TUAN[2], Y.-C. WANG[3], C.-H. CHEN[4], ***L.-J. LEE**[5], S.-F. GAU[6];
[1]Natl. Taiwan Univ., Taipei, Taiwan; [2]Natl. Tsing Hua Univ., Hsinchu, Taiwan; [3]Chi-Mei Med. Ctr., Tainan, Taiwan; [4]Chang Gung Mem. Hospital-Linkou, Taoyuan, Taiwan; [5]Natl. Taiwan Univ. Col. of Med., Taipei, Taiwan; [6]Natl. Taiwan Univ. Hosp., Taipei, Taiwan

Abstract: A microdeletion of approximately 2.4 Mb at the 8p23 terminal region has been identified in a Taiwanese autistic boy. Among the products transcribed/translated from genes mapped in this region, the reduction of DLGAP2, a postsynaptic scaffold protein, might be involved in the pathogenesis of ASD. This study used behavioral, biochemical, and morphological approaches to characterize the hippocampal function-related phenotype in *Dlgap2* mutant mice. Homozygous *Dlgap2* knockout (*Dlgap2* KO) mice exhibited impaired spatial memory in the Morris water maze test, indicating a poor hippocampal function in the absence of DLGAP2. Aberrant expressions of postsynaptic proteins, including PSD95, SHANK3, HOMER1, GluN2A, GluR2, mGluR1, mGluR5, βCAMKII, ERK1/2, ARC, BDNF, were noticed in *Dlgap2* mutant mice. Further, the spine density in middle dendritic segments was increased in *Dlgap2* KO mice, while the ratio of mushroom-type spines was decreased. We also observed a thinner postsynaptic density thickness in *Dlgap2* KO mice at the ultrastructural level. We demonstrated aberrant synaptic protein expression, altered dendritic spines, and reduced postsynaptic density in the hippocampus of *Dlgap2* KO mice, which might be linked to impaired

hippocampus-related cognitive functions such as spatial memory. Mice with *Dlgap2* deficiency, showing signs of intellectual disability, a common symptom of ASD, could be a promising animal model which may advance our understanding of ASD.

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Poster

357. Animal Models of Autism: Developmental

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 357.04

Topic: A.07. Developmental Disorders

Support: NIH Grant R01MH113926
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Title: Heterogeneous Tbr1 mutations differentially impact cortical layer formation, neuronal apoptosis, and gene expression in mice

Authors: *M. CO[1,3], R. A. BARNARD[1], J. N. JAHNCKE[3], S. GRINDSTAFF[1], L. M. FEDOROV[2], A. C. ADEY[1], K. M. WRIGHT[3], B. J. O'ROAK[1];
[1]Mol. and Med. Genet., [2]Transgenic Mouse Models Core, Oregon Hlth. & Sci. Univ., Portland, OR; [3]Vollum Inst., Portland, OR

Abstract: T-Box Brain Transcription Factor 1 (TBR1) plays essential roles in brain development, including neuronal migration, fate specification, and axon tract formation. While heterozygous loss-of-function and missense *TBR1* mutations are strongly associated with neurodevelopmental disorders, the effects of these heterogeneous mutations on brain development have yet to be fully explored. We generated mouse lines carrying *Tbr1* mutations differing by type (e.g., frameshift, missense) and genomic location, and we characterized male and female wild-type (WT), heterozygous (Het), and homozygous (Hom) mutants in parallel with the published *Tbr1* knockout (KO). Mice with the frameshift patient mutation A136PfsX80 (A136fs) showed reduced TBR1 protein in postnatal day 0 (P0) cortex by Western blot (Het mean 69.8% SD 0.2, Hom 0.0%±0.0 of WT levels), similar to TBR1 levels in KO (Het 75.2%±0.1, Hom 0.0%±0.0). In contrast, mice with the missense patient mutation K228E showed increased TBR1 (Het 227.3%±0.4, Hom 513.7%±0.8). When we performed cortical layer marker immunostaining, homozygotes of the KO and A136fs lines showed inversion of CUX1+ and CTIP2+ layer positions, while K228E homozygotes had multiple alternating CUX1+ and CTIP2+ layers. When we examined cortical apoptosis, KO homozygotes showed more cell death at P0 (465±213 cleaved caspase-3+ cells/mm^2) than A136fs (55±19 cells/mm^2) or K228E (24±6 cells/mm^2) homozygotes. Despite these discordant cortical phenotypes, *Tbr1* KO, A136fs, and K228E mutations each produced similar axon defects in P0 brain, including

anterior commissure reduction also observed in humans with *TBR1* mutations. To further resolve genotype-phenotype relationships in these *Tbr1* lines, we conducted RNA-seq in embryonic day 16.5 cortex. In our preliminary analysis, KO mice had more differentially expressed genes (DEGs) over WT (Hom 2590, Het 89, adjP<0.05) than A136fs mutants (Hom 1093, Het 15) or K228E mutants (Hom 1288, Het 13). Homozygote DEGs shared across lines included known TBR1 targets, including *Reln*, *Wnt7b*, and *Rorb*. In our gene ontology analysis of homozygotes, DEGs unique to KO mediate mitotic cell cycle, A136fs DEGs mediate extracellular matrix organization, and K228E DEGs mediate neuronal projection development. Finally, heterozygote DEGs found in at least 2/3 lines play roles in metalloprotease activity (*Adamts3*, *Tll2*), extracellular matrix (*Cxcl12*, *Col23a1*), and acetylcholine receptor activity (*Lypd6*). Future studies utilizing these *Tbr1* mutant mouse lines will decode transcription factor networks controlling cortical development and enhance our understanding of neurodevelopmental disorder etiology.

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Poster

357. Animal Models of Autism: Developmental

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Topic: A.07. Developmental Disorders

Support: Conacyt Grant 738774

Title: Sex differences in TH+ cells in Ventral tegmental area and Substantia nigra of young VPA autism-like mice model

Authors: *D. ZARATE-LOPEZ[1,4], R. GONZALEZ CASTAÑEDA[4], S. LUQUIN[4], N. IBARRA CASTAÑEDA[2,3], O. GONZALEZ-PEREZ[2], A. GALVEZ-CONTRERAS[4];
[1]Physiol. Sci. PhD program, Sch. of Med., Univ. of Colima, Colima, Mexico; [2]Neurosci. Lab., [3]Med. Sci. MD program, Sch. of Med., Univ. of Colima, Col, Mexico; [4]CUCS-Department of Neurosci., Univ. of Guadalajara, Jal, Mexico

Abstract: Background. Autism spectrum disorder (ASD) is characterized by deficits in social interaction and communication, and repetitive and restricted behaviors with broad clinical manifestations. Sex dimorphism in nonreproductive brain areas could explain the functional differences observed in autism. Midbrain dopaminergic circuits (mesolimbic and nigrostriatal) modulate social behavior and motor control, and these areas exhibit differences between sexes at early postnatal development. Dopamine disturbances, social deficits, and repetitive behavior patterns are consistent findings in ASD patients. These abnormal patterns may be due to changes in dopamine synthesis in the ventral tegmental area (VTA) and *substantia nigra* (SN). **Objective**. This work aimed to analyze the number of tyrosine hydroxylase (TH) positive cells in

VTA and SN and study autism-like behaviors with the prenatal valproic acid (VPA) autism-like model in male and female mice. **Methods**. CD1 pregnant female mice were injected on gestational day 12.5 with 500 mg/kg intraperitoneal VPA or 0.9% NaCl. Litters were assigned in two groups: VPA and control group and divided by sex. Social and repetitive behaviors were evaluated with the three-box social chamber and the marble buried test, respectively. Motor activity was assessed with the open field test. The number of TH+ cells was counted in the VTA and SN on 31-day-old mice. Student *t*-test was applied for comparisons between groups. **Results**. Male VPA mice exhibited a social impairment (t=2.30 p=0.02) and increased repetitive behaviors (t=2.04 p=0.04) as compared to the male control group. Otherwise, female VPA mice displayed similar socialization and repetitive behavior levels compared to the female control group. All groups showed similar motor activitylevels in the open field test. We found a fewer number of TH+ cells in the male VPA group as compared to the control group (t=2.50 p=0.03). We did not find statistically significant differences in the number of TH+ cells in the VTA of males and females. **Conclusion**. This study showed that the VPA autism-like model produces differential effects in social and repetitive behaviors and in the number of TH+ cells in the SN between males and females.

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Poster

357. Animal Models of Autism: Developmental

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Topic: A.07. Developmental Disorders

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Title: Unveiling cell-specific transcriptional dysregulation in adult Chd8 haploinsufficient mice

Authors: *S. A. LOZANO[1], C. P. CANALES[1], K. CICHEWICZ[1], E. SMITH[1], N. SEBAN[1], R. ORTIZ[1], J. ZHU[1], M. COREA[1], D. RAHBARIAN[1], A. CIERNIA[2], A. S. NORD[1];
[1]Univ. of California Davis Ctr. for Neurosci., Davis, CA; [2]Biochem. and Mol. Biol., Univ. of British Columbia, Vancouver, BC, Canada

Abstract: De novo mutations in a transcriptional regulator, chromodomain helicase DNA-binding protein 8 (CHD8), are thought to play a causative role in neurodevelopmental disorders (NDDs), including autism spectrum disorder (ASD) and intellectual disability (ID). Mice with a heterozygous germline loss-of-function mutation to CHD8 exhibit genomic, neuroanatomical, and behavioral pathology that aligns with clinical features of ASD and ID. Bulk RNA-sequencing on adult CHD8 mutants revealed a consistent set of genes with altered expression across cortex, hippocampus, and cerebellum, in addition to region-specific differences. Through

gene ontology (GO) enrichment analysis, we identified pathways that are dysregulated in adult brain and ultimately confirmed perturbation to synaptic function, as well as metabolic and neuroinflammatory pathways. Immune dysregulation is prevalent in human ASD patients, but immune function in CHD8 haploinsufficient mice has yet to be fully characterized. Because our bulk RNA-sequencing presented strong signatures that may link CHD8 dosage to neuroimmune function, we isolated microglia from adult CHD8 mutant cortex and performed bulk RNA-sequencing. Preliminary findings include signatures that are consistent with broad changes seen across brain regions, further analysis of these data is in progress. In order to detect additional cell-specific changes in gene expression and impart single-cell resolution on our analyses of CHD8 function, we performed single-nucleus RNA-seq on adult cortex from CHD8 mutants and wild-type mice. We found both distinct and overlapping cohorts of differentially-expressed genes (DEGs) in excitatory and inhibitory neurons, as well as signatures in oligodendrocytes and oligodendrocyte precursor cells. Validation of DEGs is ongoing, and future work will focus on mouse models with temporally-induced, cell-specific CHD8 ablation. Transcriptionally profiling these mice will illuminate cellular processes that may be disrupted in neuronal and immune cell types, and test whether effects of CHD8 haploinsufficiency are intrinsic to a given cell type or dependent on intercellular interactions. We will also continue our bulk analysis on microglia to further investigate immune-related pathways that may be tied to CHD8 dosage. Taken together, our results offer insight into cell-specific molecular phenotypes of CHD8 haploinsufficiency in the adult cortex and may point us to distinct mechanisms of ASD and NDD pathogenesis that began at earlier developmental timepoints.

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Poster

357. Animal Models of Autism: Developmental

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Title: Early developmental brain trajectories: structural volumes and white matter tracts in infant macaques from 2-26 weeks (Macaca Mulatta)

Authors: *R. VLASOVA[1], Z. A. KOVACS-BALINT[2], C. TOMLINSON[1], L. LI[3], J. BACHEVALIER[4], M. STYNER[1], M. SANCHEZ[5];

[1]Univ. of North Carolina at Chapel Hill, Chapel Hill, NC; [2]Yerkes Natl. Primate Res. Ctr., [3]Dept. of Pediatrics, Marcus Autism Ctr., [4]Emory Natl. Primate Res. Center, Dept. of Psychology, [5]Emory Natl. Primate Res. Center, Dept. of Psychiatry & Behavioral Sci., Emory Univ., Atlanta, GA

Abstract: While rhesus monkeys widely serve as a model for preclinical studies of psychiatric and neurodevelopmental disorders, much is still unknown about their neural developmental trajectories during early infancy. To fill in this gap we analyzed the structural development of cortical/subcortical regions and main white matter tracts using longitudinal DWI scans and volumetric structural MRI (sMRI) data from 31 macaques (only males) scanned at 2, 4, 6, 12, 16, 20, and 24 weeks of age (equivalent to 2-24 months in humans), and all living in complex social groups. All DWI scans underwent automated and visual quality check and were processed using UNC-Utah NA-MIC framework for DTI. Twelve white matter tracts were analyzed: cingulum, cingulum (hippocampal part), uncinate, inferior fronto-occipital, and inferior longitudinal tracts in the left and right hemispheres, as well as the corpus callosum (genu, mid-body, splenium). We modeled the longitudinal relationship between DTI fractional anisotropy (FA) in the tracts with age in days using continuous, piecewise linear models. One-knot models [$FA_{perTract}=\beta0+\beta1(age)+\beta2(age-knote)*I(age \geq knote)$] exhibited statistically significant age effects for all analyzed white matter tracts ($p<0.0001$). For all tracts, knots were located between 35 and 50 days of age; FA had a rapid increase between the first scan (at 2 weeks) and the detected knot timepoint (average slope=0.0009) and slowed down after the knot with an average slope=0.0002. Developmental changes of cortical and subcortical volumetric brain changes were also modeled using the same method, but we are still analyzing the linear growth models. Our current DTI findings suggest that the most drastic changes in tracts' myelination happen during the first 1-1.5 months after birth in rhesus monkeys, potentially the most sensitive period to environmental factors and experiences for white matter development. This period of fast structural connectivity changes takes places in parallel to important socioemotional maturation in infants and likely supports the infant's preparation and adjustment to exploration, social play and independence during the weaning period.

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Poster

357. Animal Models of Autism: Developmental

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Program #/Poster #: 357.08

Topic: A.07. Developmental Disorders

Support: Other

Title: A polygenic mouse model to study sex specific striatal phenotypes of neurodevelopmental disorders

Authors: *J. KIM[1,2], Y. VANROBAEYS[1,2], Z. PETERSON[2,3], B. KELVINGTON[1,2], M. E. GAINE[4], T. NICKL-JOCKSCHAT[2,1,3], T. ABEL[1,2];
[1]Dept. of Neurosci. and Pharmacol., Univ. of Iowa, Iowa City, IA; [2]Iowa Neurosci. Inst., [3]Dept. of Psychiatry, Univ. of Iowa, Iowa city, IA; [4]Dept. of Pharmaceut. Sci. and Exptl. Therapeut., Univ. of Iowa, Iowa City, IA

Abstract: Neurodevelopmental disorders (NDDs) are polygenic in nature and copy number variants (CNVs) enable us to study the polygenic nature of NDDs. The 16p11.2 deletion is one of the most common CNVs associated with NDD and mice with this deletion (16p11.2 del) show sex specific striatum-related phenotypes resembling sex differences in NDDs. However, the molecular mechanisms underlying the sex specific phenotypes of 16p11.2 deletion remain unknown. Previously, we found three candidate genes associated with the sex specific phenotypes of 16p11.2 del mice: MVP, Sez6l2, and TAOK2 (Kumar et al., 2018). Using the CRISPR/Cas9 technique, we introduced gene modifications in the 3 chosen genes (*Taok2*, *Sez6l2*, and *Mvp*), and we generated 3 gene hemi-deletion (3g) mice. We assessed striatum dependent phenotypes of 3g mice through behavioral tasks and RNA-seq analysis, comparing them with 16p11.2 del mice and completing sex specific analyses. Hemi-deletion of the 3 chosen genes induced sex specific behavioral alterations in striatum-dependent behavioral tasks, male specific hyperactivity and impaired motivation for reward seeking, resembling the behavioral phenotypes of 16p11.2 del mice. Moreover, RNAseq analysis revealed that 3g mice exhibit gene expression changes in the striatum similar to 16p11.2 del mice, but only in males. These results support the importance of a polygenic approach to study NDDs, identifying the individual genes within a large CNV that are actually sufficient to cause certain phenotypes.

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Poster

357. Animal Models of Autism: Developmental

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Program #/Poster #: 357.09

Topic: A.07. Developmental Disorders

Support: NSF Grant NeuroNex 1707352

Title: Behavioral and electrophysiological effects of enhancing activity of layer 5 pyramidal neurons during early postnatal development

Authors: *G. W. FOLKERT, A. M. UPRETY, L. L. MCLEAN, E. L. CRESPO, U. HOCHGESCHWENDER;
Col. of Med., Central Michigan Univ., Mount Pleasant, MI

Abstract: In neurodevelopmental disorders, neural activity is altered within the developing neocortical network. We previously showed that enhanced pyramidal firing during development,

in the otherwise normal mouse neocortex, can selectively alter adult circuit function and produce maladaptive changes in behavior.
We systematically enhanced pan-neocortical pyramidal activity levels during the early postnatal development of healthy mice using non-invasive BioLuminescent-OptoGenetic (BL-OG)-mediated activation of luminopsin 3 (LMO3) expressing neurons. Developmental hyperexcitation of Emx1-positive pyramidal neurons during postnatal days 4 - 14 led to decreased social interaction and increased grooming activity in adult animals, both of which are key symptoms of autism spectrum disorder (ASD). *In vivo*, both prefrontal neural activity and functional markers of cortico-striatal connectivity were impaired in developmentally hyperexcited adult Emx1-LMO3-positive mice, and *ex vivo* slice recordings revealed alterations to both intrinsic excitability and synaptic E/I ratio in L5 prefrontal cortex pyramidal neurons. We now want to further dissect the neural populations and their specific target areas mediating the observed behavioral and electrophysiological changes. Pyramidal neurons are distributed across all layers of the cerebral cortex with the exception of layer 1, and those in each layer are distinguished by their patterns of long-range axonal projections. Those in layer 5 integrate information between cortical areas but also project to subcortical structures involved in the generation of behavior. We carried out developmental hyperexcitation in Rbp4-LMO3 mice, thus restricting LMO3 expression to L5 projection neurons. The behavioral and electrophysiological consequences were compared to those of pan-laminar neocortical developmental hyperexcitation.

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Poster

357. Animal Models of Autism: Developmental

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Topic: A.07. Developmental Disorders

Support: National Institutes of Health National Institute of General Medical Sciences Grant SC2 GM130485

Title: Deformity and mortality in zebrafish larvae exposed to valproic acid

Authors: *S. MANKATALA[1], C. SCARAMELLA[2], J. B. ALZAGATITI[3], F. LICEA[1], C. CREIGHTON[1], L. SALAZAR[1], K. RUNNBERG[1], D. HERNANDEZ[1], G. M. WINTER[1], F. M. LEE[4], J. EMTAGE[5], D. L. GLANZMAN[4], A. C. ROBERTS[1];
[1]California State University, Fullerton, Fullerton, CA; [2]Univ. of California, Riverside, Riverside, CA; [3]Univ. of California, Santa Barbara, Santa Barbara, CA; [4]UCLA, Los Angeles, CA; [5]Caltech, Pasadena, CA

Abstract: Neurodevelopmental disorders, such as autism spectrum disorder (ASD), likely arise from atypical neural connectivity. This change in connectivity has a strong genetic basis and has

been linked to *in utero* exposure to compounds such as valproic acid (VPA) and thalidomide. Supporting the link between VPA exposure and ASD, animal models exposed to VPA exhibit social deficits and increased repetitive behaviors. Zebrafish can effectively model neurodevelopmental disorders due to their *ex utero* development, precision control of pharmacological agents, and translucency early in development. When combined with the multitude of molecular tools available in zebrafish, these biological attributes have the potential to provide insights into the molecular and physiological basis of human disorders and diseases. Despite progress in developing the zebrafish model to understand VPA exposure, there is very little agreement in the literature regarding the dose of VPA that elicits mortality and deformity. To provide further insights into the dose of VPA that larvae can tolerate, we determined the highest dosage of VPA that fails to elicit increased mortality and deformity. Consistent with studies demonstrating that higher doses of VPA are teratogenic, we found exposure to VPA (0-5 days post fertilization) at levels ≥ 45 µM significantly increased deformity. No significant increase in mortality was observed up to 75 µM VPA.

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Poster

357. Animal Models of Autism: Developmental

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Program #/Poster #: 357.11

Topic: A.07. Developmental Disorders

Support: KAKENHI 19K08065
KAKENHI 22K07611

Title: Expression of IL-17RA in the cerebral cortex of mice during postnatal development and its alteration by maternal immune activation

Authors: *T. SASAKI[1], Y. TAKEI[2];
[1]Univ. of Tsukuba, Tsukuba-Shi, Japan; [2]Univ. of Tsukuba, Tsukuba, Japan

Abstract: Clinical studies have suggested that immune responses by helper T cells 17 (Th17 cells) are involved in the pathogenesis of autism spectrum disorders (ASD), schizophrenia, and depression. However, it remains unclear how the immune response induces organic changes in the nervous system, and the contribution of Th17 cells is not well understood. Interleukin (IL-)17A is a pro-inflammatory cytokine that binds to a receptor composed of a heterocomplex of IL-17RA and IL-17RC and activates downstream pathways. Previous studies have reported that *Il17ra* is mainly expressed in the cortical plate of mouse embryonic brain at 14.5 days of embryonic period and that IL-17A increased in the placenta by maternal poly(I:C) administration

upregulates *Il17ra* mRNA expression. The expression of IL-17A and its receptors in the adult central nervous system has been reported by several research groups but continues to be controversial. In this study, we investigated the expression of *Il17ra* mRNA in the primary somatosensory cortex during postnatal development from neonatal to young adult by *in situ* hybridization. *Il17ra* mRNA was strongly expressed in layer VI (both VIa and Vb) throughout the period examined. The expression was maximal at 14 days of age and decreased through adulthood. Double staining with cellular markers showed that it was expressed in about 60% of layer VI neurons. In addition, a decrease in *Il17ra* mRNA expression was observed at 14 days of age after maternal poly(I:C) administration. This study may provide the first clue to the function of IL-17A and IL17RA in central nervous system during the postnatal period.

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Poster

357. Animal Models of Autism: Developmental

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Program #/Poster #: 357.12

Topic: A.07. Developmental Disorders

Title: Developmental and behavioral effects of valproic acid in mouse models of autism

Authors: *C. E. CREELEY, A. GONZALEZ, C. MAUCHE, J. WALTERS, L. DIXON, M. HARDY, M. EVANS;
Psychology, State Univ. of New York at Fredonia, Fredonia, NY

Abstract: Sodium valproate (Depakote®), an anticonvulsant prescribed for seizures and as an add-on medication for the treatment of bipolar disorder, has been identified as a potent teratogen that causes fetal valproate syndrome, characterized by physical malformations, developmental delays, and an increased autism risk in children exposed in utero. In animal research, early valproic acid (VPA) exposure is widely used and considered to be a robust model for environmentally-induced autism. Previous research has determined the underlying neurobiological mechanisms of VPA exposure to the developing brain include apoptotic cell death, reduced proliferation, and disruption in the development of glutamatergic and serotonergic systems, which depend on timing, dose, and duration of exposure. The objective of this research was to investigate the differential effects of two mouse models of VPA-induced autism. In the first experiment, neonatal mice were randomly assigned to treatment groups and injected with saline vehicle (n=9), 200 (n=8), or 400 mg (n=6) of VPA on postnatal days 4 through 7 (P4-7). In the second experiment, mouse pups were randomly assigned to be injected with saline (n=12), or a single dose of 200 (n=12) or 400 mg (N=16) of VPA on P14. Mouse pups were injected with VPA and then subjected to a comprehensive battery of short- and long-term physiological and behavioral testing. The testing included outcome measures of sensorimotor activity, exploration, balance/coordination, social behaviors, and learning and memory. It was predicted that both types of VPA exposure would cause significant deficits in growth and development.

Statistical results showed that only high-dose VPA exposure on P4-7 caused significant mortality, reduced body weight, changes in social behaviors, and minor cognitive deficits. The single P14 VPA exposure, however, contrary to our hypothesis, did not cause any adverse effects for any of the outcome measures. These results showing some adverse effects for early, but none for the later VPA exposure, even with a high dose are surprising, as they are not consistent with previous research that has shown the animal VPA model to be a reliable and valid model for producing VPA-induced autism-like behavioral and cognitive effects. The VPA model aims to connect neuropathology in the brain to specific adverse neurobehavioral outcomes, which we did not find a robust effect for here. However, measurement of behaviors in the VPA animal model varies between laboratories, revealing the importance of replicating methods for behavioral assessment in this animal in order to create a standard of evaluation for this highly complex human spectrum disorder.

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Poster

357. Animal Models of Autism: Developmental

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Program #/Poster #: 357.13

Topic: A.07. Developmental Disorders

Title: Prenatal exposure to valproate modifies differentially the c-Fos expression in the cerebral cortex throughout development

Authors: *C. PADILLA-GÓMEZ[1], N. HERRERA-LOZA[2], A. GARZÓN-PARTIDA[2], Á. GONZÁLEZ-GARCÍA[2], S. LUQUÍN[3], O. GONZÁLEZ-PÉREZ[4], R. GONZÁLEZ-CASTAÑEDA[3], A. GÁLVEZ-CONTRERAS[3];
[1]Univ. de Guadalajara, Guadalajara, Mexico; [3]Dept. of Neurosciences, [2]Univ. of Guadalajara, Guadalajara, Mexico; [4]Univ. of Colima, Colima, Mexico

Abstract: Background. Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder associated with an imbalance in neuronal inhibition/excitation that in turn causes hypo and hyperconnectivity in several brain regions such as somatosensory and parietal cortex, amygdala, corpus callosum, and putamen. C-Fos is a protein commonly used as a molecular biomarker of neuronal activity. In rats with autism-like behavior, it has been reported a decreased expression of C-Fos in the cochlear superior colliculus. However, C-Fos expression in the motor cortex (MC), Cingulate cortex (CG) (anterior & midcingulate), piriform cortex (PC), and striatum (Str) throughout the lifespan has not yet been investigated. **Methods.** CD1 pregnant female mice were injected intraperitoneally on gestational day 12.5 with 500 mg/kg VPA or 0.9% NaCl. Litters were divided into VPA and control groups, and divided by sex, choosing only male mice. Mice (n=5 for each time point) were killed at 31 & 91 postnatal days (P) after deep anesthesia with pentobarbital (30ul) and via intracardiac perfusion with 0.9% saline solution, followed by 4%

paraformaldehyde in 0.1 M Phosphate Buffer (PB). C-Fos expression was measured in MC, CG (anterior & midcingulate), PC, and Str (ventral & dorsal), using immunostaining to determine C-Fos positive cells. Non-parametric Mann-Whitney U test was applied between groups, setting a statistical significance at p= 0.05 in all cases. **Results.** At P31 in VPA subjects, we found lower levels of c-Fos expression in MC (*U*=116; p=0.0001), CG (*U*=234; p=0.0001) and PC (*U*=190.500; p=0.001), but higher in Str (*U*=226, p=0.004), whereas at P91 the PC (*U*=156.00; p=0.0001) and MC (*U*=176.5; p=0.0001) continued with low levels but the CG (U=257; p=0.0001) and Str (U=97.500; p=0.892) does not show statistically significance compared to controls. **Conclusion.** The prenatal exposure to VPA modifies the c-Fos expression differentially in the cerebral cortex during postnatal development.

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Poster

357. Animal Models of Autism: Developmental

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 357.14

Topic: A.07. Developmental Disorders

Support: Office of Undergraduate Research, Weber State University
Research, Scholarship, and Professional Growth Committee, Weber State University
Dept. of Health Sciences
Neuroscience Program

Title: Behavioral differences between pigmented and albino zebrafish treated with valproic acid in an animal model of human autism

Authors: M. R. CARRINGTON[1,2], S. CLARK[1,2], A. STEED[2], *J. B. HUTCHINS[3,2], E. J. SANDQUIST[4,2];
[1]Psychology, [2]Neurosci., [3]Hlth. Sci., [4]Zoology, Weber State Univ., Ogden, UT

Abstract: Autism Spectrum Disorder (ASD) is a complex neurodevelopmental disorder described by behavioral deficits in interpersonal interactions, communication, anxiety, hyperactivity, and narrowed interests. The available evidence suggests that ASD is caused by a complex mixture of genetic, neurodevelopmental, and environmental factors. In humans, maternal exposure to valproic acid (VPA) increases the risk of ASD by about threefold (Christensen et al., 2013). Our lab uses a VPA-treated zebrafish model of human autism as characterized by a number of other groups (e.g., Zimmermann et al., 2015; Meshalkina et al., 2018; Dwivedi et al., 2019). The similarities between human autism and the animal models are likely because they share similar molecular and cell level neurodevelopmental mechanisms such

as synaptic pruning. We are currently studying these changes in the zebrafish retinotectal system, and how they relate to altered behavior. Our lab is interested in aberrations of the retinotectal pathways in VPA-treated zebrafish larvae. Previous work has focused on the use of VPA-treated pigmented zebrafish. Our labeling method for the retinotectal pathway is subject to interference from melanophores. To solve this problem, we can use albinos (i.e. fish without melanophores) but albinos have known aberrations in the wiring of the retinotectal pathway. To ensure that non-pigmented fish have similar behavioral deficits to pigmented fish studied previously, we have expanded our behavioral analyses to include VPA-treated albino zebrafish. Wild-type and albino zebrafish embryos were collected and were treated with 50 μM VPA from 0 to 48 hours post fertilization (hpf). Behavioral tests modified from the Zimmermann et al. and Dwivedi et al. published protocols were then applied to 6 days post-fertilization (dpf), 30 dpf, 70 dpf, and 120 dpf larvae. These tests included measures of social behavior, locomotion, anxiety, and inattentive behavior. We have observed statistically significant differences between pigmented and albino fish at each age in each condition for each of these measures. There is a significant interaction between the pigmentation variable and the VPA treatment variable revealed by ANOVA analysis.

Disclosures: **M.R. Carrington:** None. **S. Clark:** None. **A. Steed:** None. **J.B. Hutchins:** None. **E.J. Sandquist:** None.

Poster

357. Animal Models of Autism: Developmental

Location: SDCC Halls B-H

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Program #/Poster #: 357.15

Topic: A.07. Developmental Disorders

Support: National Institutes of Health National Institute of General Medical Sciences Grant SC2 GM130485

Title: Valproic acid exposure induces hyperlocomotion in zebrafish larvae.

Authors: *G. M. WINTER[1], K. A. RUNNBERG[1], J. WISNIESKI[1], C. SCARAMELLA[2], J. B. ALZAGATITI[3], L. SALAZAR[1], D. HERNANDEZ[1], C. CREIGHTON[1], F. LICEA[1], I. N. MEJIA[1], L. ESCOBEDO[1], J. ROUVERE[1], S. MANKATALA[1], J. EMTAGE[4], F. M. LEE[5], C. GILLES[5], D. L. GLANZMAN[5], A. ROBERTS[1];
[1]California State University, Fullerton, Fullerton, CA; [2]Univ. of California, Riverside, Riverside, CA; [3]Univ. of California, Santa Barbara, Santa Barbara, CA; [4]Caltech, Pasadena, CA; [5]UCLA, Los Angeles, CA

Abstract: Zebrafish larvae are an excellent model system for understanding neurodevelopmental disorders due to key biological attributes. In particular, zebrafish larvae readily absorb small molecules from the bath and are small and translucent, key features that facilitate neural imaging. Exposure to valproic acid (VPA) is associated with autism spectrum disorder (ASD)

development in humans. Consistent with this association, VPA exposure in animal models induces social dysfunction and increased repetitive behaviors. However, conflicting reports indicate that VPA exposure can cause hyperlocomotion or hypolocomotion. These differing results likely stem from the developmental time of exposure and/or concentration of the VPA. We exposed zebrafish larvae (0-5 days post fertilization) to 15 µM VPA to determine if this treatment influenced baseline locomotion. Testing at 6 days post fertilization, we observed significant hyperlocomotion. This result suggests there are likely critical periods by which VPA can affect zebrafish locomotion.

Disclosures: **G.M. Winter:** None. **K.A. Runnberg:** None. **J. Wisnieski:** None. **C. Scaramella:** None. **J.B. Alzagatiti:** None. **L. Salazar:** None. **D. Hernandez:** None. **C. Creighton:** None. **F. Licea:** None. **I.N. Mejia:** None. **L. Escobedo:** None. **J. Rouvere:** None. **S. Mankatala:** None. **J. Emtage:** None. **F.M. Lee:** None. **C. Gilles:** None. **D.L. Glanzman:** None. **A. Roberts:** None.

Poster

357. Animal Models of Autism: Developmental

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 357.16

Topic: A.07. Developmental Disorders

Support: Jane and Aatos Erkko Foundation
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Title: Characterization of the behavior and visual system of zebrafish embryonically exposed to valproic acid

Authors: L. DEOLIVEIRA-MELLO, D. BARONIO, *P. A. PANULA;
Univy Helsinki, Univy Helsinki, U Helsinki, Finland

Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by deficits in social impairments, communication deficits and stereotypies. Sleep disturbances are among common ASD comorbidities. Prenatal exposure to valproate (VPA), a drug widely used to treat epilepsy and bipolar disorder, is an environmental risk factor for ASD and has been used to reproduce the core symptoms of this disorder in animal models. Embryonic exposure to VPA leads to molecular and neurochemical alterations in zebrafish, which persist into adulthood and accompany impaired sociability. It has been hypothesized that the behavioral deficits displayed by ASD patients might be related with impaired visual processing. Visual system functioning is essential in the interpretation of social conditions and plays an important role of various behavior responses, including circadian cycle. Thus, we investigated the visual system of zebrafish embryonically exposed to VPA and assessed behavioral responses do different visual stimuli. We used embryos, larvae and juvenile animals that had been exposed to VPA between 10 and 24

h post-fertilization (hpf). We characterized behavioral and histological phenotype of ASD zebrafish. Visual perception was analyzed by optomotor and color preference assays. 24-hour locomotor activity test was used to study sleep-like behavior. Developmental markers such as neuroligin and Sox2, and cone marker were used to evaluate retinal development by immunohistochemistry. In addition, we also analyzed the brain serotonergic system.
Although retinal development was abnormal during the first 72 hpf, our results suggest that all alterations recovered by 5 days post-fertilization. VPA-exposed and control zebrafish showed a similar behavioral phenotype during optomotor response and color preference tests. However, VPA-exposed larvae showed reduced amount of sleep-like state during the night time, which could be related with impaired light perception. VPA-exposed zebrafish also showed reduced serotonin immunoreactivity in the pineal gland and hypothalamic populations.
Our results indicate that behavioral impairments previously reported in the zebrafish VPA model of ASD, such as reduced sociability, are unlikely to be directly caused by a defective visual system. However, we could not exclude an effect of impaired sensory processing during early life, caused by an abnormal retinal development, on brain wiring. Our findings are also coherent with previous reports of altered sleep of VPA-exposed zebrafish. Finally, differences in the serotonergic system might contribute to these sleep alterations.

Disclosures: L. DeOliveira-Mello: None. **D. Baronio:** None. **P.A. Panula:** None.

Poster

357. Animal Models of Autism: Developmental

Location: SDCC Halls B-H

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Program #/Poster #: 357.17

Topic: A.07. Developmental Disorders

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Title: Early life antibiotic exposure and genetic risk in neurodevelopmental disorders: Effects on neurogenesis, gut microbiome, and behavior

Authors: *C. R. MCDERMOTT[1], A. MIRMAJLESI[2], Z. GAO[3], K. KIMBARK[4], C. NTIM[2], X. ZHANG[3], X. ZHOU[5], J. H. MILLONIG[1], M. J. BLASER[6], E. M. DICICCO-BLOOM[7]; [1]Neurosci. and Cell Biol., Rutgers Univ., Piscataway, NJ; [2]Cell Biol. and Neurosci., Rutgers Univ., New Brunswick, NJ; [3]Ctr. for Advanced Biotech. and Med., Rutgers Univ., Piscataway, NJ; [4]Biol., Lebanon Valley Col., Annville, PA; [5]Neurosci., [6]Ctr. for Advanced Biotech. and Med., [7]Neurosci & Cell Biol/ Pediatrics (Child Neurol. & Neurodevelopmental Disa, Rutgers Robert Wood Johnson Med. Sch., Piscataway, NJ

Abstract: Neurodevelopmental disorders (NDDs) affect the lives of >17% of children in the United States. While genetic factors play a major role in pathogenesis, even identical twins who share the same genetic risk exhibit incomplete concordance, suggesting a plausible role of environmental factors. To determine whether environmental factors act through certain genetic susceptibilities to produce distinct NDD outcomes, we investigate a Gene x Environment (GxE) model of NDDs. A recently identified environmental factor that has been linked to increased NDD outcomes is exposure to cephalosporin antibiotics during the first two years of life, when neurogenesis, the process by which neural precursors proliferate, migrate, and differentiate into specialized cells of the brain, predominates. Extensive research indicates that antibiotic exposure decreases the diversity of gut bacteria, which may consequently alter adult brain structure, function, and behavior. However, little is known about effects on neurogenesis, identified as a point of convergence in NDD pathogenesis based on human genetic, postmortem, and animal model studies. Our studies use a novel GxE model to determine how cephalosporin exposure alters postnatal neurogenesis in the genetically vulnerable 16p11.2 microdeletion (16pDel) mouse. This mouse models one of the most frequently observed CNVs implicated in NDDs, including ~1% of autism diagnoses. To model antibiotic exposure during infancy, wildtype and 16pDel littermates were exposed to saline (control) or the cephalosporin, cefdinir, from P5-9 and sacrificed on P13 and P21, timepoints when postnatal neurogenesis occurs in the hippocampus. Cefdinir exposure resulted in a 50% reduction of hippocampal Cyclin E (mitosis marker) in 16pDel males compared to saline-treated 16pDel males ($p=0.031$), quantified by immunoblotting. A parallel 7% reduction of *in vivo* precursor S-phase entry was detected in a separate cohort of 16pDel males, defined by EdU-labeling ($p=0.042$). To assess consequential outcomes on neurodevelopment, hippocampal lysates from P21 16pDel males were probed with dentate gyrus and CA1-3 specific marker, Calbindin. Surprisingly, we found a 53% increase of hippocampal calbindin in cefdinir-exposed 16pDel males compared to saline-exposed, perhaps compensating for the robust reduction in proliferation at P13 ($p=0.003$). These data suggest that early life cefdinir exposure alters hippocampal neurogenesis in 16pDel mice selectively, providing initial support for our hypothesis. Ongoing work will examine antibiotic effects on microbial community composition, neural gene expression, and behavioral outcomes related to NDDs.

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Poster

357. Animal Models of Autism: Developmental

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 357.18

Title: WITHDRAWN

Poster

357. Animal Models of Autism: Developmental

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 357.19

Topic: A.07. Developmental Disorders

Support: United States of America Department of Defense, U.S. ARMY MEDICAL RESEARCH Activity Award, CONGRESSIONALLY DIRECTED MEDICAL RESEARCH PROGRAM, Tuberous Sclerosis Complex Research Program W81XWH2010447

Title: Inactivating mutations in Tsc2 in inhibitory interneuron neuroprogenitors of the V-SVZ result in somatic mosaicism and the formation of SEGA-like growths and striatal nodules

Authors: *V. A. RILEY, A. M. SOKOLOV, V. N. NECKLES, J. C. HOLMBERG, D. M. FELICIANO;
Clemson Univ., Clemson, SC

Abstract: Tuberous Sclerosis Complex (TSC) is a neurodevelopmental disorder caused by inactivating mutations in the TSC1 or TSC2 genes. Mutations in TSC1 or TSC2 cause hyperactivation of mTOR and subsequent formation of hamartomas within multiple organs including the brain. In TSC patients, subependymal nodules (SENs) and subependymal giant cell astrocytomas (SEGAs) are hamartomas contiguous with the lateral ventricles of the brain. The ventricular-subventricular zone (V-SVZ) is a region along the lateral ventricles that contains late inhibitory interneuron progenitors. These progenitors generate intermediate cells that produce neuroblasts that migrate through the rostral migratory stream (RMS), before becoming mature inhibitory neurons in the olfactory bulb. TSC1/TSC2 mutations in inhibitory interneuron progenitors are hypothesized to cause SEGAs. Mice having conditional Tsc2 genes and inducible RFP were crossed to tamoxifen inducible nestin-CRE-ERT2 mice or subjected to neonatal V-SVZ electroporations with CRE and GFP plasmids. Tsc2 was conditionally removed from neonatal inhibitory interneuron neuroprogenitors which increased mTORC1 pathway activity in the V-SVZ. Loss of Tsc2 generated SEN and SEGA-like growths that protruded into the lateral ventricle. Growths were enriched in Sox2 positive cells with sporadic neuron labeling. SEGA-like lesions were composed of RFP positive but GFP negative cells indicating that lesions arose following DNA plasmid dilution. RFP positive cells had an ambiguous morphology due to their high density within SEGA-like lesions. However, giant cells which are a pathognomonic feature of TSC were identified near the SEGA-like growths including in the striatum. The striatum frequently had two categories of lesions. The first category included heterotopic nodules that consisted of cytomegalic neurons, occasional balloon-like cells, neuroblasts, and Sox2 positive cells. Nodular lesions were accompanied by the second category which included distally located elongated growths that lined the striatal vasculature. Elongated striatal lesions were predominantly comprised of Sox2 positive cells. Importantly, mTORC1 activity was elevated in both types of striatal lesions. Transcriptional and translational profiles of V-SVZ derived cells were examined to gain mechanistic insight into the molecular events responsible for SEGA-like lesion formation. Taken together, the results of this study demonstrate that loss of Tsc2 in V-

SVZ interneuron progenitors causes SEGA-like growths and striatal lesions in mice which may have relevance for the TSC patients with SEGAs that have Tsc2 mutations.

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Poster

358. Autism Mechanisms: Mouse Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 358.01

Topic: A.07. Developmental Disorders

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HU20C0290
K-21-L02-C10
IBS-R002-D1

Title: Developmental changes in ASD-related transcriptomic, synaptic, and behavioral deficits in Myt1l-mutant mice

Authors: S. KIM[1], ***H. OH**[1], S. CHOI[2], Y.-E. YOO[3], Y. NOH[1], Y. CHO[4], G. IM[2], C. LEE[2], Y. OH[1], E. YANG[5], G. KIM[1], W.-S. CHUNG[1], H. KIM[5], H. KANG[6], Y. BAE[4], S.-G. KIM[2], E. KIM[1,3];
[1]Korea Advanced Inst. of Sci. and Technol. (KAIST), Daejeon, Korea, Republic of; [2]Ctr. for Neurosci. Imaging Research, Inst. for Basic Sci. (IBS), Suwon, Korea, Republic of; [3]Ctr. for Synaptic Brain Dysfunctions, Inst. for Basic Sci. (IBS), Daejeon, Korea, Republic of; [4]Dept. of Anat. and Neurobiology, Sch. of Dentistry, Kyungpook Natl. Univ., Daegu, Korea, Republic of; [5]Col. of Medicine, Korea Univ., Seoul, Korea, Republic of; [6]Korea Inst. of Sci. and Technol. Informat, Daejeon, Korea, Republic of

Abstract: Autism spectrum disorders (ASDs) are characterized by social deficits, repetitive behaviors, and various comorbidities, including intellectual disability, anxiety, and hyperactivity. MYT1L, a zinc-finger transcription factor highly expressed in early developmental stages plays key roles in neuronal differentiation and is strongly implicated in ASD. However, it was largely unknown whether and how the deficiency of Myt1l with strong embryonic and perinatal gene expressions yields strong adult-stage ASD-related phenotypes. Here, we generated Myt1l heterozygous (Myt1l-HT) mice, and characterized their transcriptomic, synaptic/neuronal, and behavioral phenotypes across newborn, juvenile, and adult stages. Myt1l-HT mice showed age-differential ASD-like phenotypes. Myt1l haploinsufficiency leads to newborn-stage ASD-like neuronal suppression, temporary juvenile normalization, and subsequent adult-stage ASD-like deficits.

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Poster

358. Autism Mechanisms: Mouse Models

Location: SDCC Halls B-H

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Program #/Poster #: 358.02

Topic: A.07. Developmental Disorders

Support: NIH Grant R01DA020140
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Title: Sex-specific alterations in social behaviors and amygdala proteome in Foxp2 mutant mice

Authors: *L. WANG[1], N. PRAKASH[1], S. SEBAOUI[1], M. HERRERO[1], N. CAMPBELL[1], S. FELSEN[3], A. PANIGRAHI[2], N. SMITH[4], J. CORBIN[1];
[1]Ctr. for Neurosci. Res., [2]Ctr. for Cancer and Immunol. Res., Children's Natl. Hosp., Washington, DC; [3]The George Washington Univ., Washington, DC; [4]Dept. of Neurosci., Univ. of Rochester, Rochester, NY

Abstract: Innate social behaviors in animals such as aggression or mating are critical to their survival and reproductive success. These behaviors are driven by environmental cues that activate genetically hardwired neuronal circuits in the brain. Given the primacy of successful behavioral patterns for evolutionary success, the transcriptional networks controlling circuit formation, maintenance and function must have a high degree of robustness and fidelity. To understand the link between gene function and innate behavioral output, we used a mutant analyses approach in a mouse model. We focused on *Foxp2*, a transcription factor first expressed during embryonic development, which defines multiple neuronal populations involved in the pheromone-driven rodent social behavior circuit. Behavioral analyses of a whole-body heterozygous *Foxp2 knockout* (*Foxp2*$^{+/-}$) mice revealed that male *Foxp2*$^{+/-}$ mice show less territorial aggression than their wildtype counterparts while female mutant mice show increased maternal aggression. Parental care and predator avoidance were impaired only in female *Foxp2*$^{+/-}$ mice while mating and olfactory investigation of social and non-social stimuli were impaired in mutants of both sexes. To investigate molecular deficits in *Foxp2*$^{+/-}$ mice that underlie these behavioral phenotypes, proteomic analysis of the medial amygdala was carried out; revealing significant differences in the expression of proteins involved in neuronal communication, connectivity and dopamine signaling. However, the general number and distribution of Foxp2-immunopositive cells in olfactory-driven limbic circuitry of *Foxp2*$^{+/-}$ mice appears unchanged from wildtypes. This suggests that their behavioral phenotype likely arises from differences at the level of neuronal function and/or connectivity. Using a combination of approaches, including site- directed *Foxp2* mutagenesis and viral tool on selective target medial amygdala, our current

efforts are directed toward understanding the neuronal circuit mechanisms by which *Foxp2* gene function controls innate social behaviors and circuit function. Such mechanistic insights will provide a context to understand the etiology of autism spectrum disorders and other disorders involving social dysfunction in humans.

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Poster

358. Autism Mechanisms: Mouse Models

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Program #/Poster #: 358.03

Topic: A.07. Developmental Disorders

Support: NIMH 1R01 MH113948-01A1

Title: Cul3 deletion postnatally in excitatory neurons in the forebrain results in behavioral impairments and synaptic transmission deficits in the hippocampus

Authors: *P. SEKAR[1], J. WANG[1], Q. XIA[1], Z. XUAN[1], J. D. SINGER[2], C. M. POWELL[1]; [1]Dept. of Neurobio., Univ. of Alabama At Birmingham, Birmingham, AL; [2]Dept. of Biol., Portland State Univ., Portland, OR

Abstract: Autism Spectrum Disorders are debilitating neurodevelopmental disorders characterized by impairment in social interaction and communication, and restricted, repetitive behaviors. Large scale studies from patients affected with autism show multiple genes that regulate protein ubiquitination are implicated in ASD. *De novo* loss of function mutations in the gene *CULLIN3 (CUL3)* are implicated in autism. CUL3 forms an essential component of an E3 ubiquitin ligase complex required for ubiquitination of substrates, often a signal for proteasomal degradation. Studies show homozygous deletion of *Cul3* is embryonically lethal. Recent studies show heterozygous deletion of *Cul3* results in autism-like phenotypes in constitutive and conditional *Cul3* heterozygotes. To understand the function of *Cul3* in post-natal development in the brain, we crossed mice expressing Cre-recombinase under the control of CaMKII promoter and floxed *Cul3* mice that resulted in viable homozygotes. In this study, we show a delayed postnatal deletion of *Cul3* during development in forebrain excitatory neurons leads to robust behavioral differences across multiple behaviors. *Cul3* conditional homozygotes show hyper-locomotion, impaired motor co-ordination, hindlimb clasping, reduced marble burying and deficits in spatial learning and memory. Additionally, we see decreased basal synaptic transmission and expression of long term potentiation in the homozygotes and heterozygotes compared to wild type littermate controls. These studies show a role for *Cul3* during a critical postnatal window in late neurodevelopment. Our future studies are aimed at gaining mechanistic insights into *Cul3* function in the adult brain.

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Poster

358. Autism Mechanisms: Mouse Models

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Program #/Poster #: 358.04

Topic: A.07. Developmental Disorders

Support: NIH R01 MH116500

Title: Impaired neural oscillations underly learning disability in fragile X syndrome

Authors: *M. ZIMMERMAN, A. A. CHUBYKIN;
Purdue Univ., West Lafayette, IN

Abstract: Impaired Neural Oscillations underly Learning Disability in Fragile X Syndrome Michael Zimmerman[1] & Dr. Alexander Chubykin[2]
[1]Department of Biomedical Engineering, Purdue University, West Lafayette, IN 47907[2]Department of Biological Sciences, Purdue Institute of Integrative Neuroscience, Purdue University, West Lafayette, IN 47907
Autism spectrum disorder (ASD) is an extremely prevalent group of neurodevelopmental disorders that affects approximately 2% of the population worldwide, while being four times more likely in boys. A hallmark of ASD is a disruption of sensory processing and filtering that is associated with either hyper- or hyposensitivity that can impact social interaction, daily functioning, and learning and memory. A prevailing thought is that in cases of ASD there is an impairment to the excitatory/inhibitory ratio of neurons in the cortex which subsequently impacts the ability of the brain to effectively store and recall information. Currently, the underlying mechanism causing this disruption and learning disability has yet to be understood. By utilizing Fmr1 KO mice, a mouse model of Fragile X Syndrome (FX; the most prominent single gene mutation case of ASD), and an active learning, visual discrimination behavior paradigm, we find that familiarity-evoked theta (4-8 Hz) oscillations in the primary visual cortex are weaker in amplitude and shorter in duration in FX mice. Additionally, we find that although familiarity enhances the visually evoked potentials, whether a stimulus is associated with a reward impacts the oscillations. All mice in this study were given enough time to show proper discrimination training, however, the FX group required longer to become experts in the task which was expected given the learning disability symptomatic of the disorder. Through time-frequency analysis, we find that the sustained activity in the theta band is attenuated, or lost entirely. Altogether, this work demonstrates (1) that cases of FX have impaired learning and memory that is associated with changes in low frequency theta oscillations across population and single-unit dynamics, (2) these oscillations are dependent on the stimuli-reward pairing outcome as each situation shows different patterns of activity.

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Poster

358. Autism Mechanisms: Mouse Models

Location: SDCC Halls B-H

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Program #/Poster #: 358.05

Title: WITHDRAWN

Poster

358. Autism Mechanisms: Mouse Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 358.06

Topic: A.07. Developmental Disorders

Support: SFARI Investigator Award
NeuroHub Investigator Award
NSF Graduate Research Fellowship Program 1752814

Title: Degraded tactile coding in the SCN2a loss-of-function mouse model of ASD

Authors: *K. VANDEMARK[1,2], H. MONDAY[1,2], L. RODRIGUEZ[1,2], M. LEE[1], A. AHITUV[1], D. FELDMAN[1,2];
[1]Deptartment of Mol. & Cell Biol., [2]Helen Wills Neurosci. Inst., Univ. of California Berkeley, Berkeley, CA

Abstract: Autism spectrum disorder (ASD) is strongly associated with *de novo* gene mutations, one of the most common being heterozygous loss-of-function mutations in *SCN2a* (Ben-Shalom et al., 2017; Sanders et al., 2012; Satterstrom et al., 2018). *SCN2a* encodes $Na_v1.2$, a sodium channel important for action potential backpropagation in neocortical pyramidal (PYR) cells *(Bender & Trussell, 2012; Kole & Stuart, 2012). SCN2a* haploinsufficiency reduces action potential backpropagation into dendrites, impairing glutamatergic synaptic plasticity and maturation of synaptic strength (Spratt et al., 2019). How this affects neural coding and circuit function remains unknown. We tested the impact of *SCN2a* haploinsufficiency on neural coding in primary somatosensory cortex (S1), where coding deficits could relate to tactile hypo- or hyper-sensitivity in ASD. In S1 slices, L2/3 PYR cells in *SCN2a*$^{+/-}$ mice showed slowing of action potential waveforms and reduced mEPSC amplitude, consistent with impaired spike backpropagation and synaptic strengthening. Feedforward L4-L2/3 inhibition appeared to remain intact. *In vivo*, we are performing multi-site single-unit (NeuroNexus) recordings in S1 of awake head-fixed mice, to test whether whisker-evoked response strength, whisker receptive fields,

response dynamics, and somatotopic map precision are altered in *SCN2a*$^{+/-}$ mice compared to *SCN2a*$^{+/+}$ littermates. We hypothesize that these results will show impaired coding of tactile stimuli that could be related to disordered sensory processing in ASD.

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Poster

358. Autism Mechanisms: Mouse Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 358.07

Topic: A.07. Developmental Disorders

Support: Fundação para a Ciência e Tecnologia (PD/BD/137759/2018)
FCT project POCI-01- 0145-FEDER-028073 and Society in Science
The Branco Weiss fellowship, administered by Eidgenössische Technische Hochschule (ETH) Zürich

Title: Sound processing in a mouse model of Autism Spectrum Disorder

Authors: *A. M. GONÇALVES, L. JACINTO, N. SOUSA, P. MONTEIRO;
ICVS, Braga, Portugal

Abstract: Sound processing in a mouse model of Autism Spectrum DisorderAuthorsA. M. GONÇALVES[1,2], L. JACINTO[1,2], N. SOUSA[1,2], P. MONTEIRO[1,2];[1]Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho,Campus Gualtar, 4710-057 Braga, Portugal; [2]ICVS/3B's - PT Government Associate Laboratory,Braga/Guimarães, Portugal**DisclosuresA. M. Gonçalves:** None. **L. Jacinto:** None. **N. Sousa:** None. **P. Monteiro:** None.**Abstract**Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by deficits incommunication and social interaction, restricted interests and repetitive behaviours. Knowledgeabout ASD neurobiology is still scarce and currently there is no efficient treatment or cure.Although a single cause for ASD is unknown, several candidate genes have emerged from patientstudies such as *SHANK3*, a gene that encodes a protein (SHANK3) in the postsynaptic density ofexcitatory synapses. Previous studies have shown that mutant mice carrying a human ASD-mutation in the *Shank3* gene (InsG3680), exhibit repetitive behaviours, anxiety and socialinteraction deficits. Social interaction is a complex behaviour that requires the ability to combinesensory information with emotional and cognitive content. Disruptions in sensory processingsuch as auditory hypersensitivity have also been reported in ASD patients and may relate to theirsocial communication deficits. To test whether a dysfunction in primary auditory brain areas ispresent upon disruption of *Shank3*, we performed *in vivo* recordings in the primary auditorycortex (A1) of anesthetized *Shank3*-mutant mice. To test whether *Shank3*-mutant presentauditory hypersensitivity, we developed a novel behavioural test where mice can choosebetween different "soundscapes" and found that *Shank3*-mutant mice tend to prefer

quietenvironments without sounds. Overall these results seem to be in accordance with the auditoryhypersensitivity literature in ASD patients.**Funding**A.M.Gonçalves is supported by a doctoral fellowship from Fundação para a Ciência e Tecnologia(PD/BD/137759/2018). This work is supported by FCT project POCI-01- 0145-FEDER-028073 andSociety in Science, The Branco Weiss fellowship, administered by Eidgenössische TechnischeHochschule (ETH) Zürich.

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Poster

358. Autism Mechanisms: Mouse Models

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Support: SFARI grant #569293
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Title: Characterization of a novel mouse model for autism spectrum disorder

Authors: *C. A. BRADLEY[1], S. Y. KO[1], L. D'ABATE[1], J. LEE[2], J. WANG[2], X. FANG[1], A. RUTHERFORD[1], G. L. COLLINGRIDGE[2], P. W. FRANKLAND[1], S. W. SCHERER[1]; [1]The Hosp. For Sick Children, Toronto, ON, Canada; [2]Lunenfeld-Tananbaum Res. Inst., Toronto, ON, Canada

Abstract: Autism Spectrum Disorder (ASD) is a prevalent neurodevelopmental disorder characterized by social communication deficits and the display of restrictive, repetitive behaviors. Our genomic studies have implicated the *PTCHD1 / PTCHD1-AS* locus on chromosome X as a penetrant susceptibility locus in males, contributing to ASD and intellectual disability in ~ 1% of cases. Genes at this locus include protein-coding genes *PTCHD1, DDX53* and a long non-coding RNA (lncRNA) *PTCHD1-AS.* Males with deletions in *PTCHD1-AS* strongly correlate with an ASD outcome. We, therefore, generated two mouse lines removing the conserved exon 3 of *Ptchd1-as,* the first line termed *Ptchd1-as* $^{Ex3-}$, and in the second line, inserting a 2x polyA termination signal to truncate the transcript after the third exon referred to as *Ptchd1-as* ^{PolyA}. Guided closely by the human phenotype data, we have characterized the behavioral and cellular effects in *Ptchd1-as* mutant mice to look at the transcriptional consequences of a deletion within the critical region associated with high functioning autism. Both mutant mice lines recapitulate the human phenotype with mild to moderate impact on ASD-relevant tasks (repetitive grooming, social interaction; N = 10-12, $p < 0.05$ for all tasks) with no deficits in a learning task (Touchscreen pairwise discrimination; *Ptchd1-as* ^{PolyA} N = 10, 13, $p > 0.05$). Furthermore, hippocampal synaptic function was normal; altogether a phenotype indicative of a pure, high-functioning ASD. Droplet digital PCR and deep sequencing of brain mouse *Ptchd1-as* demonstrate both age and region-specific expression patterns as well as enrichment in GABAergic cell types of both interneurons (N= 3, 4, $p < 0.01$) and striatal tissues

(N = 6, $p < 0.01$). Transcriptome analysis of *Ptchd1-as* ^{PolyA} mutants show modest effects in whole-brain tissues with more differentially expressed genes observed in young mice relative to adults (N=6 per genotype and age). Quantitative PCR showed *Ptchd1-as* ^{PolyA} mutants exhibited alterations in NMDA and AMPA receptor genes in young, which were down-regulated in a subunit-dependent manner (N=6, $p < 0.05$). Further cellular and synaptic investigation aim to identify the critical molecular changes that alter social function in an otherwise overtly normal mouse.

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Poster

358. Autism Mechanisms: Mouse Models

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Topic: A.07. Developmental Disorders

Support: NIH Grant 5R03HD101767-02
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Title: Disruption of grin2B, an ASD-associated gene, produces social deficits and subpallial alterations in zebrafish

Authors: *J. ZOODSMA[1], E. KEEGAN[1], G. R. MOODY[2], A. A. BHANDIWAD[4], A. J. NAPOLI[1], H. A. BURGESS[4], L. P. WOLLMUTH[3], H. I. SIROTKIN[1];
[1]Dept. of Neurobio. & Behavior, [2]Grad. Program in Mol. and Cell. Pharmacol., [3]Dept. of Biochem. & Cell Biol., Stony Brook Univ., Stony Brook, NY; [4]Div. of Developmental Biol., Eunice Kennedy Shriver Natl. Inst. of Child Hlth. and Human Develop., Bethesda, MD

Abstract: Autism Spectrum Disorder (ASD) has complex and varied etiologies. Genome sequencing has identified many candidate genes associated with ASD, including dozens of missense and nonsense mutations in NMDA receptor subunits. *GRIN2B,* the gene encoding the GluN2B subunit, is a high-confidence ASD-associated gene with many identified disease-associated mutations in all major structural domains. How such wide-ranging alterations in GluN2B impact neurodevelopment is poorly understood, in part because knockouts of GluN2B in rodents die shortly after birth. Here, we establish the relevancy of a zebrafish model to study developmental roles of GluN2B and demonstrate that zebrafish GluN2B displays similar structural and functional properties to human GluN2B. We generated fish lacking all functional GluN2B (*grin2B*$^{-/-}$) and surprisingly found that they survive into adulthood. This prolonged survival may in part be caused by transcriptional adaptation of other NMDA receptor subunits. Zebrafish are highly social creatures. This affords the study of social preference, deficits of which are highly prevalent in ASD. Unlike wild-type fish, which develop a strong social

preference by 3 weeks post fertilization, *grin2B*$^{-/-}$ fish at this age exhibit significantly reduced social preference. This phenotype is specific for GluN2B, as removal of GluN2A or frameshift mutations in obligatory GluN1 subunit paralogues *grin1a* or *grin1b* do not generate social deficits. Notably, the lack of GluN2B does not result in a broad disruption of neurodevelopment, as *grin2B*$^{-/-}$ larvae have wild type spontaneous locomotion, exhibit learning capabilities and see no gross anatomical brain difference. Whole-brain imaging of *grin2B*$^{-/-}$ larvae revealed a reduction in inhibitory neurons in the subpallium. The zebrafish subpallium contains putative homologues to the amygdala, septum, and striatum. Abnormalities in development of these regions is linked to ASD in humans. Together, these findings highlight the unique opportunity to study, in zebrafish, the roles of GluN2B in development and disease etiology and afford a system for future examination of the role of GluN2B in the circuits that generate social preference.

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Poster

358. Autism Mechanisms: Mouse Models

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Support: SFARI Grant 569293
CIHR Foundation Grant S Scherer

Title: Determining the role of long noncoding rna ptchd1-as in autism spectrum disorder

Authors: *A. RUTHERFORD[1,2], C. A. BRADLEY[1], K. KIRIAKOPULOS[1,2], Y. HAN[1], C. SHUM[1], P. G. MAASS[1,2], S. W. SCHERER[1,2];
[1]The Hosp. For Sick Children, Toronto, ON, Canada; [2]Mol. Genet., Univ. of Toronto, Toronto, ON, Canada

Abstract: Autism spectrum disorder (ASD) is a heterogeneous condition diagnosed based on individuals having deficits in social communication, and restricted repetitive behaviors, which bring them to medical attention. A recently identified risk gene encoding a complex multi-isoform long-noncoding RNA (lncRNA), *PTCHD1-AS* (spanning ~1Mb of DNA at Xp22.11), may serve as a novel entry point into understanding the etiology of, and designing therapeutics for, ASD. Human data shows that essentially all male offspring inheriting rare deletions in *PTCHD1-AS* have ASD (female carriers of this X-linked deletion alone do not). There are also a handful of *de novo* deletions affecting *PTCHD1-AS* in males with ASD. It is unknown how *PTCHD1-AS* is acting, but deletions in *PTCHD1-AS* in patient derived cell lines were found to underlie synaptic deficits due to N-methyl-D-aspartate receptor (NMDAR) hypofunction. Therefore, we propose that the deletions in lncRNA *PTCHD1-AS* that lead to ASD cause a change in the expression of genes necessary for synaptic function. Our first aim is to assay the

expression of synaptic and NMDAR genes in whole brain tissue of both male and female *Ptchd1-as* transgenic mice, at postnatal day 6, via quantitative PCR (qPCR), and microarray. While the genes encoding the NMDAR subunits do not appear to be differentially expressed in wild type versus mutant male mice in our preliminary qPCR and microarray data, certain other genes of interest, such as olfactory receptors and known ASD risk gene *CHD2*, do (N=6). Our second aim is to knockdown and overexpress *PTCHD1-AS* in vitro using CRISPR interference and activation, and assess its impact via qPCR on the genomically-adjacent protein coding gene *PTCHD1*, which is a known susceptibility gene in males for neurodevelopmental disorders. This exploratory study will determine whether the promoter of the lncRNA is targetable separate from that of the protein coding gene, and what the relationship between the two genes might be. Understanding how this novel lncRNA is regulating gene expression could unveil a network of genes involved in the etiology of ASD and establish it as target for therapeutic intervention.

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Poster

358. Autism Mechanisms: Mouse Models

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Topic: A.07. Developmental Disorders

Support: NIH Grant R01 MH122485-01

Title: Altered dorsal striatal development: a behavioral, cellular, and molecular analysis in a prenatal stress mouse model

Authors: *M. M. EVANS[1], S. V. MAURER[1], B. W. Q. HING[1], M. A. WEBER[2], N. S. NARAYANAN[2], H. E. STEVENS[1];
[1]Psychiatry, [2]Neurol., Univ. of Iowa, Iowa City, IA

Abstract: Background: Many people with autism spectrum disorder (ASD) show enlargement of the dorsal striatum, a forebrain structure known for its roles in motor learning and habitual behaviors. ASD dorsal striatal abnormalities may contribute to restricted, repetitive behavior, a diagnostic criterion for ASD, which encompasses repetitive motor behavior, inflexibility in routine, and fixated interests. The dorsal striatum is composed mainly of medium spiny neurons (MSNs) which may be altered in early development in those with ASD. The current work investigates striatal-dependent, ASD-relevant behaviors and cellular phenotypes of the dorsal striatum in juvenile and adult CD-1 mice in a prenatal stress (PS) model, as PS is a risk factor for neurodevelopmental disorders, including ASD. Methods: CD-1 female mice were time-mated with GAD67-GFP+ CD-1 males. Starting on embryonic day 12, pregnant dams underwent repetitive restraint stress (PS; n = 15) or saline injections (SAL; n = 13) three times daily. A separate group of pregnant dams was left completely undisturbed (naïve; n = 10). Juvenile GFP+

offspring were tested on open field, rotarod, and water T maze between 3 and 5 weeks of age (n = 8-13/condition and sex). Separate GFP+ littermates were tested on the same behaviors between 8 and 12 weeks of age (n = 10-11/condition and sex), some of which then entered a 7-week interval timing task (n = 3-4/condition and sex). Immunohistochemistry for substance P, enkephalin, and GFP were used to label and stereologically count dorsal striatal MSNs in juvenile and adult offspring. GFP- male offspring at 8-12 weeks of age were used for single-cell RNA sequencing (scRNAseq) of dorsal striatum (n = 4/condition) using the 10x Genomics Chromium Single-Cell System and with processing by Cell Ranger, Seurat in RStudio, and Ingenuity Pathway Analysis. Results: Initial behavioral results show PS trend increased time spent in the open field center zone in adult males (p = 0.1062) and significantly increased distance traveled in later epochs in juvenile males (p = 0.0195), indicating decreased intrasession habituation. PS also trend increased rotarod procedural learning in adult females (p = 0.0967). Initial stereological results show PS trend increased dorsal striatal GAD67-GFP+ cell density only in adult males (p = 0.069) without changes in substance P+ cells. More behavioral, stereological, and scRNAseq data are currently in progress as described in the methods. These data demonstrate how dorsal striatal development is affected by risk factors for ASD.

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Poster

358. Autism Mechanisms: Mouse Models

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Support: ANPCYT, PICT-2016-2202
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Title: A two-hit model of autism-related behaviors in female mice: prenatal valproic acid and postnatal estradiol exposure

Authors: *A. SEIFFE[1,2], A. M. DEPINO[1,3];
[1]IFIBYNE, UBA-CONICET, Buenos Aires, Argentina; [2]DFBMC, FCEN, Univ. of Buenos Aires, Buenos Aires, Argentina; [3]DBBE, FCEN, Univ. of Buenos Aires, Buenos Aires, Argentina

Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disorder that affects 4 boys for every diagnosed girl, suggesting a higher susceptibility for males and/or resilience in females. Individuals with ASD show altered sociability, reduced communicative skills and repetitive behaviors, and the etiology is unknown. To identify the biological mechanisms involved in the sexual bias in ASD, we use a mouse model of ASD and study the effects of early exposure to

gonadal hormones that can affect brain development. We show that the injection of CF1 female mice with 17β-estradiol benzoate (E2, 50 µg) on postnatal days 2, 5 and 8 results in adult animals that express male-specific territorial behavior, have stunted ovaries, and diminished progesterone levels in plasma. Moreover, this treatment masculinizes exploration, repetitive behaviors, and depression-related behaviors, showing that gonadal neonatal exposure can affect disease-relevant behaviors. To further study the effect of sex on ASD-related behaviors, we combined the E2-mediated brain masculinization protocol with a mouse model of ASD: the prenatal exposure (at gestational day 12.5) to 600 mg/kg valproic acid (VPA). We demonstrated that VPA-exposed males have reduced sociability both as young and adults, while female littermates express normal levels of social interaction in adulthood. Thus, we hypothesized that ASD-related behaviors result from VPA altering the normal development of the male brain. To test this hypothesis, we evaluated the effects on behavior of neonatal exposure to E2 in female VPA mice. First, we found that young VPA females show alterations in juvenile play, performing less events of play solicitation and investigation than control animals. Interestingly, neonatal E2 reverts the effects on investigation (Generalized linear mixed models, $p<0.05$). In adulthood, we observed an alteration of sociability in VPA-E2 mice: they show no habituation to the stimulus mouse neither in the 3-chamber social interaction test or in the social habituation and novelty recognition test (Generalized linear mixed models, $p<0.05$). These results show that VPA can affect juvenile female behavior, but compensatory mechanisms result in adult normal sociability. This could be orchestrated by the organizational scheme given by the presence/absence of gonadal hormones in the perinatal period and modulated by the activation of the circuits by the sex steroids during puberty. Further studies of this two-hit model could help us identify possible biological mechanisms differentially affecting male and female brains, and contributing to the development of ASD-related behaviors.

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Poster

358. Autism Mechanisms: Mouse Models

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Support: Man power by Indian Council of Medical Research provided Senior Research Fellowship to Ashish Jain grant letter no (45/45/2019-PHA/BMS, dated - 23/07/2019)

Title: Valproic acid induces autism-like symptoms in Wistar offspring, irrespective of sex: A behavioral insight

Authors: *A. JAIN, B. MEDHI, Prof., A. PRAKASH, Associate;
Postgraduate Inst. of Med. Educ. and Res., Inst. of Postgraduate Med. Educ. and Res., Chandigarh, India

Abstract: Background Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder with behavioral impairments like social deficits, restricted interest in surroundings, and repetitive behavior (stacking objects). The incidence of ASD is higher in boys than girls (4:1). Valproic acid (VPA) has been shown to cause neural tube defects, congenital malformations, and autism in children post-birth. Similarly, VPA induces autism-like symptoms in rodents. Many studies have observed that only male rats show ASD features. However, the debate over including only male rodents in preclinical research is still controversial, as many researchers exclude female rats due to the lack of ASD symptoms. The study aimed to evaluate the gender bias in the VPA model of ASD. **Methods**We opted for Wistar rats (male and female) exposed to VPA at 600 mg/kg, in-utero at GD 12.5. The offspring of both control and VPA male (n=6) and female (n=6) rats were subjected to a battery of behavioral paradigms. We assessed gastrointestinal (GIT) motility (n=3), brain edema (n=3), blood-brain-barrier (BBB) permeability (n=3, Evans blue concentration), and brain histology (n=3). **Results**VPA female rats had significantly delayed latency in surface righting reflex (p=0.0128), quicker fall in wire hanging test (p=0.0223), higher feet unfolded distance (p=0.0223) and decreased head-body length and body weight, delayed locomotion in grid walking behavior, hole board and actophotometer. However, VPA female rats had significantly higher repetitive behavior (p=0.0176) than VPA male rats.We observed autism-like symptoms in both VPA male and female rats (delayed nervous reflex, motor coordination function, and developmental patterns with increased anxiety, exploratory, repetitive, and depressive behavior, social deficit, impaired BBB permeability, higher brain volume and impaired GIT motility) than the control rats. Additionally, both male (p=0.0002) and female (p=0.0002) VPA rats had similar neuronal impairment indicating poor memory performance in Barnes and Morris water maze. **Conclusions**
Our findings underscore the need to consider both sexes in preclinical ASD studies to understand sex differences better, delineate the mechanistic approach, and explore the pharmacological regimens for ASD individuals.

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Poster

358. Autism Mechanisms: Mouse Models

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Topic: A.07. Developmental Disorders

Support: Mission Enhancement Funds
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Title: Mir-7684-3p as a potential marker in autism: prenatal stress in a model of increased genetic stress susceptibility

Authors: *T. WOO[1], C. KING[2], M. CORDES[3], C. BLOOMER[4], Z. TALEBIZADEH[5], N. KIBIRYEVA[6], D. Q. BEVERSDORF[7];

[1]Univ. of Missouri, Columbia, MO; [2]Div. of Biol. Sci., Univ. of Missouri, Columbia, Columbia, MO; [3]Dept. of Psychology, Univ. of Missouri at Columbia, Columbia, MO; [4]Univ. of Kansas Med. Ctr., Kansas City, KS; [5]American Col. of Med. Genet. and Genomics, Bethesda, MD; [6]Children's Mercy Hosp. and Univ. of Missouri-Kansas City Sch. of Med., Overland Park, KS; [7]Dept Radiol, Neurol, Psychol Sci, DGS of INP, Univ. of Missouri Columbia, Columbia, MO

Abstract: Prenatal stress affects expression of a range of genes associated with the stress response pathway as well as maternal microRNA (miRNA) expression. Our previous work revealed that stress-reactive polymorphisms found on the serotonin transporter (SERT) gene may interact with environmental stressors during pregnancy to increase the risk for the development of autism spectrum disorder (ASD). In animal models, autism-associated behavior was observed in offspring born to stress-exposed mice lacking one copy of the SERT gene (SERT-het), which results in increased susceptibility to stress. Recently, we have reported differential expression (DE) of miRNAs in blood samples of prenatally stress-exposed mothers carrying the short SERT allele who have children with ASD, which may serve as a potential blood biomarker of the gene x environment (G x E) interaction in ASD. Therefore, we are interested in the role of these miRNAs as a potential maternal biomarker in the neurodevelopmental changes associated with the G x E interaction. Thus, we evaluated the profile of the maternal miRNA stress-exposed SERT-het (SERT-het/stress) mouse model at embryonic day 21 (E21) and postnatal day 60 (PD60). More than three thousand mature miRNAs were examined, and ANOVA analysis detected several DE miRNAs. Offspring from SERT-het/stress mice showed restricted social interaction and elevated repetitive behavior at PD60. 4 miRNAs at E21 and 13 miRNAs at PD60 were differentially expressed in SERT-het/stress group and corresponded with behavioral results. mir-7684, a pre-miRNA of miR-7683-3p is downregulated at PD60 while miR-7684-3p is upregulated at E21, which suggests Dicer actively cleaves mir-7684 in SERT-het/stress group. These miRNAs could be potential biomarker candidates for G x E interactions in ASD, and are highly dynamic over time. Our study provides evidence for epigenetic alterations associated with the gene x environmental interaction which we hope will lead to better understanding of the mechanisms and eventually treatments of ASD, resulting from prenatal stress exposure in genetically stress susceptible individuals.

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Poster

358. Autism Mechanisms: Mouse Models

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Title: Probiotic therapy with Lactobacillus reuteri rescues social and emotional recognition behavior in an environmental mouse model of autism

Authors: *M. E. DENYS[1], E. V. KOZLOVA[1], A. E. BISHAY[1], L. CAMPOY[1], A. HABBAL[1], C. LUNA[1], Y. KORDE[1], V. PIAMTHAI[2], A. HSIAO[2], M. CURRAS-COLLAZO[1]; [1]Molecular, Cell and Systems Biol., [2]Dept. of Microbiology and Plant Pathology, Univ. of California, Riverside, Riverside, CA

Abstract: Polybrominated diphenyl ethers (PBDEs) are neuroendocrine disrupting chemicals with adverse neurodevelopmental effects in humans. Our group has previously shown that PBDEs produce autistic-like traits in developmentally exposed mice offspring (https://doi.org/10.1007/s00204-021-03163-4). We also showed that PBDEs reduce oxytocin expression in the social brain network. Moreover, PBDEs are well established disruptors of thyroid hormone axis. Lactobacillus reuteri (LR) is a beneficial probiotic that can increase oxytocin and thyroid hormones in other ASD mouse models. In this study, C57BL/6N dams were dosed with a commercial mixture of PBDE congeners, DE-71, at 0.1 mg/kg/d (L-DE-71), or corn oil vehicle control (VEH/CON) for 10 wks (pre-conception: 4 wk; gestation: 3 wk; lactation: 3 wk). A subset of dams were gavaged with L. reuteri ATCC 6475 (gift of Biogaia) at 10^8 CFU/mL. PCR analysis showed increased gene expression of fecal LR 6475 in offspring of dams receiving LR treatment confirming efficacy of maternal LR supplementation. Pregnant dams treated with LR showed significantly elevated plasma T4 levels. Pup early maternal attachment measured by homing and maternal pup retrieval was not affected by DE-71, indicating that exposure did not affect normal maternal nor early postnatal pup behavior. Pup righting reflex was improved in L-DE-71+LR vs L-DE-71 males and females at postnatal day (PND) 6. Compared to VEH/CON, L-DE-71 exposed offspring showed abnormally elevated marble burying scores, which were normalized by LR treatment only in females. L-DE-71 produced deficient scores on a social memory recognition test (24 h retention) and was rescued by LR treatment in females at PND 30. LR also rescued deficient short-term social novelty preference test scores in males and females in adulthood. L-DE-71 exposed male and female offspring spent similar investigation times on mice subjected to 15 min restraint stress vs neutral stimulus using an emotional recognition (ER) test. In contrast, L-DE-71 + LR mice investigated restraint-subjected mice more vs neutral stimulus mice indicating an improvement of ER. These results suggest that maternal LR treatment during developmental exposure to PBDEs provides normalization of autistic-like features in a mouse model of autism.

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Poster

358. Autism Mechanisms: Mouse Models

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Title: Life-long exposure to a low-dose of Glyphosate-based herbicide RoundUp causes intestinal damage, gut dysbiosis and behavioral changes in mice

Authors: *J. R. CLARKE[1], I. DEL CASTILO[2], A. S. NEUMANN[2], F. S. LEMOS[2], M. A. DE BASTIANI[3], F. L. OLIVEIRA[2], E. R. ZIMMER[4], A. REGO[5], C. HARDOIM[6], L. M. ANTUNES[7], F. A. LARA[7], C. P. FIGUEIREDO[1];
[1]Federal Univ. of Rio De Janeiro, [2]Federal Univ. of Rio De Janeiro, Rio de Janeiro, Brazil; [3]Univ. Federal do Rio Grande do Sul, Porto Alegre, Brazil; [4]Federal Univ. of Rio Grande Do Sul (UFRGS), Federal Univ. of Rio Grande Do Sul (UFRGS), Porto Alegre, Brazil; [5]Inst. Oswaldo Cruz, Rio de Janeiro, Brazil; [6]Univ. Estadual Paulista, São Vicente, Brazil; [7]Fundação Oswaldo Cruz, Rio de Janeiro, Brazil

Abstract: RoundUp® (RUp) is a comercial formulation containing glyphosate (N-(phosphono-methyl) glycine), and is the world's leading wide-spectrum herbicide used in agriculture. Supporters of the broad use of glyphosate-based herbicides (GBH) claim they are innocuous to humans, since the active compound acts on the inhibition of enzymes which are absent in human cells. However, the neurotoxic effects of GBH have already been shown in many animal models. Further, these formulations were shown to disrupt the microbiome of different species. Here, we investigated the effects of a lifelong exposure to low doses of the GBH-RUp on the gut environment, including morphological and microbiome changes. We also aimed to determine whether exposure to GBH-RUp could harm the developing brain and lead to behavioral changes in adult mice. To this end, animals were exposed to GBH-RUp in drinking water from pregnancy to adulthood. GBH-RUp-exposed mice had no changes in cognitive function, but developed impaired social behavior and increased repetitive behavior. GBH-Rup-exposed mice also showed an activation of phagocytic cells (Iba-1-positive) in the cortical brain tissue. GBH-RUp exposure caused increased mucus production and the infiltration of plama cells (CD138-positive), with a reduction in phagocytic cells. Long-term exposure to GBH-RUp also induced changes in intestinal integrity, as demonstrated by the altered expression of tight junction effector proteins (ZO-1 and ZO-2) and a change in the distribution of syndecan-1 proteoglycan. The herbicide also led to changes in the gut microbiome composition, which is also crucial for the establishment of the intestinal barrier. Altogether, our findings suggest that long-term GBH-RUp exposure leads to morphological and functional changes in the gut, which correlate with behavioral changes that are similar to those observed in patients with neurodevelopmental disorders.

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Poster

358. Autism Mechanisms: Mouse Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 358.17

Topic: A.07. Developmental Disorders

Title: Environmental enrichment decreases socio-communicative deficits and stereotypy in BTBR mice but does not mitigate sensory deficits in a novel somatosensory paradigm: the Optimized Somatosensory Nose-poke Adapted Paradigm (OSNAP)

Authors: *M. BINDER, A. F. BORDEY;
Neurosurg., Yale Sch. of Med., New Haven, CT

Abstract: Autism spectrum disorder (ASD) commonly presents with social, communicative, and sensory deficits as well as stereotypy. While social-communicative and repetitive behaviors can be reliably assessed in murine models, there are extremely few robust, easily implementable, sensory paradigms available. The current project developed the Optimized Somatosensory Nose-poke Adapted Paradigm (OSNAP) to address this need. In addition to developing the new paradigm, we also assessed the effects of environmental enrichment on the autistic phenotype using BTBR (autistic-like) mice and C57BL/6 (control) mice. Specifically, mice were either raised in standard housing conditions (1 dam: 1 litter in a 10.5x8x5.5 inch cage) or in a physically and socially enriched condition (2 dams: 2 litters in a 30x55x30 inch enclosure). Neonatal ultrasonic vocalizations were assessed on postnatal day (PD) 6, sensory and repetitive behavior on PD 21, and social behavior on PD 24. Standard housed BTBR mice displayed increases in the quantity of ultrasonic vocalizations (USVs) produced, repetitive behaviors, social deficits, and sensory alterations relative to C57BL/6 mice. Conversely, environmentally enriched BTBR mice displayed reduced vocalizations, still different from but more similar to C57BL/6 USVs, no social deficit, and decreased stereotypy, however, the sensory deficit still persisted. Therefore, we established that the novel OSNAP paradigm is a valid, sensitive, and reliable measure of sensory behavior in mice, while also finding that environmental enrichment can significantly reduce many but not all the core deficits in ASD.

Disclosures: M. Binder: None. **A.F. Bordey:** None.

Poster

358. Autism Mechanisms: Mouse Models

Location: SDCC Halls B-H

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Program #/Poster #: 358.18

Topic: A.07. Developmental Disorders

Support: SRCEP to S Cheng
SRCEP to G Almeida
ART to J Lee

Title: Increased maternal hormone levels during mid-gestation period and abnormal embryonic cortical development in autistic BTBR T+tfJ mice

Authors: *J. LEE, S. CHENG, G. ALMEIDA;
William Paterson Univ., Wayne, NJ

Abstract: Increased maternal hormone levels during mid-gestation period and abnormal embryonic cortical development in autistic BTBR T+tf/J mice Sandy Cheng, Gabriela Almeida and Jeung Woon LeeWilliam Paterson University, Wayne NJ 07470
Autism spectrum disorder (ASD) is a developmental disorder, where genetic abnormalities and exposure to environmental agents during embryonic development have been suggested as possible causes. Using BTBR T+ tf/J (BTBR) mouse, an animal model for idiopathic ASD, we examined the maternal hormone levels during mid-gestation and embryonic cortical neurogenesis that may be associated with expression of ASD-like behaviors. Embryos at E14, E16, E18 and E20 were harvested from pregnant dams (BTBR and C57). Maternal trunk blood was also collected during harvesting of embryos. Maternal blood was tested for corticosterone, interleukin6, neuropeptide Y, and b-endorphin. Brains of BTBR and C57 embryos were fixed in paraformaldehyde, frozen-sectioned and stained for KI-67 immunoreactivity. In BTBR, the maternal blood had significantly elevated levels of corticosterone, NPY and b-endorphin during gestation period E14 to E20. BTBR had elevated level of IL-6 but not statistically different compared to control C57. BTBR embryos displayed numerous KI-67 immunoreactive cells in the cortex. These cells were mainly localized in intermediate zone and cortical plate without specific migratory pattern.The abnormal cell proliferation rate during development of E14 to E20 embryonic cortex seen in BTBR along with high levels of maternal hormones during these gestational period may be contributing factors that may predispose the BTBR with expression of ASD-like behaviors.

Disclosures: J. Lee: None. **S. Cheng:** None. **G. Almeida:** None.

Poster

358. Autism Mechanisms: Mouse Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 358.19

Topic: A.07. Developmental Disorders

Support: DOD award TS190074

Title: Increased mTOR activity in layer 2/3 pyramidal neurons of the medial prefrontal cortex leads to communicative deficits

Authors: *L. ZHANG, M. BINDER, A. BORDEY;
Neurosurg., Yale Sch. of Med., New Haven, CT

Abstract: The Mechanistic Target of Rapamycin complex 1 (mTORC1) is a molecular integrator that controls fundamental processes involved in cell development. As a result, several neurodevelopmental disorders characterized by increased mTORC1 activity are associated with abnormalities in neuron development and a spectrum of neurological deficits, including cognitive deficits and autism. However, the mechanistic connection between hyperactive mTORC1 and specific neurological deficits remains unclear. Here, we selectively increase mTORC1 activity in layer (L) 2/3 pyramidal neurons of the medial prefrontal cortex (mPFC) that is a higher order center altered in several mTORopathies. To achieve this, we used bilateral in utero electroporation of a constitutively active form of Rheb, a canonical mTORC1 activator. We found that postnatal day (P) 13 neonates with hyperactive mTORC1 in the mPFC displayed a significant increase in the quantity of ultrasonic vocalizations upon maternal separation, but no social deficits. At the cellular level, L2/3 pyramidal neurons with hyperactive mTORC1 displayed abnormalities in connectivity. Collectively, the data suggest that L2/3 pyramidal neurons of the mPFC are involved in communication information processing and increased mTORC1 activity in these neurons leads to communication deficits, one of the criteria for an autism diagnosis.

Disclosures: L. Zhang: None. **M. Binder:** None. **A. Bordey:** None.

Poster

358. Autism Mechanisms: Mouse Models

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 358.20

Topic: A.07. Developmental Disorders

Support: ERC DisConn 802371

Title: Chemogenetic excitation of pyramidal neurons during early development: a longitudinal behavioral and imaging study

Authors: *A. STUEFER[1,6], L. BALASCO[6], S. BERTOZZI[2], L. COLETTA[1,6], A. HAYWARD[1], F. ROCCHI[1,6], C. MONTANI[1], F. ALVINO[1], A. GALBUSERA[1], M. ALDRIGHETTI[1], S. GINI[1], V. CURCIC[1], F. PAPALEO[3], G. IURILLI[4], L. CANCEDDA[5], A. ARMIROTTI[2], Y. BOZZI[6,7], A. GOZZI[1];
[1]Functional Neuroimaging Lab., Inst. Italiano di Tecnologia, Rovereto, Italy; [2]Analytical Chem. Facility, [3]Genet. of Cognition Lab., Inst. Italiano di Tecnologia, Genova, Italy; [4]Systems Neurobio. Lab., Inst. Italiano di Tecnologia, Rovereto, Italy; [5]Brain Develop. and Dis. Lab., Inst. Italiano di Tecnologia, Genova, Italy; [6]CIMeC - Ctr. for Mind/Brain Sci., Univ. degli Studi di Trento, Rovereto, Italy; [7]CNR Neurosci. Inst., Pisa, Italy

Abstract: Autism and related developmental disorders encompass a wide range of etiologically heterogeneous conditions characterized by social and communicative impairment, as well as restricted and repetitive behaviors. Supported by evidence of altered inhibitory function in a substantial proportion of ASD subjects, one possible unifying mechanism underlying autism could be the onset of excitatory-inhibitory (E-I) imbalance as a consequence of genetic or developmental insult. To probe this hypothesis, we chemogenetically manipulated E-I balance in the cortex of infant mice and measured how this alteration affects brain connectivity as well as various domains of behavior throughout the life span of the mice. Cell-type specific increase of neuronal excitability was obtained via expression of DREADD receptors in Vglut1-cre mice and following chronic CNO treatment during the first two postnatal weeks. Subsequently, longitudinal behavioral tests and resting state fMRI were performed at multiple developmental stages, i.e. late infancy, adolescence and adulthood. Mice also underwent open field, habituation-dishabituation social interaction, y-maze, novel object recognition, rotarod and whisker nuisance tests. We found that chemogenetically manipulated mice exhibited impaired social behavior as assessed with direct social interaction habituation-dishabituation test at multiple timepoints, but not anxiety-like phenotypes, or impairments in working and long-term memory, motor coordination or tactile sensitivity. These effects were associated with atypical functional connectivity in socio-behaviorally relevant networks in adulthood as measured with rsfMRI. These initial results suggest that developmental hyperexcitability can lead to social dysfunction and functional dysconnectivity alteration reminiscent of hallmark findings in developmental disorders.

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Poster

358. Autism Mechanisms: Mouse Models

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Program #/Poster #: 358.21

Topic: A.07. Developmental Disorders

Support: NIH R01MH124808
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NIH R01NS099429

Title: Critical period plasticity of cortical functional connectivity is disrupted by a novel syndromic intellectual disability/autism spectrum disorder mutation

Authors: *S. CHEN[1], R. M. RAHN[2], A. R. BICE[3], S. H. GAINES[4], J. D. DOUGHERTY[5], J. P. CULVER[6];

[1]Genetics, Radiology, [2]Genetics, Psychiatry, Radiology, [3]Radiology, Washington Univ. in St. Louis, St. Louis, MO; [4]Radiology, Washington Univ. in St. Louis, St Louis, MO; [5]Genetics, Psychiatry, [6]Radiology, Physics, Imaging Sci., Washington Univ. in St. Louis, St. Louis, MO

Abstract: Neurodevelopmental disorders (NDDs) such as Autism Spectrum Disorder (ASD) and Intellectual Disability (ID) have hundreds of newly-discovered genetic causes, which are often mutations in genes encoding synaptic proteins and transcription factors. Such NDDs are characterized by impaired motor, language and cognitive development. MYT1L syndrome is a newly-identified, rare NDD. The disorder is caused by a loss-of-function mutation in *MYT1L*, a zinc finger TF gene, and is associated with many types of developmental anomalies, including ID and ASD. However, it remains unclear how this genetic mutation leads to ID/ASD and other learning impairments seen in patients.
Because experience-dependent plasticity is necessary for learning, one possible hypothesis of learning impairments in NDDs is the inability of the brain to modulate its functional connections to reflect learning. Monocular deprivation (MD) is a classic manipulation used to study experience-dependent plasticity of neuronal properties in the visual cortex, such as mini-EPSC characteristics and firing rate dynamics. However, it remains unknown how cortical network resting-state functional connectivity (rsFC) responds to MD, and whether NDDs may impair such plasticity. Therefore, we sought to characterize response in rsFC of healthy mice to critical period MD and examine critical period plasticity in a mouse model of MYT1L syndrome. To this end, we used wide-field optical imaging to collect calcium resting-state FC data in C57BL/6J wildtype control and Myt1L mice hemizygous for the *Thy1*-GCaMP6f allele at four timepoints during visual critical period: baseline (postnatal day [P]24), MD1 (P25), MD2 (P26) and MD3 (P27). In control mice, rsFC patterns re-organized rapidly during critical period MD. Myt1L mice, in contrast, exhibited changes on a longer timescale as well as different patterns of network reorganization compared to their wildtype littermates, suggestive of impaired critical period plasticity.

Disclosures: **S. Chen:** A. Employment/Salary (full or part-time):; Washington University in St. Louis. **R.M. Rahn:** None. **A.R. Bice:** A. Employment/Salary (full or part-time):; Washington University in St. Louis. **S.H. Gaines:** A. Employment/Salary (full or part-time):; Washington University in St. Louis. **J.D. Dougherty:** A. Employment/Salary (full or part-time):; Washington University in St. Louis. **J.P. Culver:** A. Employment/Salary (full or part-time):; Washington University in St. Louis.

Poster

358. Autism Mechanisms: Mouse Models

Location: SDCC Halls B-H

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Program #/Poster #: 358.22

Topic: A.07. Developmental Disorders

Support: NIH Grant R56 AG041250
NIH Grant R01 CA166590

Title: Hypothalamic TrkB.FL overexpression improves metabolic outcomes in the BTBR mouse model of autism spectrum disorder

Authors: *J. M. ANDERSON[1], A. A. BOARDMAN[2], R. BATES[2], X. ZOU[2], W. HUANG[2], L. CAO[2];
[1]Dept. of Neurosci., [2]Dept. of Cancer Biol. and Genet., The Ohio State Univ., Columbus, OH

Abstract: BTBR *T+ Itpr3tf*/J (BTBR) mice are used as a model of autism spectrum disorder (ASD), displaying similar behavioral and physiological deficits observed in patients with ASD. Our recent study found that implementation of an enriched environment (EE) in BTBR mice improved metabolic and behavioral outcomes. Brain-derived neurotrophic factor (*Bdnf*) and its receptor tropomyosin kinase receptor B (*Ntrk2*) were upregulated in the hypothalamus, hippocampus, and amygdala, suggesting that BDNF-TrkB signaling plays a role in the EE-BTBR phenotype. Here, we used an adeno-associated virus (AAV) vector to overexpress the TrkB full-length (TrkB.FL) BDNF receptor in the BTBR mouse hypothalamus to assess whether BDNF-TrkB signaling is responsible for the improved metabolic and behavioral phenotypes associated with EE. Normal chow diet (NCD)-fed and high fat diet (HFD)-fed BTBR mice were randomized to receive either bilateral injections of AAV-TrkB.FL or AAV-YFP as control and were subjected to metabolic and behavioral assessments up to 24 weeks post-injection. Both NCD and HFD TrkB.FL overexpressing mice displayed improved metabolic outcomes, characterized as reduced percent weight gain and increased energy expenditure. NCD TrkB.FL mice showed improved glycemic control, reduced adiposity, and increased lean mass. In NCD mice, TrkB.FL overexpression altered the ratio of TrkB.FL/TrkB.T1 protein expression and increased phosphorylation of PLCγ in the hypothalamus. In inguinal white adipose tissue (iWAT) and gonadal white adipose tissue (gWAT), TrkB.FL overexpression altered expression of genes involved in energy metabolism and thermogenesis, including *Pgc1α* and *Adrb3*. In HFD mice, TrkB.FL overexpression increased phosphorylation of PLCγ. TrkB.FL overexpression in the hypothalamus did not improve behavioral deficits in either NCD or HFD mice. Together, these results suggest that enhancing hypothalamic BDNF-TrkB.FL signaling improves metabolic health in BTBR mice.

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Poster

358. Autism Mechanisms: Mouse Models

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Program #/Poster #: 358.23

Topic: A.07. Developmental Disorders

Support: the Austrian Research Promotion Agency (Early Stage Grant 'Molecular Control' FFG-878286)
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Erasmus MC Department of Immunology
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NWO-ALW (grant 824.02.001; C.I.D.Z.)

Title: Bi-allelic PAX5 mutations as a Mendelian cause of ASD, intellectual disability, restlessness, and impaired sensorimotor learning

Authors: F. M. P. KAISER[1,2], S. GRUENBACHER[2], M. ROA OYAGA[1], E. NIO[1], N. VRIELER[3], A. DE AMICI[1], Q. SUN[2], W. VAN DER ZWAAG[4], E. KREIDL[2], L. M. ZOPF[5], V. A. S. H. DALM[1], J. PEL[1], C. GAISER[1], R. VAN DER VLIET[1], L. WAHL[1], A. RIETMAN[1], L. HILL[2], I. LECA[2], K. TACHIBAN[6], C. DE ZEEUW[1], M. BUSSLINGER[2], ***A. BADURA**[1]; [1]Erasmus MC, Rotterdam, Netherlands; [2]Res. Inst. of Mol. Pathology (IMP), Vienna BioCenter (VBC), Vienna, Austria; [3]Neurobio., Hebrew Univ., Jerusalem, Israel; [4]Spinoza Ctr. for Neuroimaging, Amsterdam, Netherlands; [5]Vienna BioCenter Core Facilities (VBCF), Vienna BioCenter (VBC), Vienna, Austria; [6]Inst. of Mol. Biotech. of the Austrian Acad. of Sci. (IMBA), Vienna BioCenter (VBC), Vienna, Austria

Abstract: Large-scale sequencing studies have implicated *PAX5* haploinsufficiency as a potential risk factor for autism spectrum disorder (ASD); however, a causal relationship between *PAX5* and ASD does not exist. We have identified a patient with biallelic mutations in *PAX5* that presented with ASD, impaired sensorimotor learning, intellectual disability, and restlessness. Using CRISPR/Cas9 genome editing, we have generated a mouse model with the patient-specific mutations and assessed behavioral and motor abnormalities in a broad battery of tests. Pax5-mutant mice displayed behavioral deficits in all ASD domains, impaired motor control, and poor sensorimotor learning. Both the patient and mouse model showed aberrant cerebellar foliation in high resolution MRI imaging, but no abnormalities in the cerebellar microarchitecture. PAX5 deficiency caused profound hypoplasia of the substantia nigra and ventral tegmental area due to loss of GABAergic neurons and thus affected central midbrain hubs in motor function and reward processing. Heterozygous Pax5-mutant mice exhibited similar anatomical and behavioral abnormalities. Lineage-tracing with novel Pax5-driven Cre and mCherry-reporter mice identified Pax5 as a crucial regulator of cerebellar morphogenesis and midbrain GABAergic neurogenesis. We demonstrate new roles of Pax5 in brain development and describe the underlying mechanism of a novel neurodevelopmental syndrome, thus connecting PAX5 with ASD and core deficits in motor control, sensorimotor learning, and cognition as well as hyperactivity.

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Poster

359. Comparative Anatomy: Development and Evolution

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 359.01

Topic: A.10. Development and Evolution

Support: NSF BCS-1846201

Title: A comparison of cannabinoid receptor 1-immunoreactive axon density in the amygdala among nine primate species

Authors: *D. N. JONES[1], K. N. HIRTER[1], E. L. MUNGER[1], C. C. SHERWOOD[2], W. D. HOPKINS[3], P. R. HOF[4], M. RAGHANTI[1];
[1]Dept. of Anthrop., Kent State Univ., Kent, OH; [2]Dept. of Anthrop., George Washington Univ., Washington, DC; [3]Dept. of Comparative Med. Michael E. Keeling Ctr. for Comparative Med. and Res., Univ. of Texas, Bastrop, TX; [4]Nash Family Dept. of Neurosci. and Friedman Brain Inst., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: The amygdala is a sensory integration center that plays an important role in emotional learning, behavior, and motivation. Cannabinoid signaling in the amygdala modulates aspects of anxiety, aggression, and fear in rodents via cannabinoid receptor 1, however little is known about cannabinoid signaling in the amygdala of humans and nonhuman primates. Primates are behaviorally diverse, with closely related species often displaying distinct social styles characterized by varying degrees of social tolerance and agonistic tendencies. Such behavioral differences are thought to be associated with neurochemical differences among species. Given what is known about the functional role of cannabinoid signaling in the amygdala, we tested whether relatively tolerant species, such as humans, bonobos, and marmosets, possess relatively higher cannabinoid receptor 1-immunoreactive (CB1R-ir) axon density in the basolateral amygdala. We used immunohistochemistry and stereological methods to compare CB1R-ir axon density among 47 primates representing nine species: humans (n=5), chimpanzees (n=6), bonobos (n=2), baboons (n=6), rhesus macaques (n=5), Japanese macaques (n=6), pigtail macaques (n=6), marmosets (n=5), and capuchins (n=6). The basolateral amygdala is comprised of the lateral, basal, and accessory basal nuclei. Stereological data for each nucleus was collected separately. After ruling out sex differences within each species, we used repeated measures ANOVA to evaluate species differences. The interaction ($F_{16,76} = 5.061$, $p<.001$) and main effects of species ($F_{8,38} = 8.007$, $p<.001$) and area ($F_{2,76} = 59.616$, $p<.001$) were all significant. However, the observedspecies differences did not support our hypothesis related to social tolerance nor did the data conform to a phylogenetic pattern. Instead, we found that while some closely related species differed from each other in a nucleus-dependent manner, some distantly related species shared unexpected similarities. Our results highlight the need for additional comparative work on the cannabinoid system from a molecular and genetic perspective. We discuss the implications of our observations with special focus on primate brain evolution and its connection to primate social style.

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Poster

359. Comparative Anatomy: Development and Evolution

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Program #/Poster #: 359.02

Topic: A.10. Development and Evolution

Support: NIGMS P20GM103446
NIGMS 5P20GM103653
NICHD 1R21-HD-101964-01A1
NICHD 7R21-HD101964-02
Auburn Start-up funds

Title: Translating time across the lifespan of primates identifies event variations in humans

Authors: *M. J. BRYANT[1], T. M. LEE[2], K. OFORI[3], B. RIGBY DAMES[4], C. FALCONE[5], C. J. CHARVET[1];
[1]Col. of Vet. Med., [2]Auburn Univ., Auburn Univ., Auburn, AL; [3]Delaware State Univ., Dover, DE; [4]Univ. of Bath, Bath, United Kingdom; [5]Scuola Internazionale Superiore di Studi Avanzati, Trieste, Italy

Abstract: Much research relies on model species, and the creation of translational tools from model systems to humans is needed to enhance biomedical research. In particular, the generation of cross-species age alignment tools will bridge the gap across model systems and humans. To that end, we collected a comprehensive dataset, consisting of 489 time points, captured across behavioral, structural, and transcriptional scales. These included time points from transcriptional variation extracted from machine learning models. These data were collected across the lifespan of humans and eight other primate species including apes (e.g., orangutans, chimpanzees) and monkey species (i.e., marmoset, macaques). We aligned ages by applying a linear model to these time points (expressed in days after conception) versus an event scale, which is an ordering of time points averaged across species. Aligning ages across the studied species revealed possible heterochronies (relative accelerations or decelerations in timing) of select biological pathways. In particular, the timing of deciduous tooth eruption, cranial and colossal growth, and carpal ossification appeared to deviate relative to the timing of other biological programs in human and non-human primates. These heterochronies account for diverse adaptations in locomotor and dietary abilities among primate species, and enhance our ability to translate ages across species.

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Poster

359. Comparative Anatomy: Development and Evolution

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Program #/Poster #: 359.03

Topic: A.10. Development and Evolution

Support: FONDECYT 1210069
FONDECYT 1210169

Title: Main stages of the ontogeny of the chicken visual tecto-isthmo-fugal pathway take place independently of retinal influences

Authors: R. REYES-PINTO[1], T. VEGA-ZUNIGA[2], H. LUKSCH[3], G. J. MARÍN[1], *J. MPODOZIS[1];
[1]Facultad de Ciencias, Univ. de Chile, Santiago, Chile; [2]Inst. of Sci. and Technol. Austria (IST Austria), Klosterneuburg, Austria, Austria; [3]Tech. Univ. of Munich, Freising, Germany

Abstract: The tectofugal pathway, a highly conserved retino-midbrain-fugal visual system, is the main visual pathway in birds and many mammals. In birds the tectofugal projection arises from the tectal ganglion cells (TGCs), which possesses large fields of specialized dendritic endings distributed in specific superficial tectal layers. TGCs receive synaptic contact from retinal fibers and from axon terminals of the nucleus isthmi parvocellularis (Ipc). The Ipc exerts a strong control on the propagation of visual activity from the TGCs to higher visual areas. TGCs axons projects bilaterally to the thalamic nucleus rotundus (Rt), which in turn project to the visual dorsal ventricular ridge (vDVR), a trilaminar complex in the ventrolateral pallium. Interconnections between layers of the vDVR follow a "columnar/ recurrent" arrangement comparable to that of the columnar circuitry of the mammalian sensory cortex. We sought to describe the ontogenetic course of the establishment of the chicken tecto-isthmo-fugal circuitry, as well as to assess the possible influence of the retinal afferences in this process. Neurotracing “ex-vivo” experiments revealed a highly organized Rt-vDVR projection as early as E8, and a columnarly organized system of axons connecting the vDVR layers from E10 onwards. Immunohistochemical assays suggest that both system of projection probably emerges in conjunction, as early as E6-E8. In contrast, Ipc axonal terminals, as well as TGCs dendritic endings exhibit a mature morphology only at about E18, which is the early stage at which a mature pattern of retinotectal connectivity can be found. Retinal ablations at E4 altered the cytoarchitecture and volume of the TeO and Rt, however, they did not modify the arrangement of the thalamo-palial-intrapalial circuitry assessed at several developmental stages, including E17. Similarly, these ablations did not alter the expression pattern of specific molecular markers in the Rt and vDVR. Thus, we found that the development of the chicken tectofugal pathway seems to proceed in two phases: First, the tecto-thalamo-pallial-intrapalial circuitry is established, and subsequently, a mature retino-isthmo-tectal projection is attained. In addition, we found that the establishment and subsequent maintenance of the Rt-E and E-M circuitry is independent of the early retinal inputs, suggesting that the transmission of retinal activity through this pathway would only be possible from E18 onwards. We conclude that in *Gallus gallus*, in contradistinction to mammals, the establishment of visual sensory pathways seems to be largely independent of bottom-up, spontaneous or visually elicited, influences

Disclosures: **R. Reyes-Pinto:** None. **T. Vega-Zuniga:** None. **H. Luksch:** None. **G.J. Marín:** None. **J. Mpodozis:** None.

Poster

359. Comparative Anatomy: Development and Evolution

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 359.04

Topic: A.10. Development and Evolution

Title: A new multimodal map of the pigeons meso- and hyperpallium

Authors: *C. HEROLD[1], M. N. CAPPALLO[1], N. PALOMERO-GALLAGHER[1,3], O. GÜNTÜRKÜN[4], M. AXER[3,5], K. AMUNTS[2,3];
[1]C. and O. Vogt Inst. for Brain Research, Med. Fac. and Univ. Hosp. Düsseldorf, Heinrich Heine Univ. Düsseldorf, Düsseldorf, Germany; [2]C. and O. Vogt Inst. for Brain Research, Med. Fac. and Univ. Hosp. Düsseldorf, Heinrich Heine Univ. Düsseldorf, Duesseldorf, Germany; [3]Inst. of Neurosci. and Med. INM-1, Res. Ctr. Juelich, Juelich, Germany; [4]Dept. of Psychology, Inst. of Cognitive Neuroscience, Biopsychology, Ruhr-University Bochum, Bochum, Germany; [5]Univ. of Wuppertal, Wuppertal, Germany

Abstract: In birds, the dorsal pallium developed into the hyperpallium and thus is considered homologous to the mammalian cortex. Based on the connectivity profile of the hyperpallium, earlier studies suggested that it can be divided into a four-layered, small rostral part, involved in somatosensory information processing and a much larger part, visual in nature. Both parts receive functionally separated input from thalamic nuclei with a high number of fibers targeting the interstitial part of the hyperpallium apicale (IHA) that resembles layer IV of the cortex, while the visual part also receives thalamic input in the dorsal hyperpallium (HD), which resembles together with the hyperpallium intercalate (HI) layer II/III of cortical organization. The overlaying layer hyperpallium apicale (HA) is thus comparable to cortical layer V/VI. To most of the hyperpallial rostro-caudal extent, ventrally, the mesopallium is located. It belongs to the so-called dorsal ventricular ridge (DVR) and its homology to the exact pallial parts (layers, cells, lateral or ventral pallial origin) is still a matter of debate. However, it has been shown that the mesopallium harbors a cortex-like fiber architecture and comparable to HI/HD, the mesopallium is integrated into a canonical circuit to process sensory information. Thereby, it is indicated that trigeminal, visual-tectofugal and auditory information is processed in individual parts along the rostro-caudal axis of the mesopallium.Within our multi-modal approach that includes cellular Nissl-stained, 3D-Polarized-Light- Imaging and neurotransmitter-receptor binding studies, we can now separate the two large pallial structures based on cyto-, fiber- and receptor-architectonic analysis into several sub-structures along the rostro-caudal axis that indeed show different in- and outputs. Thus, we will present a new map of the hyper- and the mesopallium that allows us a more detailed comparison to visual and sensory cortical areas/layers of the mammalian brain. In the future, this will provide us with the basis to resolve questions about the functional similarities of pallial bird brain areas that evolved in between 320 million years of separate evolution to

mammals. Surely, this will help us to gain additional knowledge of how these little birds reached out to become such high-flyers developing the same functional organization principles like the mammalian forebrain.

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Poster

359. Comparative Anatomy: Development and Evolution

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 359.05

Topic: A.10. Development and Evolution

Title: Impact of malocclusion traits on the quality of life, anxiety, and depression among the Mongolian population

Authors: Z. BODIKHUU[1], M. MUNKHTSETSEG[1], G. TUMUR-OCHIR[2], B. LKHAGVASUREN[3], G. GANBURGED[1], ***T. JADAMBA**[4];
[1]Mongolian Natl. Univ. of Med. Sci., Ulaanbaatar, Mongolia; [2]Brain and Mind Res. Institute, Mongolian Acad. of Sci., Ulaanbaatar, Mongolia; [3]Intl. Univ. of Hlth. and Welfare Narita Hosp., Narita, Japan; [4]Brain and Mind Res. Institute, MAS, Ulaanbaatar, Mongolia

Abstract: Background: The purpose of our population-based, cross-sectional study was to assess the association between malocclusion and the quality of life (QoL), anxiety, and depression. **Methods:** The study was conducted between July and October, 2020, in Mongolia. Clinical examinations were carried out by a trained orthodontist. Using a millimeter ruler, excessive and reverse overjet were recorded as abnormal. Crowding was recorded for the incisor and posterior segments. Anterior diastema was diagnosed when there was a space of at least 1 mm between incisors. Facial profile (straight, convex, and concave) was determined by vision using soft tissue reference points. Each participant completed the World Health Organization Quality of Life (WHO-QoL-BREF), Hospital Anxiety and Depression Scale (HADS), and orthodontic questionnaires. **Results:** The study consisted of 436 participants, aged between 13 and 65 years (mean age=39.6±14.8), and the majority were females (68.1%, n=297). The prevalence of malocclusion, in general, was (85.1%, n=371). In terms of the prevalence of the malocclusion traits: abnormal overjet was (56.2%, n=245), crowded dentition was (27.1%, n=118), and diastema was (17.2%, n=75). Participants with malocclusions had increased depression scores (p=0.008). Participants with diastema had decreased QoL in physical and social domains (p=0.022, p=0.020). Moreover, reverse correlations were found between depression scores and QoL in the psychological, social, and environmental domains within the population with malocclusion traits (p=0.035, p=0.0039, p=0.002). **Conclusions:** We found that the prevalence of malocclusion was 85.1% in the general population. Participants with malocclusion have decreased QoL, which in turn is associated with increased depression scores.

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Poster

359. Comparative Anatomy: Development and Evolution

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 359.06

Topic: A.10. Development and Evolution

Title: Controllability of the infant brain

Authors: *H. SUN, A. DUFFORD, W. DAI, D. SCHEINOST;
Yale Univ., New Haven, CT

Abstract: The architecture of the infant brain develops extensively during early life and increasingly supports coordinated control of brain activities. However, less is known about how the brain structural network develops to support brain dynamics in infancy. Network control theory provides a mathematical framework to understand these dynamics (Gu et al. 2015; Deng and Gu 2020). Controllability of a region refers to its ability to manipulate the network towards a certain dynamical state. Here, we applied network control theory to the infant structural connectome to investigate how different brain regions facilitate brain dynamics in early life. This study used diffusion MRI data of 642 infants from the Developing Human Connectome Project. The infants were born at a median of 39.14 weeks gestational age at birth (GA) [range: 23-42.29] and scanned at a median of 40.43 weeks postmenstrual age at scan (PMA) [range: 26.71-45.14]. After preprocessing steps, a neonatal-adapted AAL atlas was applied to each individual to parcellate the brain into 90 regions. The individual structural connectome was constructed by calculating the sum of tracts connecting each pair of regions using DSI-Studio. To quantify the controllability of each brain region, we employed two measurements: the average controllability (AC) to measure the ability to drive nearby brain state transition and the modal controllability (MC) to measure the ability to drive distant brain state transition. Regions with the highest average and modal controllability were concentrated in the frontal cortex and temporal lobe. Infants with higher whole-brain AC also show higher MC ($r=0.50$, $p<0.001$), suggesting that brain networks that support nearby brain state transitions also support those transitions with a longer distance. Both whole-brain AC and MC are more significantly correlated with PMA ($r=-0.80$, $p<0.001$; $r=-0.44$,$p<0.001$), compared to GA ($r=-0.67$,$p<0.001$; $r=-0.42$, $p<0.001$).
In conclusion, we implemented network control theory to quantify controllability at both regional and whole-brain levels in a large sample of newborn infants. Controllability was highest in frontal and temporal regions and correlated with age at birth and age at scan, indicating the role of birth in influencing the controllability of the infant brain.

Disclosures: **H. Sun:** None. **A. Dufford:** None. **W. Dai:** None. **D. Scheinost:** None.

Poster

359. Comparative Anatomy: Development and Evolution

Location: SDCC Halls B-H

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Program #/Poster #: 359.07

Topic: A.10. Development and Evolution

Support: NSF # 2123061

Title: Mechanics of interplay between gyrification and connectivity in the developing brain

Authors: *M. RAZAVI;
Mechanical Engin., Binghamton Univ., Vestal, NY

Abstract: A connection between abnormal gyrification (surface morphology) and underlying connectivity (neuronal fibers) has been observed in several brain disorders such as ASD, schizophrenia, bipolar. However, the mechanics and underlying mechanisms of this correlation have not been explored and explained yet. Therefore, there is a vital need to discover the role of axonal fibers of the brain's connectivity on the formation and modulation of folding patterns in the developing human brain. The lack of knowledge of the physical interplay between cortical folding and connectivity is a critical barrier to the fundamental understanding of the relationship between cortical folding, brain connectivity, and brain function in different neurodevelopmental stages. This study aimed to delineate how the interplay between the differential tangential growth of cerebral cortex and the tension of axonal fibers induces and regulates the folding patterns in a developing human brain. To achieve this aim, image-based multiscale mechanical models on the basis of the embedded nonlinear finite element method was employed to investigate a set of growth and folding scenarios. Our results showed that the differential growth between cortical and subcortical layers is the main inducer of cortical folding. In addition, the gyrification of the cortex pulls the areas with a high density of stiff axonal fiber bundles towards gyri rather than sulci; therefore, axonal fiber bundles induce symmetry breaking, and regulate the folding patterns. In particular, the spatial distribution of axonal fiber bundles is the determinant factor to control the locations of gyri and sulci, the observation that has been made by imaging studies, but not elucidated fundamentally. As another result, the special type of gyral folds, called 3-hinge gyral folds, developed in the growth models similar to the natural brain folds. The 3-hinge gyral folding is the conjunction of gyri crest lines from three different orientations. Our results clearly indicated that the density of fiber bundles after folding is higher in the 3-hinge gyral folds in agreement with prior brain tractography studies. In conclusion, we propose that brain connectivity might be the main regulator of folding patterns responsible for the formation of regular cortical folding patterns. This study provides a deeper understanding of cortical folding and mechanics of the linked impaired connectivity and gyrification which is the key to interpreting the normal development of the human brain during the early stages of growth.

Disclosures: M. Razavi: None.

Poster

359. Comparative Anatomy: Development and Evolution

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 359.08

Topic: A.10. Development and Evolution

Support: NSF IOS 1457291

Title: Analyzing the relationship between skull morphology and brain morphology in domestic dogs

Authors: *S. A. BARTON[1], M. KENT[2], E. E. HECHT[1];
[1]Human Evolutionary Biol., Harvard Univ., Cambridge, MA; [2]Col. of Vet. Med., Univ. of Georgia, Athens, GA

Abstract: Linking variation in brain anatomy to variation in perception, cognition, and behavior is one of the major goals of neuroscience. Domestic dogs offer a unique and powerful opportunity to address this issue because different breeds have been selected for different perceptual, cognitive, and behavioral capacities. However, dogs have also been selectively bred for appearance, including substantial variation in head shape across breeds. It is possible that this extreme artificial selection on external skull morphology might have had effects on the size and shape of the endocranial cavity and therefore the brain. To assess this question, we examined T1-weighted MRI images of 67 dogs from 33 breeds, plus an additional 12 dogs of mixed or unknown breeds. Scans were opportunistically collected from a veterinary MRI scanner from dogs who were referred for neurological examination but were not found to show any neuropathology. Images were segmented into gray matter, white matter, and CSF. Gray matter segmentations were registered to a bilaterally symmetric, study-specific template using ANTS and then modulated by the Jacobian determinant of the warpfield, an index of where and how much each subject's scan had to deform to come into alignment with the template. We also measured the maximum left-right, superior-inferior, and anterior-posterior dimensions of the endocranial cavity. Several cortical and subcortical regions were significantly correlated with variation in endocranial cavity dimensions. We discuss the potential relevance of these results for brain and behavior differences across dog breeds.

Disclosures: S.A. Barton: None. **M. Kent:** None. **E.E. Hecht:** None.

Poster

359. Comparative Anatomy: Development and Evolution

Location: SDCC Halls B-H

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Program #/Poster #: 359.09

Topic: A.10. Development and Evolution

Support: NIH Grant RF1 MH124605-01

Title: A common coordinate framework for quantitative cell census in developing mouse brains

Authors: *F. A. KRONMAN[1], J. LIWANG[1], R. BETTY[1], S. MANJILA[1], J. MINTEER[1], L. PUELLES[2], J. GEE[3], J. ZHANG[4], L. NG[5], Y. KIM[1];
[1]Dept. of Neural and Behavioral Sci., Penn State Col. of Med., Hershey, PA; [2]Dept. of Human Anat. and Psychobiology, Univ. Murcia Fac of Med., Murcia, Spain; [3]Univ. of Pennsylvania, Philadelphia, PA; [4]3Bernard and Irene Schwartz Ctr. for Biomed. Imaging, Dept. of Radiology, New York Univ., New York, NY; [5]Allen Inst. For Brain Sci., Seattle, WA

Abstract: Recent advances in brain cell type mapping with data integration in standard spatial framework promote collaboration via multi-team science approaches to unravel details of cell type diversity in the mouse brain. However, unlike in the adult mouse brain, a lack of standard 3D reference atlases in developing mouse brains significantly limits data integration of different studies to understand brain development. Relying on 2D distorted male histology samples to delineate anatomical regions, existing developmental mouse brain reference atlases fail to meet both technological standards of the modern 3D imaging as well as the standards of equitable inclusion of female subjects in science. Here, we present multimodal 3D developmental common coordinate frameworks (DevCCFs) that account for differential brain morphology during mouse brain development. We generated DevCCFs composed of a morphological average of male and female mouse brains at postnatal day (P)4, P14, and P56. We used light sheet fluorescent microscopy and magnetic resonance imaging to create undistorted morphologically averaged templates at 10um isotropic resolution. Moreover, we are establishing iterative 3D anatomical parcellation at each age defined by a developmental ontology. Iterative parcellations allow modifications in the DevCCFs as anatomical standards continue to be understood based on community knowledge of the developing brain. Thus, the DevCCFs can be used to analyze and integrate signals from various imaging modalities at different scale to promote open, collaborative, and inclusive neuroscience.

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Poster

359. Comparative Anatomy: Development and Evolution

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Program #/Poster #: 359.10

Topic: A.10. Development and Evolution

Support: NIH grant 2R01MH113257-06

Title: Late prenatal to elderly developmental trajectory of the expression of SMI-32 in macaque motor cortex

Authors: *F. KAMARA[1], T. SPADORY[2], P. RAKIC[3], A. DUQUE[4];
[1]Yale Med. Sch., New Haven, CT; [2]Neurosci., Yale Univ., New Haven, CT; [3]Dept. of Neurosci., Yale Univ. Sch. Med, Dept of Neurosci., New Haven, CT; [4]Neurosci., Yale Univ. Sch. Med., New Haven, CT

Abstract: Previous studies, in human and non-human primates, have used staining with monoclonal antibody SMI-32, which recognizes non-phosphorylated neurofilament protein, to study different aspects of cellular, laminar, and regional organization within the cortex. SMI-32 positive pyramidal neurons in human neocortex were found to be highly vulnerable to degeneration in Alzheimer's disease (AD), and a drastic reduction in SMI-32 immunoreactivity has been described in the cortical layers where tangle-bearing neurons are localized. Rhesus macaques are commonly used as models of human aging and disease; however, many of these studies were conducted only in adults. Here, evidence is presented to demonstrate that the expression of SMI-32 in the soma, dendrites, and axons of the small proportion of neurons in the motor cortex that express SMI-32, including the prominent Betz cells, drastically varies with age. The present study was carried out at 7 distinct developmental ages, from late gestation to elderly (19 years of age). All materials used are from collection 6 of the MacBrain Resource Center housed at Yale University (https://medicine.yale.edu/neuroscience/macbrain/collections/), which contains coronal series of sections spaced 1 mm apart from the rostral to the caudal tips of the macaque brain, and all materials are publicly available. The most salient findings are that prenatally, in addition to somata and proximal dendrites, long spans of subcortically projecting axons are strongly labeled, including the axon initial segment. As time passes, staining further from the soma takes place and most staining in the proximal dendrites and axons deteriorates and becomes fragmented or disappears. Later in life, distal parts of the dendrites are labeled while the most proximal parts are not. Betz cells' somata become smaller and many are reduced to stumps. SMI-32 expression in the axons traveling the posterior limb of the internal capsule, and in those destined to go across the corpus callosum, appears fragmented and is observed only in later postnatal life. These observations open new avenues of interpretation for the results of previous studies and contribute to a better understanding of the cellular processes at play during the primate life span, including normal aging and the formation of neurofibrillary tangles common in AD.

Disclosures: F. Kamara: None. **T. Spadory:** None. **P. Rakic:** None. **A. Duque:** None.

Poster

359. Comparative Anatomy: Development and Evolution

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Program #/Poster #: 359.11

Topic: A.10. Development and Evolution

Title: Age-associated patterns of myelin in axons of the corpus callosum in Virunga mountain gorillas (Gorilla beringei beringei)

Authors: *M. LOPEZ[1], C. CRAIN[2], R. MUVUNYI[4], A. MUDAKIKWA[4], M. CRANFIELD[5], K. GILARDI[5], C. SHERWOOD[6], K. A. PHILLIPS[3];
[1]Trinity Univ., [3]Psychology, [2]Trinity Univ., San Antonio, TX; [4]Rwanda Develop. Board, Kigali, Rwanda; [5]Gorilla Doctors, Davis, CA; [6]Anthrop., George Washington Univ., Washington, DC

Abstract: Compared to other great apes, Virunga mountain gorillas display accelerated life history strategies. These strategies are believed to be due to their dietary ecology, as their largely herbivorous diet displays continuity across seasons. The abundance of foliage diminishes energetic risk, leading to a shorter age of weaning, earlier first birth, and shorter interbirth intervals. In addition, Virunga mountain gorillas show relatively accelerated brain development, attaining adult brain mass around 3-4 years. To better understand variation in brain structure of primates, we quantified microstructural characteristics of the corpus callosum across the lifespan in Virunga mountain gorillas. Measurements of myelin thickness, density, and myelin fraction were obtained from electron microscopy digital photographs of the five regions of the corpus callosum of eight mountain gorillas ranging in age from infancy (1.2 years) to late adulthood (>40 years). We hypothesized that, in line with known data regarding brain growth cessation in mountain gorillas, there would be a period of rapid growth of myelin through 4 years, at which point the measures of myelin would decrease with age. Across the whole sample, distribution of myelin thickness showed a right skew. The most frequently occurring myelin thickness was 0.20µm; myelin thickness was infrequently >0.58µm. Our results indicate a pattern of rapid expansion of myelin in juveniles, but display no clear pattern of age-related myelin changes in adults. Additional regional differences were seen in myelin thickness across regions, such that in juveniles, myelin thickness in region I was diminished compared to regions II, III or IV. These differences, particularly since they were only present in juveniles, could reflect different developmental trajectories of the prefrontal lobe compared to other areas of the cortex. In the splenium, the overall distribution of myelination thickness and myelin fraction shows a similar pattern as chimpanzees. However, compared to chimpanzees, Virunga mountain gorillas show accelerated development of the splenium.

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Poster

359. Comparative Anatomy: Development and Evolution

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Program #/Poster #: 359.12

Topic: A.10. Development and Evolution

Support: U.S. Department of Education Award Number P382A150041

Title: The Density of Nuclear Pores in Neurons of Adult Mouse and Macaque Cortex

Authors: *D. GEORGE[1], N. B. KASTHURI[2], A. MASELLI[1];
[1]Biol. Sci., Chicago State Univ., Chicago, IL; [2]Neurobio., Univ. of Chicago, Chicago, IL

Abstract: By using volume electron microscopy (EM) connectomics to study connections between individual neurons, we can observe important structural information and discover interesting insights into neurodegenerative diseases. While volume EM has been extensively used to map connections between neurons, it has been less widely used to study the internal composition and ultrastructure of neurons. In this study, we use volume EM to reconstruct individual neuronal nuclear pores of excitatory neurons in two data sets: adult mouse and macaque cortex. We manually labeled the nuclear membrane and individual nuclear pores, using the free software VAST (Volume Annotation and Segmentation Tool). Nuclear pores allow for the communication and transit of molecules between the nucleus and cytoplasm. A thorough investigation of the distribution and cross-species comparison of nuclear pores is not present in the literature, but such an investigation would provide insight into whether nuclear pore distribution is determined by nucleocytoplasmic dynamics and whether these dynamics are conserved between the model species used in this investigation. In addition to presenting a proof-of-concept study for using Volume EM and the VAST software to map the number and location of nuclear pores in an adult mouse and primate brain, we sought to introduce a pixel analysis of the segmentations generated for both datasets. The pixel analysis will provide greater confidence for the cross-species nuclear pore results, while also serving to potentially assist in the expansion of this nuclear pore study to include a mitochondrial perspective. Consequently, the future of this investigation will seek to present a mitochondrial aspect to the analysis of the distribution of nuclear pores.

Disclosures: D. George: None. **N.B. Kasthuri:** None. **A. Maselli:** None.

Poster

359. Comparative Anatomy: Development and Evolution

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Program #/Poster #: 359.13

Topic: A.10. Development and Evolution

Support: AMED JP22dm0307006

Title: Non-invasive T1w/T2w myelin mapping of nonhuman primates: interspecies comparison of cortical structure of macaque, night monkey, and marmoset.

Authors: *T. IKEDA[1], J. A. AUTIO[1], A. KAWASAKI[1], C. TAKEDA[1], T. OSE[1], M. TAKADA[2], D. C. VAN ESSEN[3], M. F. GLASSER[3,4], T. HAYASHI[1];
[1]Ctr. for Biosystems Dynamics Res., RIKEN, Kobe, Japan; [2]Ctr. for the Evolutionary Origins of Human Behavior, Kyoto Univ., Inuyama, Aichi, Japan; [3]Dept. of Neurosci., [4]Dept. of Radiology, Washington Univ. Sch. Med., Saint Louis, MO

Abstract: Neuroimaging studies using nonhuman primates (NHPs) provide insights for understanding the structure, function, and evolution of the human brain. High-quality MRI and cortical surface-based analysis methods in humans (Glasser et al. 2013, 2016) were recently extended to NHPs including macaques (Donahue et al. 2018; Autio et al. 2020) and marmosets (Ose et al. 2022), potentially enabling direct interspecies comparisons (Hayashi et al. 2021). Here we applied this species-harmonized method to night monkeys (9 *Aotus lemurinus*) and compared their cortical organization with that in macaques (22 *Macaca mulatta* and 10 *Macaca fascicularis*) and marmosets (20 *Callithrix jacchus*). MRI data were collected using a 3T MRI scanner and multi-array RF coils designed for NHPs (Autio et al., 2020). Structural images were acquired using 3D T1 and T2-weighted (T1w and T2w) scans under deep anesthesia and were analyzed using a NHP version of Human Connectome Project (HCP) pipeline, which corrected intensity bias, transferred into a standard space for each species, segmented brain structures, estimated cortical inner (white) and outer (pial) surfaces, registered the surfaces across subjects using folding patterns, followed by generation of myelin maps with T1w/T2w ratio (Glasser and Van Essen 2011). Cortical surfaces and maps of thickness, curvature, and myelin were resampled to a 32k mesh and averaged across subjects. Despite the significant difference in gyrification pattern, these three species share a similar pattern of cortical thickness and myelination: thin and heavily myelinated in early sensory areas and thick and lightly myelinated in association areas. Cortical maps of myelin and its gradient were analyzed in detail to parcellate three functionally different regions: a heavily myelinated MT+ complex, a moderate to heavily myelinated auditory cortex, and a lightly myelinated parietal region, which corresponds to Brodmann area 7 in macaque (BA7). Quantitative comparisons of areal size revealed that MT+ complex and auditory cortex are significantly larger in night monkey (2.4% and 2.5% relative to the total cortex surface, respectively) than marmoset (1.2% and 1.5%) or macaque (0.9% and 0.6%). In contrast, the relative size of BA7 was larger in the macaque (3.2%) than in night monkey (2.3%) or marmoset (2.0%). These findings suggest that enlarged cortical areas for auditory and motion perception in the night monkey may reflect cortical adaptations to the nocturnal environment. Our T1w/T2w myelin mapping enables us to quantitatively compare cortical organization across Old World and New World monkeys.

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Poster

359. Comparative Anatomy: Development and Evolution

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Program #/Poster #: 359.14

Topic: A.10. Development and Evolution

Support: NIH F32 NRSA FHD103481A

Title: Translating the timing of developmental benchmarks in Monodelphis domestica to facilitate generalization of experimental findings in rodents

Authors: *C. BRESEE, J. LITMAN-KLEPER, C. J. CLAYTON, L. A. KRUBITZER; UC Davis, Davis, CA

Abstract: The gray short-tailed opossum, *Monodelphis domestica*, is the most used marsupial model species, but is still vastly under-utilized in comparison to rodents. Mice and other rodents are in many ways exceptionally good models, with their small size, short generations, docility, and ubiquity. Though much of neuroscience is aimed at understanding human brains, one could describe modern neuroscience as the study of the mouse brain, since the majority of studies in mammals are done in mice [1]. In order to understand human brains, we must 1) understand the mammalian brain in general, 2) have outgroups with which to contrast results from euarchontoglires, and 3) not become so specialized in mouse neuroscience that findings do not apply to primates. *M. domestica* is an alternative model species that both presents many of the advantages of mice (small, short generations, docile, and accessible). In addition, it is the only marsupial to have its genome sequenced . These advantages help fill in gaps with mouse research; allowing comparative evolutionary studies that are difficult or impossible if restricted to rodents. We present data to facilitate the use of *M. domestica* in comparative developmental neuroscience studies. First, we present diagrams describing externally observable morphological characteristics typifying important postnatal developmental benchmarks, from observations of 22 pups from 6 litters at 25 timepoints. Second, we present timepoints at which various neurological structures develop, comparing data between mice, rats, and *M. domestica*, from primary literature and the Translating Time dataset [2]. These comparisons reveal that, though opossums are born at embryonic day (E) 14, their nervous systems are similar to that of an E10-E14 rodent, resulting in pups accessible to manipulation during many developmental events. These data are designed to facilitate wider adoption of M. domestica as a model species. The table allows researchers generalizing results from rodents to look up the age at which manipulations should be done in M. domestica. The staging diagrams are then used to confirm when pups are the appropriate age to include in experiments. References: [1] Manger PR, Cort J, Ebrahim N, Goodman A, Henning J, Karolia M, Rodrigues S, and Štrkalj G (2008) Is 21st century neuroscience too focused on the rat/mouse model of brain function and dysfunction? Frontiers in Neuroanatomy, V2, 5 [2] Clancy B, Darlingron RB, Finlay BL (2001) Translating developmental time across mammalian species. Neuroscience 105(1):7-17

Disclosures: C. Bresee: None. **J. Litman-Kleper:** None. **C.J. Clayton:** None. **L.A. Krubitzer:** None.

Poster

359. Comparative Anatomy: Development and Evolution

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 359.15

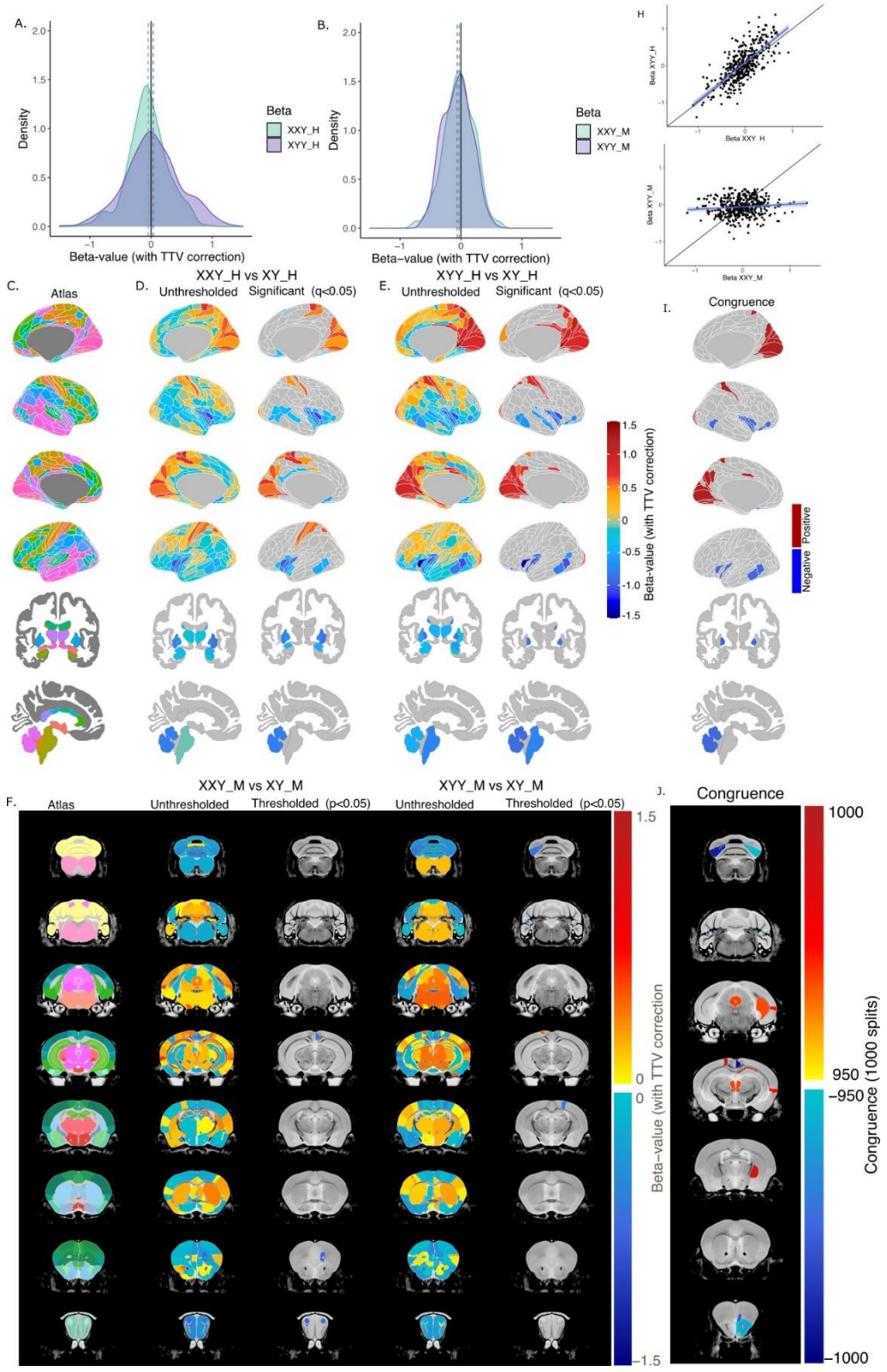
Topic: A.10. Development and Evolution

Support: Fonds de Recherche du Quebec en Sante
NIH Grant

Title: Sex chromosome trisomy significantly alters human, but not mouse brain anatomy

Authors: *E. GUMA[1], A. BEAUCHAMP[4], S. LIU[2], E. LEVITIS[3], L. CLASEN[2], E. TORRES[2], J. BLUMENTHAL[5], F. M. LALONDE[6], L. R. QIU[4], H. HRNCIR[7], A. MACKENZIE-GRAHAM[8], X. YANG[7], A. P. ARNOLD[9], J. P. LERCH[10], A. RAZNAHAN[11];
[1]Natl. Inst. of Mental Hlth., [2]Natl. institute of Mental Hlth., Bethesda, MD; [3]Natl. institute of Mental Hlth., Behtesda, MD; [4]Univ. of Toronto, Toronto, ON, Canada; [5]Natl. Inst. of Mental Hlth., Bethesda, MD; [6]Natl. Inst. of Mental Hlth., Reston, VA; [8]UCLA, [7]UCLA, Los Angeles, CA; [9]Univ. of California Los Angeles, Univ. of California Los Angeles, Los Angeles, CA; [10]Oxford Univ., Oxford, United Kingdom; [11]NIMH/NIH, NIMH/NIH, Bethesda, MD

Abstract: Sex chromosome aneuploidies (SCA) (characterized by abnormal X- and/or Y-chromosomes count) are associated with altered neuroanatomy and increased risk for psychopathology. Studying animal models such as the Sex Chromosome Trisomy (SCT) mouse model may provide mechanistic insight into SCA effects on the brain. We investigate the effects of SCA on human and mouse brains, leveraging comparative neuroimaging-derived brain volume measures. T1w structural magnetic resonance images (MRI) were gathered from humans with SCA and controls (XXY_H: n=99, XY_H: n=82 & XYY_H: n=34, XY_H: n=37). T2w scans were acquired in adult gonadal male mice from the SCT model (XXY_M: n=17 & XYY_M: n=20, XY_M: n=18). We (1) quantify the effects of SCA on total tissue volume (TTV) & regional brain volume; (2) assess the spatial convergence of effects for each SCA in each species; (3) assess similarity of effects between species for a set of homologous regions. We find that SCA alters human TTV (sig. dec. in XXY_H: β=-0.94,$p<0.001$: non-sig. inc. in XYY_H: β=0.42,p=0.12), but not mouse (XXY_M and XYY_M β>-0.31,$p>0.4$]). Highly similar effects of XXY & XYY on regional brain volume in humans (r=0.7) including significant volume increases in the occipital, parietal, and frontal cortex, and volume decreases in the insula, temporal cortex, cerebellum, brainstem, and basal ganglia (TTV & age corrected). Mice lacked statistically-significant effects of SCA on regional volume, although bootstrap analysis revealed subtle effects of XXY & XYY on regional volume (r=0.002). Convergent volume increases between XXY & XYY mice were observed in the parietal association cortex (inc. in humans), right (R) dorsal pallidum (dec. in humans), while volume decreases were found in R cingulate cortex (dec. in humans), R primary somatosensory cortex (inc. in humans), R orbital area (dec. in humans), and the left cerebellum (dec. in humans). In sum, SCA effects are larger in humans than mice. Some subthreshold similarities and dissimilarities exist between species, pointing to potential cross-species vulnerability of some regions to SCA.



Disclosures: E. Guma: None. **A. Beauchamp:** None. **S. Liu:** None. **E. Levitis:** None. **L. Clasen:** None. **E. Torres:** None. **J. Blumenthal:** None. **F.M. Lalonde:** None. **L.R. Qiu:**

None. **H. Hrncir:** None. **A. Mackenzie-Graham:** None. **X. Yang:** None. **A.P. Arnold:** None. **J.P. Lerch:** None. **A. Raznahan:** None.

Poster

359. Comparative Anatomy: Development and Evolution

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 359.16

Topic: A.10. Development and Evolution

Title: Breastfeeding and Booze: how maternal drinking behavior impacts murine offspring brain and behavioral development.

Authors: *R. F. PEREZ, Jr.[1], K. E. CONNER[2], M. NABATANZI[1], M. ERICKSON[1], K. J. HUFFMAN[1,2];
[1]Psychology, [2]Interdepartmental Neurosci. Program, Univ. of California, Riverside, Riverside, CA

Abstract: Offspring alcohol exposure during gestation alters brain and behavioral development. Thus, the CDC advises against maternal alcohol consumption during pregnancy. However, little emphasis has been placed on educating new parents about alcohol consumption while breastfeeding. This is partly due to a paucity of research on lactational ethanol exposure effects in children; although, it has been shown that infants exposed to ethanol via breastmilk frequently present with reduced body mass, low verbal IQ scores, and altered sleeping patterns (Tay et al., 2017; May et al., 2016). As approximately 36% of breastfeeding mothers in the US consume alcohol (May et al., 2016) continued research in this area is critical. Our study employed a novel murine lactational ethanol exposure (LEE) model, where offspring were administered ethanol via nursing from postnatal day (P) 6 through P20. Wellness metrics (blood ethanol concentration, BEC; and plasma osmolality, pOsm) were measured at weaning (P20) for dams and pups. Experimental dams had higher BEC levels when compared to controls, as did LEE pups at P20. Notably, all dam and pup pOsm levels were normal. We investigated potential sex specific phenotypes via multiple ANOVA. Offspring body weights were lower for male and female LEE offspring at P20 and P30. Offspring brain weights were lower for male and female LEE offspring at P20; however, only male LEE brain weights were lower at P30. Cortical lengths of male and female LEE mice were smaller only at P30, compared to controls. Neocortical architecture, as observed in Nissl-stained tissue, revealed a decrease for male and female LEE frontal cortex thickness compared to controls; however, no differences were observed in prelimbic, somatosensory, auditory, and visual cortices. Dendritic spines in neocortex were visualized using Golgi-Cox staining and spine densities were computed; no significant differences were observed for any age, sex, or condition. However, results of behavioral tests indicated main effects of treatment for LEE mice, with increased time spent in the uncovered arms of the elevated plus maze and decreased time spent immobile in the forced swim test. No differences were observed in the accelerated rotarod, three-chambered sociability task, and Suok bar test. In summary, our data describe potential adverse brain and behavioral developmental outcomes due to LEE. Thus,

women should be advised to refrain from consuming alcohol during breastfeeding until additional research can better guide recommendations of safe maternal practices in early infancy.

Disclosures: R.F. Perez: None. **K.E. Conner:** None. **M. Nabatanzi:** None. **M. Erickson:** None. **K.J. Huffman:** None.

Poster

359. Comparative Anatomy: Development and Evolution

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 359.17

Topic: A.10. Development and Evolution

Support: James S. McDonnell Foundation, Grant/Award Number: 220020516
National Institute of Child Health and Human Development, Grant/ Award Number: R01HD084362-01A1

Title: Nature and nurture: neurodevelopment and parental care in the prairie vole (Microtus ochrogaster)

Authors: *M. NABATANZI[1], R. F. PEREZ, Jr.[1], R. T. BOTTOM[2], L. A. KRUBITZER[4], K. J. HUFFMAN[3];
[1]Dept. of Psychology, [2]Interdepartmental Neurosci. Program, [3]Dept. of Psychology and Interdepartmental Neurosci. Program, Univ. of California, Riverside, Riverside, CA; [4]Ctr. for Neurosci. and Dept. of Psychology, Univ. of California, Davis, Davis, CA

Abstract: How genetic and environmental influences contribute to offspring development varies among species. Parental rearing styles in mammals reflect a source of variation, although the question remains whether this is a “nurture” phenomenon or whether there is a genetic “nature” predisposition for parenting style that is passed on to offspring. Therefore, investigating how different parenting styles may impact offspring is critical to understanding variation in postnatal development. Prairie voles (*Microtus ochrogaster*) exhibit a natural variation in parenting style, demonstrating either high (HC) or low (LC) contact with their pups; this appears to result in differences in pup brain and behavioral development. In a previous report, we found biological differences between newborn HC and LC voles. As this was before substantial parental contact, these differences were likely primarily due to genetic variation. In the current study, we test the effects of one week of experience with either LC or HC parenting styles on offspring outcome measures by comparing results from newborn P1 voles (from Bottom et al., 2020) to P7 voles. This way, we can begin to discern the contribution of genetics and experience to the contact-level phenotypes. To do this, pups born from confirmed HC or LC dams and sires were sacrificed at P7, and neuroanatomical measures were used to determine whether HC and LC offspring differed at P7 and how these features compared to those in HC and LC voles at P1. We examined contact-related differences in neocortical gene expression, using *in situ* RNA hybridization, cortical thickness, and patterns of intraneocortical connections using post-mortem

tracing methods. Variations were found between HC and LC mice at P7 and alterations were present in the age comparison analysis. This study demonstrates that phenotypic differences between HC and LC pups appear to arise from both variations in genetic predisposition as well as experiential events.

Disclosures: M. Nabatanzi: None. **R.F. Perez:** None. **R.T. Bottom:** None. **L.A. Krubitzer:** None. **K.J. Huffman:** None.

Poster

359. Comparative Anatomy: Development and Evolution

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 359.18

Topic: A.10. Development and Evolution

Title: Transgenerational changes in neuroanatomy and behavior caused by prenatal ethanol exposure in a mouse model

Authors: *K. E. CONNER[1], R. F. PEREZ, Jr.[2], D. J. ROHAC[4], K. J. HUFFMAN[3]; [1]Neurosci., [2]Psychology, [3]Psychology/Neuroscience, Univ. of California, Riverside, Riverside, CA; [4]Psychology, Univ. of California Riverside, Riverside, CA

Abstract: Background: Fetal Alcohol Spectrum Disorders (FASD) encompass a range of biological and behavioral phenotypes in offspring exposed to ethanol via maternal consumption during pregnancy. Many of these have been identified in our laboratory's CD-1 mouse model for prenatal ethanol exposure (PrEE). Previously, we found that PrEE results in abnormal neocortical gene expression, ectopic intraneocortical connectivity, altered neocortical anatomy, and disrupted behavior in the first filial (F1, directly exposed) generation (El Shawa et al., 2013, Abbott et al., 2016). Additionally, work by our laboratory suggests that PrEE can induce phenotypic change (neocortical gene expression, connectivity) that passes transgenerationally (Abbott et al., 2018, Bottom et al., 2022) most likely from epigenetic modifications. In the current study, to further explore potential heritable effects of PrEE, we replicate the exposure conditions of our PrEE model and explore neuroanatomical detail and behavior in F2 (indirectly exposed) and F3 (not exposed) generations. Of note, any phenotypes observed in F2 PrEE mice do not reflect true heritability as reproductive germ cells were present in the F1 embryo during exposure, representing an indirect exposure. However, phenotypes persisting to F3 constitute heritable change, most likely from epigenetic alteration. Comparative analyses of gross measurements such as body weight, brain weight, cortical length, and structural measures from selected neocortical areas, thalamic nuclei, and subcortical structures were evaluated in F1, F2, and F3 PrEE and control newborn mice. Results: All generations of PrEE offspring had decreased body weights and brain weights/lengths compared to controls. These effects were present despite decreased litter sizes in all PrEE generations. Neuroanatomical measures in F1, F2, and F3 newborn PrEE mice demonstrated altered neocortical thickness and subcortical volumes. Additionally, PrEE resulted in disrupted sensorimotor integration, motor control,

increased anxiety, and altered social behavior that persisted to all generations. Our data suggest that phenotypic variation from prenatal ethanol exposure can have long-term effects in exposed (F1) individuals as well as persistent transgenerational effects in F2 and F3 offspring.

Disclosures: K.E. Conner: None. **R.F. Perez:** None. **D.J. Rohac:** None. **K.J. Huffman:** None.

Poster

359. Comparative Anatomy: Development and Evolution

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 359.19

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

Support: NSF Grant NSFNCS-FR 1926781

Title: Percolation Theory Identifies Critical Constraints on White Matter Tract Development

Authors: *R. M. RAZBAN[1], K. A. DILL[1], L. R. MUJICA-PARODI[2];
[1]Laufer Ctr. for Physical and Quantitative Biol., [2]Dept. of Biomed. Engin., Stony Brook Univ., Stony Brook, NY

Abstract: To better understand fundamental constraints on the global structural organization of the brain, we apply percolation theory as a quantitative measure of global communication across a network. The largest present sub-network across which all sets of regions are able to communicate defines a giant cluster. By novel analytic solution, we prove that if constructed tracts are constrained to emanate from those regions already in the giant cluster, a giant cluster grows smoothly without requiring a critical point. The predicted structure is highly consistent with brain data, among human diffusion MRI across adults (UK Biobank, *N*=19,380), adolescents (Adolescent Brain Cognitive Development Study, *N*=15,593) and neonates (Developing Human Connectome Project, *N*=758), as well as mouse viral tracing (Allen Institute). This suggests a fundamental mechanism for neurodevelopment in which the earliest tracts also become the longest and densest.

Disclosures: R.M. Razban: None. **K.A. Dill:** None. **L.R. Mujica-Parodi:** None.

Poster

359. Comparative Anatomy: Development and Evolution

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 359.20

Topic: A.10. Development and Evolution

Title: Cell lineage-mediated synaptic-specialized compartment in the dorsal pallium in medaka, Orizias latipes

Authors: *Y. ISOE[1], R. NAKAMURA[2], S. NONAKA[4], Y. KAMEI[5], T. OKUYAMA[6], N. YAMAMOTO[7], H. TAKEDA[3], H. TAKEUCHI[8];
[1]Harvard Univ., Harvard Univ., Cambridge, MA; [2]Univ. of Tokyo, Bunkyo, Japan; [3]Univ. of Tokyo, Bunkyo-Ku, Tokyo, Japan; [4]Natl. Inst. for Basic Biol., Okazaki, Japan; [5]Natl. Inst. For Basic Biol., Natl. Inst. For Basic Biol., Aichi, Japan; [6]Inst. for Quantitative Biosci., Inst. for Quantitative Biosci., Tokyo, Japan; [7]Nagoya Univ., Nagoya, Japan; [8]Tohoku Univ., Tohoku Univ., Sendai, Japan

Abstract: The dorsal telencephalon in vertebrates, the pallium, embraces various anatomical subdivisions whose functions are critical for animals' survival. The lateral pallium (amygdala in mammals) and medial pallium (hippocampus in mammals) are considered to be conserved among vertebrates including teleosts. But it is not known whether mammalian and primate dorsal pallium (isocortex) corresponds to the dorsal pallium (Dd) in teleosts or not. In the current paper, we used medaka fish (Oryzias latipes) as a model organism since its telencephalon has a clear compartment in the dorsal pallium (named Dd2), and molecular genetic tools and genomic analysis are available. Here we focused on clonal units, a sub-population of cells derived from the same neural stem cell, in the adult brain since the post-hatch neurogenesis continues throughout life. First, our systematic clonal structural analysis shows that the pallial anatomical regions are composed of multiple clonal units in an exclusive way. Then, we investigated the transcriptional regulations in clonal units by an assay of transpose-accessible chromatin using sequencing (ATAC-seq), which suggested that clonal units in Dd2 possess a very unique open chromatin structure which was quite different from other pallial regions. Gene ontology analysis shows that many synapse-related genes were strongly regulated in Dd2. Also we observed high density of synapses in Dd2 by immunostaining. We performed RNA-seq on Dd2 and confirmed specific synaptic genes are up- and down-regulated in Dd2. Lastly we figured out a couple of transcription factors (TF) (bHLH and T-box) as candidate regulators which bind to common elements in Dd2-specific open chromatins. Taken together, we found that at least medaka fish has a unique anatomical compartment in the dorsal pallium that was enriched with specialized synapses whose epigenetic regulations were orchestrated by combination of TFs' expression in cell lineages. This is the first study in teleosts implying that the species-specific dorsal pallial compartment could play a unique role in the neural network of the telencephalon. This study will shed a light on the mechanism of how brain regions have been evolved by cell lineages in terms of structure and functions.

Disclosures: Y. Isoe: None. **R. Nakamura:** None. **S. Nonaka:** None. **Y. Kamei:** None. **T. Okuyama:** None. **N. Yamamoto:** None. **H. Takeda:** None. **H. Takeuchi:** None.

Poster

359. Comparative Anatomy: Development and Evolution

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 359.21

Topic: A.10. Development and Evolution

Support: JSPS KAKENHI Grant Number 20K15597

Title: Projection of the diencephalic visual relay nucleus to the telencephalon and presumed higher order, visually-related intra-telencephalic fiber connections in a goby, teleost

Authors: H. HAGIO[1,2], *N. YAMAMOTO[2];
[1]Inst. for Advanced Research, Nagoya Univ., Nagoya, Japan; [2]Lab. of Fish Biology, Grad. Sch. of Bioagricultural Sci., Nagoya Univ., Nagoya, Japan

Abstract: Two ascending visual pathways to the telencephalon (cerebral cortex) have been found in mammals. One of the pathways is called the geniculate system, in which retinal input is relayed to the striate cortex by the lateral geniculate nucleus in the diencephalon. The other is called the extrageniculate system, in which retinal information reaches the extrastriate cortex via the superior colliculus in the mesencephalon and then the lateral posterior nucleus-pulvinar complex in the diencephalon. Similarly, two pathways have been also found in birds, reptiles, amphibians and cartilaginous fishes. In actinopterygians, cypriniform fishes (such as the goldfish and the carp) possess two visual pathways, while only an extrageniculate-like pathway was identified in holocentrid fishes (such as the squirrelfish) that belong to acanthopterygians. We investigated visual pathways of a different acanthopterygian or gobiiform fish, the yellowfin goby (Gobiiform fish represent a taxon that emerged soon after the divergence of holocentrids) by tract-tracing methods. We found that goby possess extrageniculate-like pathway in which retinal inputs reach the lateral, dorsal, and central parts of the dorsal telencephalic area via the optic tectum and then the nucleus prethalamicus (PTh). Thus, our finding suggests that the disappearance of one visual pathway is not a phenomenon specific for holocentrid fish alone; presumably, it is a feature shared in acanthopterygians. We also elucidated similarities in neural connections between teleosts and mammals. Our studies showed retinotopic organization of the retino-tecto-diencephalic pathway as with mammals. We furthermore revealed subnuclei of the diencephalic visual nucleus (PTh) project to different regions of the telencephalon, similarly to the situation of the lateral posterior nucleus-pulvinar complex of mammals. Our studies by injections into many visual telencephalic regions suggested that goby possesses visually-related intra-telencephalic fiber connections. These connections might perhaps represent the presence of higher order visual system, like the association cortex, in teleosts.

Disclosures: H. Hagio: None. **N. Yamamoto:** None.

Poster

359. Comparative Anatomy: Development and Evolution

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 359.22

Topic: A.10. Development and Evolution

Support: Serrapilheira Foundation Serra-1709-16981
CNPq PQ 2017 312837/2017-8

Title: What is the shape of a cortex? A systematic coarse-graining analysis shows cortices are all approximations of a universal self-similar shape

Authors: *B. MOTA;
Physics Inst., Univ. Federal Do Rio De Janeiro, Rio de Janeiro, Brazil

Abstract: The mammalian cerebral cortex can take on a bewildering diversity of shapes and sizes within and across species, whilst maintaining archetypal qualities that make it instantly recognisable as a ``brain". Here we present a new way of expressing the shape of a cortex explicitly as the hierarchical composition of structures across spatial scales. In computational simulations, as one successively removes sulci and gyri smaller than a specified scale, the cortices of 11 primate species are gradually coarse-grained into less folded brains until lyssencephaly (no folding). We show that this process, in all cases, occurs along a common scale-free morphometric trajectory, indicating that these cortices are not only approximately fractal in shape, but also strikingly approximations of the \textit{same} archetypal fractal shape. These results imply the existence of a single universal gyrification mechanism that operates in a scale-free manner on cortical folds of all sizes, and that there are surprisingly few effective degrees of freedom through which cortical shapes can be selected for by evolution. Finally, we demonstrate that this new understanding can be applied to show that the aging process affects cortical morphology in a highly scale-dependent way. To our knowledge, this is the most parsimonious universal description of the brain's shape that is at the same time mechanistically insightful and in full agreement with empirical data across species and individuals.

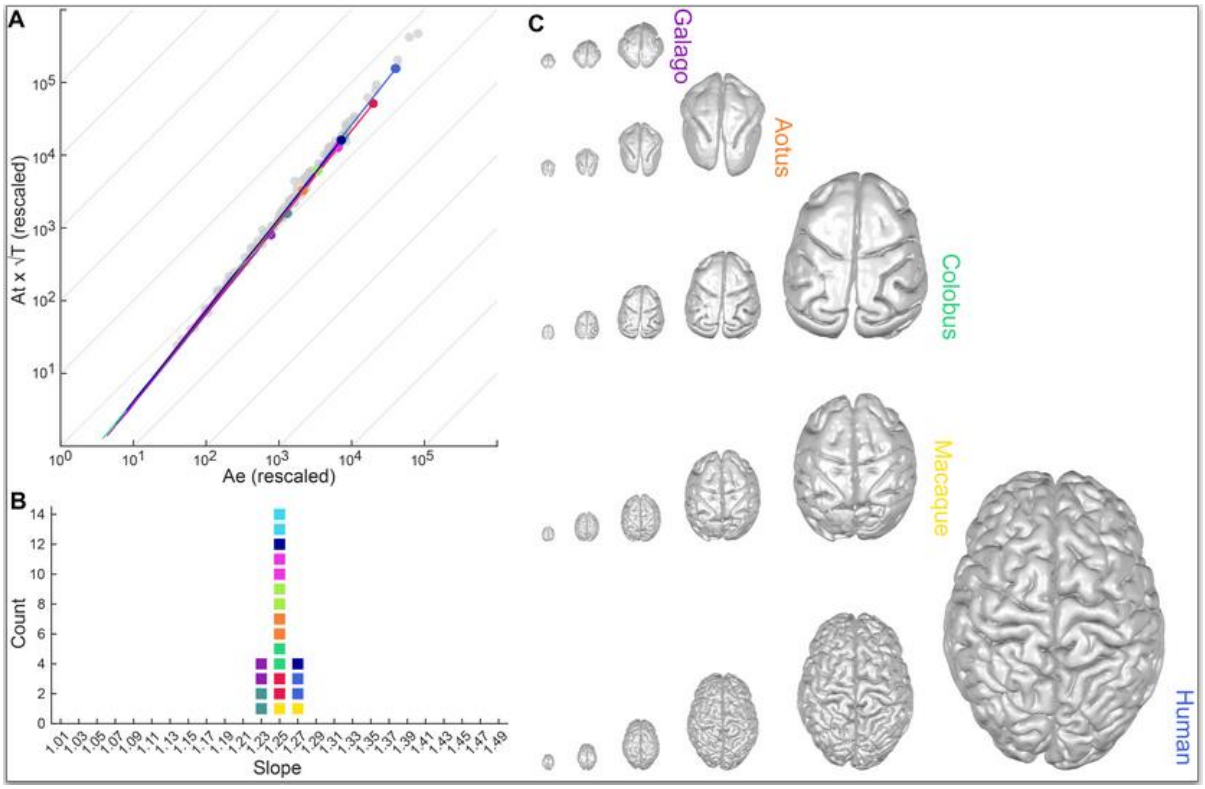


FIGURE: Morphometric trajectories over coarse-graining for cortices of 11 primate species recapitulate scaling law between total area At, exposed area Ae and cortical average thickness T. Each color represents a species, with the initial value indicated by a point. The best-fit slope for the iterates of each species' cortex cluster tightly around the theoretical expectation of 1.25. Gray points correspond to cortices for a wide range mammalian.

Disclosures: B. Mota: None.

Poster

359. Comparative Anatomy: Development and Evolution

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 359.23

Topic: I.03. Anatomical Methods

Support: NeuroCure

Title: Fiber Counts and Architecture of the Human Dorsal Penile Nerve

Authors: *E. TUNÇKOL[1], L. PURKART[1], L. EIGEN[1], I. VIDA[3], M. BRECHT[1,2];
[1]Bernstein Ctr. for Computat. Neurosci., [2]NeuroCure Cluster of Excellence, Humboldt Univ. zu Berlin, Berlin, Germany; [3]Charite, Berlin, Berlin, Germany

Abstract: The human penis transmits behaviorally highly relevant sensory information via the dorsal penile nerve, but we have no detailed understanding of the penile sensory innervation. To address this issue, we analyzed the architecture and quantitative composition of the dorsal penile nerves of six male subjects from a body donation program. Nerve fibers were visualized by immune-fluorescence staining with an anti-neurofilament-H antibody, a marker expressed by peripheral sensory axons. We also identified myelinated axons by Luxol fast blue staining. In order to visualize nerve bundles as they travel along the shaft of the penis we performed micro CT scans after counterstaining penes with Iodide. Our results show that the dorsal penile nerve is organized in 10-50 nerve bundles, running as part of the vascular nerve cord mediodorsally in the shaft of the penis, but the bundles are not tightly packed. This penile nerve organization is similar to that observed in other mammalian species, but differs from other sensory nerves. About half of the dorsal penile nerve fibers were myelinated and a human hemi-penis contained a total of 8495 ± 1877 (mean ± SD) axons. Thus, the number of sensory axons in human dorsal penile nerve is higher than in other species described so far. The large fraction of unmyelinated nerve fibers suggests that conduction speed is not a crucial aspect of penile sensory transmission.

Disclosures: E. Tunçkol: None. **L. Purkart:** None. **L. Eigen:** None. **I. Vida:** None. **M. Brecht:** None.

Poster

360. Nicotinic Acetylcholine Receptors: Trafficking, Regulation, and Function

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 360.01

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: Infectious Disease Society of America
Idaho State University INBRE-4

Title: Study of interactions between herpes virus 1 and nicotinic acetylcholine receptors.

Authors: *S. YEASMIN, K. SHARMA, A. RANJIT, A. A. HABASHI, M. K. SCHULTE; Idaho State Univ., Idaho State Univ., Pocatello, ID

Abstract: This project is focused on investigating the hypothesis that part of the neurotrophic effect of Herpes virus1 (HSV1) may be linked to an interaction of herpes virus 1 glycoprotein D (HSV1gD) with nicotinic acetylcholine receptors (nAChRs). nAChRs are ligand-gated ion channel receptors with important roles in both the central and peripheral nervous systems. The critical roles nAChRs play in the autonomic nervous system and central regulation of inflammation, along with data suggesting an interaction of SARS-Cov2 with these receptors, has led to increased interest in their role in viral pathogenesis. The rabies virus has long been known to interact with nAChRs, potentially through similarities in structure to the LY6 family of receptors, a similarity shared with alpha bungarotoxin (alpha-btx.). HSV1 is another endemic neurotropic virus that has been linked to neurological diseases including encephalitis, and neurodegenerative diseases like Alzheimer's disease. HSV1gD is the key glycoprotein with known interactions to host receptors. In addition, there are known interactions between viruses such as SARS-Cov2 and the resurgence of HSV in humans. Interestingly, nicotine has also been shown to promote a similar resurgence of HSV. We performed an In-silico study to find structural homology with the known alpha7 receptor antagonist, alpha-btx. and HSV1gD. These studies identified structural homology between HSV1gD and alpha-btx., a homology shared with other LY6 proteins. Some sequence homology was present but was limited. To determine if this structural homology provided a sufficient basis for receptor interaction, we conducted Surface Plasmon Resonance (SPR) using fragments of HSV1gD that included the homologous loop region. We also conducted functional studies to determine the effect of HSV1gD on alpha7 nAChRs expressed in *Xenopu*s oocytes by using two-electrode voltage clamp (TEVC) electrophysiology. A negative control peptide was constructed based on a similar surface loop of HSV1gD that was not structurally homologous to alpha-btx. binding loop. Data from SPR studies indicate that the HSV1gD peptide interacts with the Acetylcholine Binding protein from *Lymnaea stagnalis* in a 1:1 binding model with a KD value of 1.07×10^{-7} M. Functional studies on alpha7 nAChRs in *Xenopus* oocytes showed inhibition of the acetylcholine-induced response for all homologous peptides. IC50 values for inhibition of these responses by HSV1gD peptides on alpha7 receptors ranged from ~1.5 µM to 100 µM. These findings may help to understand the mechanism of action of HSV1 on the nAChRs and explore how herpes virus1 plays a role in Alzheimer's disease.

Disclosures: **S. Yeasmin:** None. **K. Sharma:** None. **A. Ranjit:** None. **A.A. Habashi:** None. **M.K. Schulte:** None.

Poster

360. Nicotinic Acetylcholine Receptors: Trafficking, Regulation, and Function

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 360.02

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: BYU Internal funding award

Title: In silico screening to identify positive allosteric modulators specific for the alpha3beta2 nicotinic acetylcholine receptor subtype

Authors: *S. SUDWEEKS[1], J. CHRISTENSEN[1], B. HARRISON[2], J. JACKSON[2], K. LIU[2], C. ULRICH[2], C. ULRICH[2], J. WELLS[2], M. STEVENS[1], R. TORGESEN[1], C. D. CALLISON[3]; [1]Cell Biol. & Physiol., [2]Neurosci., [3]Neurosci. Ctr., Brigham Young Univ., Provo, UT

Abstract: Neuronal nicotinic acetylcholine receptors (nAChR's) are non-specific cation channels that play an essential role in the nervous system as neurotransmitter receptors. There are 18 different nicotinic subunits (including both muscle and neuronal forms) that have been cloned, including alpha 1-10, beta 2-4, gamma, delta, and epsilon. Different pentameric combinations of these subunits can create different subtypes of nAChR receptors. Single-cell RT-PCR analysis from rat hippocampal interneurons identified a possible unique subunit co-expression pattern that does not appear to be commonly expressed in other areas of the brain: alpha3 with beta2. This subunit combination was the most highly expressed alpha/beta combination detected in rat hippocampal interneurons. Alpha3 has traditionally thought to be expressed mainly in the peripheral nervous system along with the beta4 subunit and has very low levels of expression in the central nervous system overall, so it's abundance in rat hippocampal interneurons warranted further examination. Co-injection of human alpha3 and beta2 nAChR subunit mRNA into Xenopus laevis oocytes resulted in functional nicotinic receptor currents, with at least 2 distinguishable subtypes based on different ratios of injected alpha3:beta2 subunits. Since this subunit combination appears to be unique to hippocampal interneurons, it represents a potential hippocampus specific neuronal nAChR drug target. We decided to identify potential drug molecules that could act as positive allosteric modulators (PAM) on alpha3 beta2 nAChRs. PAMs can potentiate nAChR function by binding to sites that are distinct from the acetylcholine binding site. We hypothesized that a PAM binding site could exist at the interface between the alpha3 and beta2 subunits - thus requiring specificity for both the alpha3 and the beta2 subunits - analogous to a previously identified PAM site at the alpha:beta interface on alpha4 beta2 receptors. When we started these studies, no published structure for the α3 subunit was available so we used Swissprot to create a structural model of the alpha3 sequence based on the published α4 subunit (PDB ID 6CNJ) as a reference structure. We used the freely available computer program AutoDock Vina and the publicly available ZINC compound database to

screen hundreds of thousands of compounds to see if they would be predicted to bind allosterically to α3β2 nAChR's. We have identified several compounds that show specificity for the alpha3:beta2 subunits compared to alpha4:beta2 and alpha3:beta4 subunit combinations, indicating that a hippocampus specific nAChR PAM may be feasible.

Disclosures: **S. Sudweeks:** A. Employment/Salary (full or part-time):; Brigham Young University. **J. Christensen:** None. **B. Harrison:** None. **J. Jackson:** None. **K. Liu:** None. **C. Ulrich:** None. **C. Ulrich:** None. **J. Wells:** None. **M. Stevens:** None. **R. Torgesen:** None. **C.D. Callison:** None.

Poster

360. Nicotinic Acetylcholine Receptors: Trafficking, Regulation, and Function

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 360.03

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Title: N-myc transcription factor increases the expression of alpha-7 nicotinic acetylcholine receptor promoting cell viability in neuroblastoma cell line

Authors: *L. TARABEY, V. OCHOA;
CUSM, Colton, CA

Abstract: Neuroblastoma is the most common extracranial solid tumor in children. It accounts for more than 7% of malignancies in patients less than fifteen years of age and makes up 15% of oncology deaths. Neuroblastoma is a cancer derived from primordial neural crest cells under conditions of inappropriate migration, maturation, and or differentiation. These extracranial tumors are formed in the adrenal medulla, sympathetic ganglia, and abdomen. Current treatments for neuroblastoma include surgery, chemotherapy and radiotherapy. Interestingly, neuroblastoma tumors are heterogenous with some children having low-risk tumors that regress entirely while others have high-risk tumors with widespread metastasis and poor outcomes. The expression of MYCN, a gene in a family of oncogenic drivers, in neuroblastoma tumors has been associated with high-risk neuroblastoma and worse outcomes. Children with high-risk neuroblastoma account for approximately half of all patients diagnosed with neuroblastoma. Despite current interventions, children with high-risk neuroblastoma have a long-term survival rate of less than 50%. Currently, there are not many targeted therapies for high-risk neuroblastoma as there is little known about the mechanism driving this cancer. By identifying the mechanism driving tumor progression, a treatment to specifically target the different steps of this cancer can be created. Signaling through neuronal nicotinic acetylcholine receptors (nAChRs) is involved in many neuronal processes of neural tissue, including the proliferation of neural crest cells. These receptors are a diverse group of ligand-gated pentametric channels can exist as homopentamers or heteropentamers of α or β subunits. The only mammalian homopentamer is the α7 receptor that when activated is suggested to promote neural cell survival. Furthermore, expression of nAChR has been shown to be regulated by MYCN. Our lab has shown that MYCN over-

expressing neuroblastoma tumors have upregulated expression of α7, α5, and α3 nAChR subunits with α7 expression having the greatest increase in MYCN expressing cells. Furthermore, through an MTT growth assay, we illustrate that the activation of nAChRs in MYCN over expressing cells have an increased cell number compared to control cells not over expressing MYCN. These results suggest that targeting the interaction between nAChRs and MYCN can become a potential therapy of high-risk neuroblastoma.

Disclosures: L. Tarabey: None. **V. Ochoa:** None.

Poster

360. Nicotinic Acetylcholine Receptors: Trafficking, Regulation, and Function

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 360.04

Title: WITHDRAWN

Poster

360. Nicotinic Acetylcholine Receptors: Trafficking, Regulation, and Function

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 360.05

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

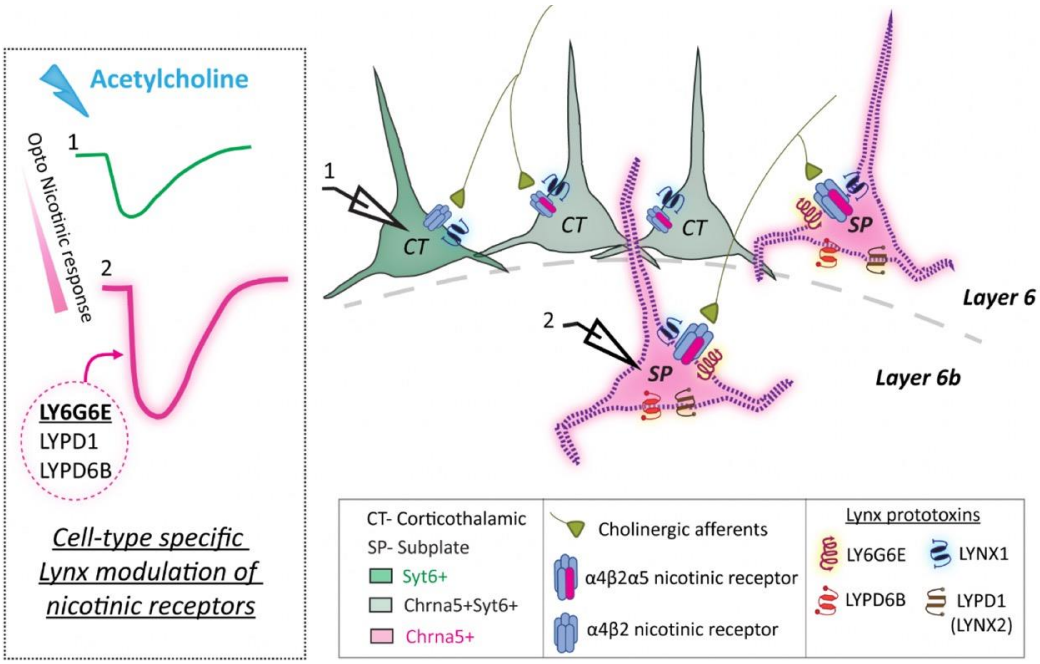
Support: CIHR grant MOP-89825 (EKL)
CIHR grant PRJ-153101 (EKL)
Canada Research Chair in Developmental Cortical Physiology (EKL)
Ontario Graduate Scholarships (SV)

Title: Chrna5 marks acetylcholine super-responder subplate neurons with specialized expression of nicotinic modulator proteins

Authors: *E. K. LAMBE[1], T. CHEN[1], Y. LIU[1], E. E. TURNER[2], S. TRIPATHY[1], S. VENKATESAN[1];
[1]Univ. of Toronto, Toronto, ON, Canada; [2]Ctr. for Integrative Brain Res., Seattle Children's Res. Inst., Seattle, WA

Abstract: Attention depends on cholinergic excitation of prefrontal neurons. Knockout/knockdown studies indicate nicotinic alpha5 subunits encoded by Chrna5 are required for this response, but their native cellular roles and molecular interactions are unknown. Here, we probe endogenous cholinergic regulation of prefrontal Chrna5-expressing neurons (Chrna5+) using compound transgenic mice. Chrna5+ neurons show high sensitivity to acetylcholine, with a

subpopulation clearly different from nearby, well-characterized cells labeled by Synaptotagmin6 (Syt6+). Transcriptomic analysis reveals this distinct Chrna5+ population as subplate neurons, a diverse group of firstborn cells that have eluded previous transgenic characterization. Intriguingly, Chrna5+ subplate neurons express a distinct profile of GPI-anchored lynx prototoxins, suggesting specialized regulation of their cholinergic responses. In brain slices, endogenous nicotinic responses can be bidirectionally altered by perturbing GPI-anchored lynxes with phospholipase C activation or exogenous application of recombinant Ly6g6e prototoxin. Our work reveals cell-type specific Chrna5 and Lynx modulation leading to exquisite cholinergic sensitivity of prefrontal subplate neurons in adulthood.



Disclosures: **E.K. Lambe:** None. **T. Chen:** None. **Y. Liu:** None. **E.E. Turner:** None. **S. Tripathy:** None. **S. Venkatesan:** None.

Poster

360. Nicotinic Acetylcholine Receptors: Trafficking, Regulation, and Function

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 360.06

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIDA Grant DA041378

Title: Inappropriate activation of alpha2 nAChR-expressing OLM cells in developing mouse brains disrupts normal OLM cell function in later life

Authors: *S. NAKAUCHI, H. SU, K. SUMIKAWA;
Univ. of California, Irvine, Irvine, CA

Abstract: Early postnatal nicotine exposure, a rodent model of smoking during pregnancy, affects hippocampal synaptic plasticity and memory. We investigated the role of α2 nAChR-expressing OLM (α2-OLM) cells in LTP in unexposed and postnatal nicotine-exposed mice. We found that reduced α2 nAChR-dependent activation of OLM cells in α2 heterozygous knockout mice prevented LTP, whereas enhanced α2 nAChR-dependent activation of OLM cells in heterozygous knockin mice expressing hypersensitive α2 nAChRs facilitated LTP. Both optogenetic and chemogenetic activation of α2-OLM cells facilitated LTP as nicotine did. However, in postnatal nicotine-exposed mice, expressing chemogenetic hM3Dq receptors in α2-OLM cells, LTP was facilitated and both nicotinic and chemogenetic activation of α2-OLM cells prevented rather than facilitated LTP. These results demonstrate a critical role of α2-OLM cell activation in LTP as well as altered α2-OLM cell function in postnatal nicotine-exposed mice. To determine whether nicotine-mediated α2 nAChR activation in developing brains causes facilitated LTP and altered nicotinic modulation of LTP in adolescence, we used homozygous knockin mice expressing hypersensitive α2 nAChRs as a way to selectively activate α2-OLM cells. In the knockin mice, postnatal exposure to a low dose of nicotine, which had no effect on LTP in wild-type mice, is sufficient to cause facilitated LTP and altered nicotinic modulation of LTP as found in wild-type mice exposed to a higher dose of nicotine. Thus, the nicotine-mediated activation of α2 nAChRs on OLM cells in developing brains disrupts the α2-OLM cell-mediated control of LTP in adolescence that might be linked to impaired memory.

Disclosures: S. Nakauchi: None. **H. Su:** None. **K. Sumikawa:** None.

Poster

360. Nicotinic Acetylcholine Receptors: Trafficking, Regulation, and Function

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 360.07

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: ERA-NET Neuron
Austrian Science Fund FWF Project I 3778-B27

Title: The human specific nAChR-subunit dupα7 reduces α7 nAChR function in hiPSC-derived cortical neurons

Authors: G. I. SOZTEKIN[1], U. MASKOS[2], S. E. HUCK[1], ***P. SCHOLZE**[1];
[1]Med. Univ. of Vienna, Wien, Austria; [2]Inst. Pasteur, Paris Cedex 15, France

Abstract: Neuronal nicotinic acetylcholine receptors (nAChR) are pentameric ligand-gated cation channels composed of five out of twelve subunits (9α and 3β) and can assemble into a multitude of different homo- or hetero-pentameric receptors. The subunit α7, which is encoded by the gene *CHRNA7*, assembles into homomeric receptors consisting of five α7-subunits in

most species. Humans, however, contain an additional nAChR-gene, since at some late time point of evolution the gene *CHRNA7* was duplicated and fused with the gene FAM7A forming a new human specific gene called *CHRFAM7A*. This gene encodes for the subunit dupα7, which lacks the agonist binding-site, but shares most of the other structural elements with α7. Since *CHRNA7* and *CHRFAM7A* are located on a very unstable region in the genome at chromosome 15, both can even today still be duplicated or deleted frequently, resulting in humans carrying copy number variations (CNVs) of either of the two genes. In literature, *CHRNA7* CNVs have been associated with several disorders including cognitive and psychiatric disorders including schizophrenia. We have however little knowledge of the consequence of *CHRFAM7A* CNVs. α7- and dupα7-subunits are believed to co-assemble. We hypothesize that dupα7, when integrated into the receptor, acts as a dominant negative regulator and may reduce α7 nAChR function. In order to study this effect more closely, we differentiated cortical neurons from human induced pluripotent stem cells (hiPSCs) and analyzed them functionally using Fura-2 Ca^{2+}-Imaging and Patch-Clamp electrophysiology. We used two lines of neuronal precursor cells: one, which lack the *CHRFAM7A* gene, and a second which overexpresses the dupα7-subunit after lentiviral infection. With patch-clamp recordings in the voltage-clamp mode, hiPSC-derived neurons lacking *CHRFAM7A* responded to depolarizing voltage steps by inward-directed sodium currents and outward-directed potassium currents. After at least 4 weeks of differentiation, these currents formed the basis for trains of action potentials, indicating the presence of mature neurons. We observed an increase in the frequency of miniature excitatory post-synaptic currents upon combined applications of α7 nAChR specific compounds. Fura-2 Ca^{2+}-Imaging revealed that in the presence of TTX, PNU-282987 or choline, when combined with PNU-120596, induced an increase of intracellular Ca^{2+}. On the other hand, hiPSC-derived neurons with overexpressing CHRFAM7A showed reduced levels of intracellular Ca^{2+} in the presence of those compounds. In conclusion, we were able to confirm that dupα7 subunits reduce α7 nAChR function in hiPSC-derived cortical neurons.

Disclosures: **G.I. Soztekin:** None. **U. Maskos:** None. **S.E. Huck:** None. **P. Scholze:** None.

Poster

360. Nicotinic Acetylcholine Receptors: Trafficking, Regulation, and Function

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 360.08

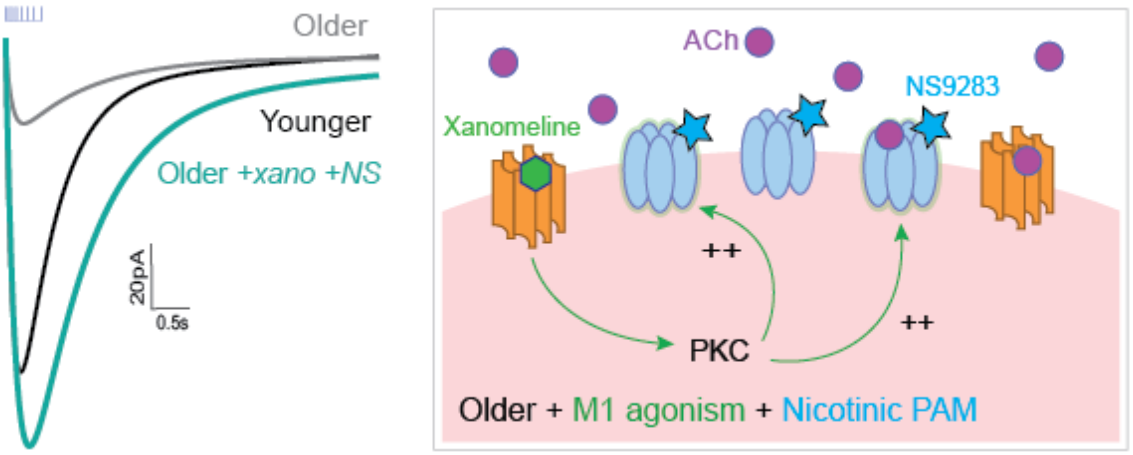
Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: CIHR MOP 89825
PJT-153101 (EKL)
CIHR CGS D (S POWER)

Title: Loss of endogenous nicotinic receptor signaling in middle age: Novel rescue by xanomeline

Authors: *S. K. POWER[1], S. VENKATESAN[1], E. K. LAMBE[1,2,3];
[1]Physiol., [2]Obstetrics and Gynecology, [3]Psychiatry, Univ. of Toronto, Toronto, ON, Canada

Abstract: Cholinergic synapses in prefrontal cortex are vital for attention but undergo pre- and postsynaptic changes during adulthood with unclear integrated consequences. To investigate the functional impact of age-related changes, we probe cholinergic synapses optogenetically in brain slices from adult mice across a broad age range. Results show a clear decline in cholinergic neurotransmission in middle adulthood and a switch in receptor mechanisms with loss of the rapid nicotinic current and preservation of the slow excitatory muscarinic current. Improving rapid nicotinic signaling by allosteric modulation with NS9283 is successful in young adults but declines with age, highlighting loss of nicotinic receptor availability. To improve availability, we harness the intact signalling pathways of excitatory muscarinic receptors. M1 agonist and cognitive-enhancer xanomeline significantly restores nicotinic responses in older mice in a muscarinic- and PKC-dependent manner. Furthermore, xanomeline-restored nicotinic responses gain greater sensitivity to allosteric enhancement by NS9283. With this combined treatment, neurons from older mice respond to endogenous acetylcholine with the strength, speed, and receptor mechanism of young adults. Our results demonstrate a new and efficient strategy to rescue age-related nicotinic signaling deficits, illustrating a previously unknown pathway by which xanomeline enhances cognitively-essential prefrontal brain circuits.



Disclosures: **S.K. Power:** None. **S. Venkatesan:** None. **E.K. Lambe:** None.

Poster

360. Nicotinic Acetylcholine Receptors: Trafficking, Regulation, and Function

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 360.09

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIH Grant R01DA044760
NIH Grant P30CA14599
NIDA Grant T32DA043469

Title: Trapping of nicotinic acetylcholine receptor ligands as assayed by invitro cellular studies and invivo PET imaging

Authors: *A. P. GOVIND[1], H. J. ZHANG[2], M. ZAMMIT[2], C.-M. KAO[2], S. MITCHELL[2], N. HOLDERMAN[2], M. BHUIYAN[2], R. FREIFELDER[2], X. ZHUANG[3], J. MUKHERJEE[4], C.-T. CHEN[2], W. N. GREEN[1,5];
[1]Neurobio., [2]Dept of Radiology, [3]Dept of Neurobio., Univ. of Chicago, Chicago, IL; [4]Dept of Radiological Sci., Univ. of California, Irvine, CA; [5]Marine Biol. laboratory, Woods Hole, MA

Abstract: Distribution of nicotine and other nicotinic acetylcholine receptor (nAChR) membrane-permeant ligands, such as the anti-smoking drug varenicline (Chantix), in the brain is not well studied. Previously we found that ligands, like varenicline, with high pKa and high-affinity for α4β2-type nicotinic receptors (α4β2Rs) are trapped in intracellular acidic vesicles whereas Nicotine, with lower pKa and α4β2R affinity, is not trapped. Combining *in vitro* ligand binding and *invivo* imaging with PET, ligands, we present additional evidence of trapping of nAChR ligands in α4β2R-containing Golgi satellites (GSats). Two PET ^{18}F-labelled imaging ligands were chosen: [^{18}F]2-FA85380 (2-FA) with varenicline-like pKa and affinity and [^{18}F]Nifene with nicotine-like pKa and affinity. [^{18}F]2-FA PET-imaging kinetics were very slow consistent with 2-FA trapping in α4β2R-containing GSats. In contrast, [^{18}F]Nifene kinetics were rapid, consistent with its binding to α4β2Rs but no trapping. Specificity of [^{18}F]2-FA and [^{18}F]Nifene to bind to β2-containing receptors were demonstrated using β2 subunit knockout mice or by acute nicotine injections. Chloroquine, which dissipates GSat pH gradients, reduced [^{18}F]2-FA distributions while having little effect on [^{18}F]Nifene distributions both *in vivo* and *invitro* consistent with only [^{18}F]2-FA trapping in GSats. By combining *in vitro* and *in vivo* imaging, we mapped both the brain-wide and subcellular distributions of weak-base nicotinic receptor ligands. We conclude that ligands, such as varenicline, have higher intracellular residence time due to trapping in α4β2R-containing GSats, which results in very slow and continued release long after nicotine is gone after smoking.

Disclosures: A.P. Govind: None. **H.J. Zhang:** None. **M. Zammit:** None. **C. Kao:** None. **S. Mitchell:** None. **N. Holderman:** None. **M. Bhuiyan:** None. **R. Freifelder:** None. **X. Zhuang:** None. **J. Mukherjee:** None. **C. Chen:** None. **W.N. Green:** None.

Poster

360. Nicotinic Acetylcholine Receptors: Trafficking, Regulation, and Function

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 360.10

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NINDS Intramural Program Grant NS003135 to ZMK

Title: Picrotoxin blocks nicotinic receptors on dopamine neuron axon terminals

Authors: *S. G. BRILL-WEIL[1,2], P. F. KRAMER[1], F. H. CLEVER[1], Z. M. KHALIQ[1];
[1]NIH/NINDS, Bethesda, MD; [2]Program in Neurosci., Harvard Univ., Boston, MA

Abstract: Picrotoxin is a widely used $GABA_A$ receptor antagonist and is a common tool for isolating circuit components in slice and *in vivo*. Structurally, the $GABA_A$ receptor is part of the cys-loop receptor family, along with nicotinic acetylcholine receptors (nAChRs), glycinergic receptors, and 5-HT_3 receptors. Due to this close structural similarity, drugs that target one receptor could have off-target effects on the others. In culture and expression systems, it has been shown that picrotoxin blocks nAChR-mediated currents. Yet, picrotoxin continues to be used for studying neural circuits that involve both GABAergic and cholinergic signaling. One such circuit is the striatum. The striatum is comprised primarily of GABAergic neurons in addition to a relatively small population of cholinergic interneurons that exert robust control over the circuitry. In particular, previous work has highlighted the impact that both GABAergic and cholinergic signaling have on striatal dopaminergic axon excitability and dopamine release. Here we show that picrotoxin acts in a dose-dependent manner to decrease nAChR-mediated evoked Ca^{2+} influx in the axon terminals of SNc dopaminergic neurons through non-$GABA_A$-mediated mechanisms. This decrease in axonal Ca^{2+} occurs without a corresponding decrease in acetylcholine (ACh) release, as measured using the fluorescent ACh sensor $GRAB_{ACh3.0}$. Additionally, using direct axonal recordings, we show that depolarizations evoked by transient applications of exogenous ACh were blocked by picrotoxin. Taken together, our results demonstrate that picrotoxin directly blocks nAChRs on dopaminergic axons and support the conclusion that picrotoxin should be used cautiously as a $GABA_A$ antagonist.

Disclosures: S.G. Brill-Weil: None. **P.F. Kramer:** None. **F.H. Clever:** None. **Z.M. Khaliq:** None.

Poster

360. Nicotinic Acetylcholine Receptors: Trafficking, Regulation, and Function

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 360.11

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Title: Visinin-like protein-1 alters a4ß2 nicotinic acetylcholine receptor function and isoform expression

Authors: *M. WELTZIN[1], S. SUAREZ[1], H. DANIELSON[2];
[1]Univ. of Alaska Fairbanks, Fairbanks, AK; [2]Picker Engin. Program, Smith Col., Northampton, MA

Abstract: Neurological conditions including sleep-related hypermotor epilepsy (SHE), nicotineaddiction, and Alzheimer's disease, are associated with disruptions in brain cholinergic tonecaused in part by dysregulation of α4β2 nicotinic acetylcholine receptors (nAChRs). The α4β2subtype is one of the most prevalent nAChRs within the central nervous system and expressesas two isoforms with high sensitivity (HS, [(α4β2)2β2)]) and low sensitivity (LS, [(α4β2)2α4)]) toagonists. Visinin-like protein-1 (VILIP-1) is a calcium sensor protein that has been shown toenhance acetylcholine (ACh) sensitivity and surface expression of α4β2 nAChRs. VILIP-1putatively interacts in the residue region 302-339 of the α4 subunit, which overlaps with thelocation of an intracellular loop SHE associated mutation in the α4 subunit (α4(R336H)). Wehypothesize that VILIP-1 can modulate α4β2 function and isoform expression, and that theα4(R336H) SHE mutation modifies this interaction. To test our hypothesis, *Xenopus laevis*oocytes were injected with biased ratios of individual α4 and β2 subunit cRNA to drive receptorisoform expression. VILIP-1 functional interaction was assessed by injecting a range of VILIP-1cRNA ratios with respect to the α4 subunit. Using two-electrode voltage clampelectrophysiology, ACh potency was measured by generating concentration response curves,and receptor function was assessed using the maximum receptor response (IMAX). Isoformexpression was functionally evaluated by application of Sazetidine-A, which is an α4(+)/β2(-)interface selective agonist used to measure the HS-phase of α4β2 nAChR responses. Our resultsshow that VILIP-1 does not modify ACh potency of either α4β2 nAChR isoform. However, wesee attenuation of LS-isoform ACh peak currents with increasing the VILIP-1 concentration. Inoocytes containing five-times more VILIP-1 than α4 cRNA, VILIP-1 decreases peak currents 17-fold in wildtype α4β2 nAChRs 2 days post cRNA injection. Interestingly, in α4(R336H) mutantcontaining nAChRs, peak currents are attenuated by approximately 330-fold under these samecRNA injection ratios. Results could not be attributed to decreases in expression of the morefunctional LS-isoform, as VILIP-1 enhances the functional expression of the wildtype and mutantLS-isoforms. Our findings of VILIP-1 driven functional affects and modulation of α4β2 nAChRisoform expression have potential therapeutic implications to rebalance cholinergic tone.

Disclosures: **M. Weltzin:** None. **S. Suarez:** None. **H. Danielson:** None.

Poster

361. Calcium Channels and Related Signaling

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 361.01

Topic: B.03. Ion Channels

Title: Activity-dependent clustering of neuronal L-type calcium channels by CaMKII

Authors: *Q. YANG[1], L. HU[1], J. QUAY[2], R. J. COLBRAN[3];
[1]Dept Molec Physiol & Biophysics, [2]Chem. and Physical Biol., Vanderbilt Univ., Nashville, TN; [3]Dept Molec Physiol & Biophysics, Vanderbilt Brain Institute, Vanderbilt Kennedy Ctr., Vanderbilt Univ. Sch. Med., Nashville, TN

Abstract: L-type voltage-gated Ca^{2+} channels (LTCCs) are key initiators of excitation-transcription (E-T) coupling, a process that can be induced by Ca^{2+} increases within a local LTCC nanodomain. The formation of LTCC/Ca^{2+} nanodomains to initiate E-T coupling may involve the clustering of the major neuronal LTCC α1 subunits ($Ca_V1.2$, $Ca_V1.3$). Indeed, we found that a neuronal depolarization that induces CREB Ser133 phosphorylation also increases $Ca_V1.3$ LTCC clustering in cultured hippocampal neurons. Our previous work showed that binding of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) to an N-terminal RKR motif in $Ca_V1.3$ is required for LTCC-mediated E-T coupling. We co-expressed $Ca_V1.3$ containing two different epitope tags, with or without CaMKII in HEK cells. Co-immunoprecipitations (co-IPs) from the cell lysates revealed that CaMKII not only interacts with $Ca_V1.3$, but also assembles multimeric $Ca_V1.3$ LTCC complexes in an activity-dependent manner. Moreover, pharmacological activation of LTCCs in HEK cells co-expressing CaMKII increased the clustering of surface localized $Ca_V1.3$ channels. The N-terminal CaMKII-binding RKR motif is conserved in $Ca_V1.2$, and we found that CaMKII promotes the activity-dependent co-clustering of $Ca_V1.3$ and $Ca_V1.2$ in HEK cells. Additionally, the beta2a auxiliary subunit, which also directly binds to CaMKII, facilitates CaMKII-dependent $Ca_V1.2$-$Ca_V1.3$ clustering in HEK cells, relative to the beta3 auxiliary subunit that cannot bind CaMKII. Our ongoing studies are examining the role of CaMKII in $Ca_V1.3$ clustering and $Ca_V1.2$-$Ca_V1.3$ co-clustering in cultured neurons. Taken together, our work suggests that CaMKII mediates activity-dependent LTCC clustering that may be essential for the initiation of a specific long-range signal from LTCCs in the plasma membrane to the nucleus.

Disclosures: Q. Yang: None. **L. Hu:** None. **J. Quay:** None. **R.J. Colbran:** None.

Poster

361. Calcium Channels and Related Signaling

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 361.02

Topic: B.03. Ion Channels

Support: Intramural grant from Rajiv Gandhi Centre for Biotechnology, Government of India
Fellowships from Council of Scientific and Industrial Research, Government of India

Title: Assay for L-type voltage gated calcium channels in HEK-293 cells

Authors: *R. OMKUMAR, A. G. MOHANAN, A. R. CHANDRIKA;
Rajiv Gandhi Ctr. For Biotech., Thiruvananthapuram, India

Abstract: Voltage gated calcium channels (VGCCs) are pursued as drug targets for various neurodegenerative conditions. High throughput drug screening targeting VGCCs depends on patch-clamp electrophysiology or fluorophore-based calcium imaging that require powerful

equipment and specialized expertise thus leading to cost escalation. Moreover, VGCC needs to be transfected into cell lines such as HEK-293 cells. We report the presence of L-type VGCC (L-VGCC) subunit protein, Cav1.2, and its accessory protein subunits, α2δ and β, in HEK-293 cells and the application of simple methods for its assay. Endogenous expression of the channel in HEK-293 cells overcomes the need for transfection. L-VGCC in HEK-293 cells was activated either by the agonist, BayK8644 or by KCl-mediated depolarization. Activity was detected using the calcium sensing probe, GCaMP6m by live imaging. L-VGCC activity caused enhancement in GCaMP6m fluorescence that returned to baseline corresponding to channel-closure. Activity was also shown using a methodology involving the end-point detection of the calcium dependent interaction of α-CaMKII with its ligand, NMDA receptor subunit GluN2B sequence. Activation by BayK 8644 or by depolarization was blocked by the specific L-VGCC antagonist, nifedipine. This methodology further simplifies the assay as it eliminates the need for real time imaging. Finding the presence of endogenous L-VGCC in HEK-293 cells and detection of its activity with simple methods offer commercially viable assays for L-VGCC-targeted drug screening.

Disclosures: R. Omkumar: None. **A.G. Mohanan:** None. **A.R. Chandrika:** None.

Poster

361. Calcium Channels and Related Signaling

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 361.03

Topic: B.03. Ion Channels

Support: NIH Grant AG064554
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NIH Grant MH109091
NIH Grant NS099573

Title: Contributions of L-Type Calcium, NMDA and AMPA channels to Synaptically-Evoked Depolarizations in the Mouse Neocortex Layer 2/3 - GEVI voltage imaging

Authors: M. ZHU[1], A. S. EDELSTEIN[2], B. L. BARBEAU[2], D. D. LOVIC[2], ***S. D. ANTIC**[2]; [1]Neurosci., [2]UConn Hlth., Farmington, CT

Abstract: Contributions of L-Type Calcium, NMDA and AMPA channels to Synaptically-Evoked Depolarizations in the Mouse Neocortex Layer 2/3 – GEVI voltage imaging
Mei Hong Zhu, Abigail S. Edelstein, Brianna L. Barbeau, Darko D. Lovic and Srdjan D. Antic
UConn Health School of Medicine, Farmington, CT, USA
We sought to estimate the contribution of L-type voltage-gated calcium channels (VGCC) to cortical depolarizations engulfing thousands of pyramidal cells simultaneously. Extracellular stimuli were delivered in one cortical layer and evoked depolarizations were recorded in several cortical layers simultaneously through population voltage imaging. Voltage imaging allows monitoring of subthreshold synaptic depolarizations, to which calcium imaging is relatively

blind. In brain slices of mice (Cux2-ASAP2s, age 40-80 days, both sexes), we stimulated Layer 5 or Layer 2/3 (glass electrode), and recorded voltage depolarizations (voltage imaging, 1 kHz) in the neuropil made of L2/3 pyramidal neurons (GEVI was restricted to L2/3 pyramids). We found that L2/3 cortical depolarizations were ~4-fold stronger (in amplitude) and ~3-fold shorter (in duration, half-width) depending on stimulus location: L2/3 vs. L5 (n=5). In experiments in which stimuli were delivered in L5 and responses measured in L2/3 of the same cortical column, a block of L-type VGCCs caused ~20% amplitude reduction (n=6). A combined block of both L-Type VGCC and NMDAr yielded only a small additional amplitude decrease (additional 10%). Much stronger reductions in the cortical depolarization peak amplitude (additional 30%) were achieved by the AMPAr inhibitor DNQX, 10 μM (n=6). Scientific rigor was achieved by making recordings in the same brain slice in 4 subsequent conditions (1. Baseline; 2. Blocked VGCC; 3. Blocked VGCC+NMDAr; and 4. Blocked VGCC+NMDAr+AMPAr). Our measurements have estimated: [1] the amount of direct depolarization from stimulating electrode to be around 35%; [2] the amount of VGCC activation in L2/3 pyramids (~20%); and the contributions of [3] NMDA (~10%) and [4] AMPA (~35%) receptor currents to the synaptically-evoked population voltage signal in L2/3 pyramidal neurons.

Disclosures: M. Zhu: None. **A.S. Edelstein:** None. **B.L. Barbeau:** None. **D.D. Lovic:** None. **S.D. Antic:** None.

Poster

361. Calcium Channels and Related Signaling

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Program #/Poster #: 361.04

Topic: B.03. Ion Channels

Support: NINDS Grant T32NS086750
NINDS Grant 1R01NS125271

Title: NMDAR Ca^{2+} flux and LTCC voltage-dependent conformational change cooperate locally and sequentially to signal to the nucleus

Authors: H. G. KHALED[1], N. MANDELBERG[2], X. WANG[2], R. W. TSIEN[2];
[1]Ctr. for Neural Sci., New York Univ., New York, NY; [2]New York Univ. Sch. of Med., New York, NY

Abstract: Enduring plasticity depends on neurons' ability to couple electrical signals at the surface membrane to gene expression in the nucleus, a process called excitation-transcription (E-T) coupling. L-type voltage gated Ca^{2+} channels (LTCCs) are critical for E-T coupling. Switching on LTCC initiates a signaling cascade relying upon calcium/calmodulin-dependent kinase II (CaMKII) and leading to activation of transcription factors such as cAMP response element binding protein (CREB), critical for neural plasticity and long-term memory. Previous studies of LTCC have often made the implicit assumption that they act as Ca^{2+} sources, parallel

to the N-methyl-d-aspartate receptor (NMDAR), and generate synaptic plasticity that is best studied with NMDAR blocked. On the contrary, we have recently demonstrated that excitatory neurons use two distinct signals to synergistically mediate CaMKII mobilization to dendritic spines and activity-dependent gene transcription: not just a local rise in Ca^{2+}, but also a voltage-dependent conformational change (VΔC) of LTCC. However, much remains unknown about how LTCC VΔC might cooperate with other postsynaptic Ca^{2+} sources such as NMDAR to drive excitation-transcription coupling. We find that NMDAR activation is sufficient to drive synergy between Ca2+ elevation and LTCC VΔC in cultured cortical neurons when LTCC Ca^{2+} flux is pharmacologically blocked. LTCC VΔC is required for NMDA-induced CaMKII mobilization and pCREB increases, which is blocked by the addition of LTCC-blocker nimodipine to prevent the conformational change. We clarified the spatial range of cooperation using Ca2+ chelators to block either long-range (EGTA) or short-range (BAPTA) Ca^{2+} spread. Only BAPTA successfully suppressed NMDA-induced rises in pCREB, suggesting that NMDAR and LTCC cooperate locally at the synapse. We investigated the temporal scale of LTCC VΔC-NMDAR Ca^{2+} synergy by measuring the effects of blocking LTCC VΔC at intermediate time points after NMDAR activation. Our results show that CaMKII mobilization increases independently of LTCC VΔC at early time points (15s) but not later ones (30-60s). These results suggest that NMDAR Ca^{2+}-LTCC VΔC synergy functions in series rather than in parallel, whereby NMDAR plays a dominant role initially while LTCC is more influential later on. This may translate to distinct roles for each channel in synaptic plasticity: NMDAR in driving CaMKII mobilization, and LTCC in sustaining it. Together, our findings provide molecular basis for how NMDAR and LTCC may cooperate to drive long-term synaptic changes.

Disclosures: H.G. Khaled: None. **N. Mandelberg:** None. **X. Wang:** None. **R.W. Tsien:** None.

Poster

361. Calcium Channels and Related Signaling

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Program #/Poster #: 361.05

Topic: B.03. Ion Channels

Support: NINDS 1R01NS125271
NINDS T32NS086750

Title: Novel technique for optically controlling L-type calcium channel functionshows local signaling from dendritic spine to nucleus in conjunction with NMDA receptors.

Authors: *N. MANDELBERG[1], R. W. TSIEN[2];
[1]New York Univ. Sch. of Med., New York Univ. Sch. of Med., New York, NY; [2]NYU Grossman Sch. of Med., New York Univ. Neurosci. & Physiol., New York, NY

Abstract: Neurons perform a remarkable range of tasks by changing their physiology in response to external stimuli. L-type voltage gated Ca^{2+} (Ca_V1) channels are critical for plasticity

because of their privileged role in regulating transcription in response to depolarization. However, it is not generally clear how activation of synapses up to hundreds of microns away from the cell body controls nuclear transcription. The coupling mechanism between synaptic activity and activation of gene expression predetermines the types of synaptic or electrical activity that can affect neurons' genetic programs. We developed a technique to optically isolate dendritic or somatic Ca_V1 activity using a photolabile Ca_V1 channel antagonist. We show that Ca_V1 channels act from dendritic spines to drive CaMKII-dependent activation of the powerful transcription factor CREB, synergizing with N-methyl-*D*-aspartate receptors (NMDARs), even in the absence of spikes. These same synaptic molecules enable both mEPSPs and action potentials to signal to the nucleus. We find that Ca_V1 channels cooperate with NMDARs to drive signaling to the nucleus from dendritic spines, and that activity from even a handful of spines can have impact on a neuron's transcriptional program.

Disclosures: N. Mandelberg: None. **R.W. Tsien:** None.

Poster

361. Calcium Channels and Related Signaling

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Program #/Poster #: 361.06

Topic: B.03. Ion Channels

Support: NS055251
R25GM083270
R01HL126887

Title: Voltage-gated CaV2.2 calcium ion channels in skin mediate capsaicin-induced heat hypersensitivity via interleukin -1α signaling

Authors: *A.-M. N. SALIB[1], M. J. CRANE[2], A. M. JAMIESON[2], D. LIPSCOMBE[1];
[1]Neurosci., [2]Dept. of Mol. Microbiology and Immunol., BROWN UNIVERSITY, Providence, RI

Abstract: Voltage-gated calcium ion (Ca_v) channels are essential for the transmission of sensory stimuli, detected by peripheral nerve endings, at synapses in the spinal cord and brain. Our lab recently discovered a novel, critical role for $Ca_v2.2$ channels (N-type currents) in peripheral Trpv1 nociceptor nerve endings in skin. Specifically, $Ca_v2.2$ channel activity is essential for the rapid, transient, and robust hypersensitivity to heat, but not to mechanical stimuli, that follows intradermal capsaicin (DuBreuil et al., 2021). We have shown capsaicin-induced, $Ca_v2.2$ channel-dependent intracellular Ca2+ signals in Trpv1 nociceptor endings and have evidence for ATP and P2X7 receptor involvement in the development of heat hypersensitivity. Here, we present data on the identity of inflammatory mediators released in response to intradermal capsaicin that are dependent on the activity of $Ca_v2.2$ channels. We show that $Ca_v2.2$ channel activation is necessary for the release of early inflammatory mediators underlying behavioral

hypersensitivity to heat, but not to mechanical stimuli. Within 15 minutes of an intraplantar capsaicin injection mice exhibit maximal heat and mechanical hypersensitivity. Coincident with these behavioral responses, we measured associated increases in interleukin-1α (IL-1α) levels in the interstitial fluid of the injected hindpaw. In $Ca_v2.2$ KO mice, capsaicin-induced heat hypersensitivity was reduced and IL-1α levels in hindpaw interstitial fluid were lower by ~60%, as compared to wild type controls (6-8 animals per group; p =0.0400). Direct intradermal injection of recombinant IL-1α in wild type mice induced heat hypersensitivity when compared to vehicle controls (n= 6 per group, p =0.0050). Further, co-injecting capsaicin with an IL-1α neutralizing antibody (Anti-mIL-1α-IgG) occluded capsaicin-induced heat hypersensitivity observed 15 minutes post-injection (n= 9 per group; p= 0.0043). Our data show that $Ca_v2.2$ channel activation in skin is necessary for neuroimmune signaling cascades that trigger the release of IL-1α, and that IL-1α plays a critical role in the development of heat hyperalgesia.

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Poster

361. Calcium Channels and Related Signaling

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Program #/Poster #: 361.07

Topic: B.03. Ion Channels

Support: DFG
SFB 1506

Title: In vivo electrophysiological analysis suggests a complex role of Cav2.3 channels in modulating burst activity of dopaminergic substantia nigra neurons

Authors: *G. GSCHWEND[1], L. DENTLER[1], J. SCHIEMANN[2], T. SCHNEIDER[3], G. SCHNEIDER[4], D. KAETZEL[1], B. LISS[1,5];
[1]Inst. of Applied Physiology, Ulm Univ., Ulm, Germany; [2]Ctr. for Integrative Physiol. and Mol. Medicine, Saarland Univ., Homburg, Germany; [3]Inst. for Neurophysiology, Univ. of Cologne, Cologne, Germany; [4]Dept. of Computer Sci. and Mathematics, J. W. Goethe Univ., Frankfurt am Main, Germany; [5]Linacre and New College, Univ. of Oxford, Oxford, United Kingdom

Abstract: Dopamine releasing neurons (DAN) within the midbrain are important for a variety of brain functions and their dysfunction can lead to complex diseases like Parkinson's (PD) or Schizophrenia. They are arranged in two overlapping nuclei, the substantia nigra (SN) and the ventral tegmental area (VTA). In vivo, within the intact basal ganglia network, DA midbrain neurons mainly display two general types of firing patterns: tonic irregular single-spike activity (~1-10 Hz) or phasic burst activity with higher frequencies. These activity patterns of DA neurons are crucial for their physiological function and their distinct pathophysiology. In PD, SN DAN are particularly affected by progressive degeneration, while VTA DAN remain largely

intact. The cause for this differential vulnerability is still unclear, but cell type specific ion channel activity, activity-related calcium homeostasis, and metabolic stress are important factors. We have previously identified R-type voltage-gated Ca2+ channels (Cav2.3) as crucial contributors to somatic activity-related Ca2+ signaling in mouse SN DAN, and to their preferential degeneration in an in vivo PD mouse model, suggesting Cav2.3 inhibition as a novel strategy for PD-therapy. However, the physiological roles of Cav2.3 channels - and of their chronic inhibition or inactivation - in SN DAN remain unclear.
Here we addressed Cav2.3 channel function in SN DAN in vivo, by comparing spike-patterns of isoflurane-anesthetized adult wildtype (WT, N=17) and Cav2.3 knockout (KO, N=10) mice. We combined stereotactically guided extracellular single-unit recordings with juxtacellular neurobiotin-labeling, as well as anatomical and immunohistochemical identification (double-staining for tyrosine hydroxylase and for calbindin-d28k, a marker for less-vulnerable DA neurons). By activity-pattern analysis, using spike sorting approaches, autocorrelation histograms, and a probabilistic spiking model for rhythmic bursting and tonic activity, we found that the overall spike-frequencies of SN DAN were similar in WT (4.1±1.8 Hz, mean±SD) and KO (4.3±1.5 Hz) within the 8 min recording-intervals (p=0.7, MWU-test). However, the number of SN DAN displaying burst activity, was about 43% lower in KO mice (~24%), compared to WT (~42%; p=0.09, Chi2-test), while the number of spikes per burst was significantly higher (WT: 2.7±0.5; KO: 4.3±1.2; p=0.01, MWU).
Our findings suggest a complex role of Cav2.3 channels for burst activity of SN DAN, but not for their overall firing-frequency. Reduced metabolically demanding burst activity of SN DAN in Cav2.3 KO mice offers a possible mechanism for their reduced vulnerability in the PD-model.

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Poster

361. Calcium Channels and Related Signaling

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Program #/Poster #: 361.08

Topic: B.03. Ion Channels

Support: Austrian Science Fund (FWF) DOC30-B30
NFB grant LSC19-017
Austrian Science Fund (FWF) F44150

Title: Consequences of $\alpha_2\delta$ subunit mutations linked to brain disorders on neuronal calcium channel trafficking and synapse composition

Authors: *S. HADDAD[1], C. ABLINGER[1], R. STANIKA[2], M. HESSENBERGER[2], M. CAMPIGLIO[1], N. ORTNER[3], G. OBERMAIR[2,1];
[1]Med. Univ. of Innsbruck, Innsbruck, Austria; [2]Karl Landsteiner Univ. of Hlth. Sci., Krems, Austria; [3]Univ. of Innsbruck, Innsbruck, Austria

Abstract: The roles of auxiliary $\alpha_2\delta$ subunits of voltage-gated calcium channels in modulating membrane expression and calcium current properties are widely recognized. In addition, recent literature suggests an important role of $\alpha_2\delta$ proteins in synapse formation and differentiation. Therefore, it is not surprising that $\alpha_2\delta$ proteins have been linked to various neurological and neuropsychiatric disorders, emphasizing their importance for brain connectivity. Here we aimed to investigate human mutations in $\alpha_2\delta$ proteins by addressing their synaptic functions, besides their role as channel subunit, to shed light on the underlying pathophysiological mechanisms. We characterized two mutations, the autism-associated mutation p.Arg351Thr in $\alpha_2\delta$-1 (CACNA2D1) and the epilepsy-related mutation p.Arg596Pro in $\alpha_2\delta$-2 (CACNA2D2), cloned into mouse cDNA, by employing primary cultured hippocampal neurons and tsA201 cells as homologous and heterologous expression system, respectively. First, we quantified plasma membrane trafficking and analyzed potential consequences on synapse composition. Second, to determine potential effects on the biophysical channel properties, we performed electrophysiological recordings after expression in tsA201 cells of either $Ca_V2.1$ or $Ca_V1.3$ together with wildtype or mutated $\alpha_2\delta$ and auxiliary β subunits. Live-cell labelling of cultured hippocampal neurons transfected with 2HA-tagged $\alpha_2\delta$ subunits revealed a strong and highly significant reduction in somatic, dendritic and axonal membrane expression as well as in synaptic targeting of both mutants. However, only neurons transfected with $\alpha_2\delta$-2_p.Arg596Pro showed a significantly reduced mismatched localization of postsynaptic $GABA_A$Rs opposite glutamatergic nerve terminals, a previously identified trans-synaptic function of $\alpha_2\delta$-1 and $\alpha_2\delta$-2 splice variants lacking exon 23. Similarly, electrophysiological analysis indicated that current density of $Ca_V1.3$ was not compromised by $\alpha_2\delta$-1_p.Arg351Thr, contrary to co-expression of $\alpha_2\delta$-2_p.Arg596Pro, which resulted in a strongly reduced current density together with a ~9 mV right shift in the current-voltage relationship compared to co-expression of wild type $\alpha_2\delta$-2. Surprisingly however, both mutations did not affect the current properties of heterologously expressed presynaptic $Ca_V2.1$ channels despite the strong reduction in membrane expression, which was also evident in tsA201 cells. Taken together, our data show that disease-associated $\alpha_2\delta$ mutations can alter channel-dependent as well as synaptic functions of $\alpha_2\delta$ proteins, both of which may contribute to pathophysiological mechanisms.

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Poster

361. Calcium Channels and Related Signaling

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Program #/Poster #: 361.09

Topic: B.03. Ion Channels

Support: FWF DOC30-B30
FWF F44150
NfB LSC19-017
FWF FG18

Title: Loss of autism-associated $\alpha_2\delta$-3 affects synaptic function

Authors: *C. ABLINGER[1], A. SAH[2], N. J. ORTNER[2], H. SEITTER[2], S. GEISLER[2], M. CAMPIGLIO[1], M. MISSLER[3], N. SINGEWALD[2], G. J. OBERMAIR[4,1];
[1]Med. Univ. Innsbruck, Innsbruck, Austria; [2]Dept. of Pharmacol. and Toxicology, Inst. of Pharmacy, CMBI, Univ. of Innsbruck, Innsbruck, Austria; [3]Westfaelische Wilhelms Univ. of Muenster, Muenster, Germany; [4]Dept. of Physiol., Karl Landsteiner Univ. of Hlth. Sci., Krems an der Donau, Austria

Abstract: Genome wide association studies (GWAS) have linked CACNA2D3, the gene encoding for the auxiliary calcium channel subunit $\alpha_2\delta$-3, to autism spectrum disorders. Hence, $\alpha_2\delta$-3 knockout (KO) mice may serve as a model for studying neurodevelopmental disorders. We further hypothesize that defects in synapses or synaptic connections may underlie potential behavioral phenotypes.
To test this, we analyzed the behavioral phenotype of adult $\alpha_2\delta$-3 wildtype, heterozygote, and knockout mice using open field and forced swim tests (n=7-17 mice), assessed brain structure in serial Nissl-stained sections (n=3 mice) and analyzed synaptic protein expression using western blot analysis of synaptosomal lysates (n=4 mice). Furthermore, we tested synaptic functions in cultured hippocampal neurons (obtained from P0 mice) using presynaptic calcium imaging (n=3 cultures; 25-30 cells). Finally, we analyzed neuronal excitability, miniature excitatory postsynaptic potentials (EPSPs) (n=5-6 mice; 14-26 cells) as well as short- and long-term plasticity in slice electrophysiology (n=4-5 mice; 7-16 cells).
Behavioral characterization revealed a mild anxiogenic phenotype together with increased active coping in KO mice compared to littermate controls, suggesting an involvement of $\alpha_2\delta$-3 in neurological disorders. Structural brain analysis in Nissl-stained sections illustrated no gross morphological changes in $\alpha_2\delta$-3 KO brains. To study consequences on synaptic functions we employed synGCaMP6f targeted to presynaptic boutons of cultured hippocampal neurons. Knockout of $\alpha_2\delta$-3 resulted in a reduction of calcium transients after stimulation with 1 action potential (AP), 3 APs, and 10 Aps. Biochemical analysis of synaptosomal lysates demonstrated a strong reduction of synaptic protein expression in $\alpha_2\delta$-3 KO brains, most strikingly of striatal NMDAR2B. Therefore, we next analyzed plasticity and the intrinsic excitability of striatal neurons in slice electrophysiology, revealing a hypo-excitability in $\alpha_2\delta$-3 KO neurons. Both miniature and evoked EPSP amplitude were significantly reduced, and short-term plasticity (paired-pulse ratio) and long-term plasticity (LTP) were affected by loss of $\alpha_2\delta$-3.
Our results illustrate a novel important synaptic role of $\alpha_2\delta$-3 in regulating pre- and postsynaptic function and protein expression, neuronal excitability, and synaptic plasticity. Together our findings support the link of CACNA2D3 ($\alpha_2\delta$-3) to neurodevelopmental disorders including autism. To ultimately assess the suitability of $\alpha_2\delta$-3 KO mice as a model for studying autism, we will test for signs of autism-like behavior and examine synaptic ultrastructure and wiring.

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Poster

361. Calcium Channels and Related Signaling

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Program #/Poster #: 361.10

Topic: B.03. Ion Channels

Support: NINDS T32 NS086749 Training Grant

Title: Differential effects of calcium channel mutations on (R)-roscovitine analog-mediated slowed deactivation

Authors: *S. ALDRICH[1], R. LAGHAEI[3], M. H. CHENG[2], I. BAHAR[2], S. MERINEY[1]; [1]Neurosci., [2]Computat. and Systems Biol., Univ. of Pittsburgh, Pittsburgh, PA; [3]Pittsburgh Supercomputing Ctr., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Insufficient release of neurotransmitter from motor neurons is a cause of debilitating muscle weakness in multiple types of neuromuscular disease, including Lambert-Eaton Myasthenic Syndrome and Spinal Muscular Atrophy. Neurotransmitter release from motor neurons is controlled by the Ca_V2 family of voltage-gated calcium channels (VGCCs), which open in response to an action potential, causing an influx of calcium that triggers vesicle fusion. The drug GV-58, an analog of the CDK inhibitor (R)-roscovitine, has been found to rescue neuromuscular transmission in animal disease models by selectively prolonging the opening of Ca_V2 VGCCs, thereby enhancing calcium influx during action potentials and increasing the probability of vesicle fusion. Our main objective is to understand where (R)-roscovitine analogs bind to VGCCs and the mechanism by which they prolong channel opening. This information would aid in the design of improved analogs, as well as providing insight into the structural basis of VGCC gating. To that end, we used patch clamp electrophysiology to measure the effects of the (R)-roscovitine analog KK-20 on the deactivation kinetics of wild-type, singly mutated, doubly mutated, and chimeric calcium channels. We show that, of the four six-segment heterologous domains of VGCCs, domain III segments 4-6 (but not 1-3) of the Ca_V2 channel sequence are sufficient for the (R)-roscovitine analog KK-20 to prolong opening of Ca_V1/Ca_V2 chimeric channels, narrowing down the likely binding site to this region. We further present several exploratory single-residue mutations of Ca_V2-specific inner pore residues, selected based on computer models of drug binding in Ca_V2 channels, that have differential effects on KK-20's open-state-prolonging effect and concentration-dependence of binding. Finally, we present the results of a preliminary investigation into the hypothesis (based on our site-directed mutagenesis findings) that (R)-roscovitine analog binding disrupts residue interactions that couple voltage sensor deactivation with pore closure.

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Poster

361. Calcium Channels and Related Signaling

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Program #/Poster #: 361.11

Topic: B.03. Ion Channels

Support: NIH Grant 1 R35 GM141802

Title: Early exposure to general anesthesia leads to increased T-current densities and long-lasting hyperexcitability of thalamocortical networks in mice

Authors: *V. P. TADIC[1], S. M. TODOROVIC[2];
[2]Anesthesiol., [1]Univ. of Colorado Anschutz Med. Campus, Aurora, CO

Abstract: It has been shown that the exposure to general anesthetics (GA) during the critical periods of development in rodent and mammalian brains can cause a widespread neurodegeneration, including in the thalamus. However, lasting effects of an early exposure to GA to neuronal function is not well studied. Here, we focused on the thalamocortical (TC) network which consists of mutually interconnected neurons of somatosensory cortex, ventrobasal (VB) thalamic nuclei, and reticular thalamic nucleus (NRT). It is well known that rhythmic oscillations of TC network are important for normal sensory processing, cognitive functions and consciousness. We studied here whether there is an altered excitability of intact TC network, as well as plasticity of ion channels in neurons of VB thalamus in adult mice that were treated with GA in early life. Mice pups were exposed at postnatal day (PND) 7 to either GA (treated group) or mock anesthesia (control group) for 6 hours (n=54). Being commonly used in clinical practice, Sevoflurane was the anesthetic of choice (3% for the first 2h of exposure; 2.4% for the other 4h) and mock anesthesia for the control group consisted of regular air supplemented with 30% O2. Approximately at PND 65 - PND 80, the surviving animals from both groups (n=34) were implanted with cortical electroencephalogram (EEG) electrodes and recordings were obtained 15 minutes before and 45 minutes after the intraperitoneal (IP) application of gamma-butyrolactone (GBL), a drug used in research for its ability to induce a characteristic spike-wave discharge (SWD) pattern on EEG. We found that there was a significant increase in cumulative SWD duration of about 2-fold in first the 30 minutes after the GBL application ($p<0.001$) in treated (n=15) vs. control (n=19) groups. In ensuing voltage-clamp recordings, we studied properties of T-type calcium currents (T-currents) of VB neurons (n=15 control; n=25 treatment). We demonstrated that peak T-current densities were significantly increased in the GA group compared to the control group (about 40%). In addition, the half-maximal inactivation voltage (V50) showed a significant depolarizing shift of about 3 mV in the GA group ($p<0.05$), without significant changes in voltage-dependent activation.Based on our experiments, we conclude that there is a chronic hyperexcitability of TC networks in mice that were exposed to GA during early life, with the possible contribution of altered biophysical properties and increased densities of T-currents in VB neurons. We propose that targeting T-type calcium channels may ameliorate chronically hyperexcitable TC networks in mice exposed to GA during brain development.

Disclosures: V.P. Tadic: None. **S.M. Todorovic:** None.

Poster

361. Calcium Channels and Related Signaling

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Program #/Poster #: 361.12

Topic: B.03. Ion Channels

Title: Two alternatively spliced exons at the C-terminus of CACNA1G, the spinocerebellar ataxia-42 gene, modulate the activity of the low-voltage activated Cav3.1 calcium channel

Authors: R. WANG[1], Z. WANG[1], D. MOAKLEY[2], C. ZHANG[2], Y. YU[1], ***M. RUGGIU**[1]; [1]Biol. Sci., St. John's Univ., Jamaica, NY; [2]Dept. of Systems Biol. & Dept. of Biochem. and Mol. Biophysics, Columbia Univ. Med. Ctr., New York, NY

Abstract: Spinocerebellar ataxias (SCAs) are a heterogeneous group of neurodegenerative disorders, characterized by brainstem and cerebellum degeneration accompanied by loss of balance and coordination, and by slurred speech. They are autosomal dominant genetic diseases with >40 genetically distinct SCA subtypes. Mutations in the *CACNA1G* gene, which encodes the alpha-1G subunit of the low-voltage-activated T-type calcium channel Cav3.1, cause SCA42. Cav3.1 plays crucial roles in cardiac and smooth muscle cells and neurons by influencing the transmembrane potentials and regulating intracellular Ca^{2+} signaling, and *Cacna1g* null mice display severe motor coordination defects, aberrant action potentials in the brain, disrupted sleeping patterns, absence epilepsy, and abnormal electrical conductance in the heart. Different splice variants of Cav3.1 have been described, and alternative splicing of Cav3.1 can alter channel kinetics, localization, and cytosolic Ca^{2+} trafficking, thus creating a complex and diverse system of electrical conduction and signal transduction. In this work we analyzed the function of two exons, termed E34 and E35, which are found immediately after domain IV of the intracellular C-terminus, and whose function is largely unknown. These exons are alternatively spliced in all possible combination in mouse tissues, giving rise to four different splice variants. Alternative splicing of E34 and E35 is developmentally regulated, as the two exons are preferentially included in nerve tissue postnatally, while they are mostly skipped in embryonic tissues. To investigate the mechanism of E34 and E35 splicing regulation, we generated minigene reporters and discovered that inclusion of E34 and E35 is specifically promoted by the neuron-specific splicing factors Nova1, Nova2, and Ptbp2. To examine the physiological properties of these splice variants, we recorded channel activity by two-electrode voltage clamp in *Xenopus* oocytes, and whole cell patch clamp in HEK293 cells. We discovered that including either or both E34 and E35 in Cav3.1 may facilitate Ca^{2+} influx. We also investigated the effects of the calcium-binding protein calmodulin on the activity of the different channel splice variants; our data show that calmodulin confers current facilitation on E34 and/or E35-including Cav3.1 isoforms and shifts the inactivation curve. Taken together, our data indicate that alternative splicing at E34 and E35 of Cav3.1 may regulate neuron excitability and modulate the intrinsic firing pattern by controlling Ca^{2+} influx through the channel.

Disclosures: R. Wang: None. **Z. Wang:** None. **D. Moakley:** None. **C. Zhang:** None. **Y. Yu:** None. **M. Ruggiu:** None.

Poster

361. Calcium Channels and Related Signaling

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 361.13

Topic: B.03. Ion Channels

Support: FWF SFB F-44
DFG

Title: Complex changes of voltage-gated Ca^{2+} currents in vulnerable dopaminergic substantia nigra neurons with age and in the A53T α-synuclein Parkinson's disease mouse model

Authors: *A. GAIFULLINA[1], C. POETSCHKE[1], N. WIEDERSPOHN[1], N. WATTAD[2], T. GERADA-GANAN[1], G. SARIGU[1], T. P. SNUTCH[3], J. A. GOLDBERG[2], B. LISS[1];
[1]Inst. of Applied Physiol., Ulm Univ., Ulm, Germany; [2]Dept. of Med. Neurobio., The Hebrew Univ. of Jerusalem, Jerusalem, Israel; [3]Dept. of Psychiatry, Michael Smith Lab, UBC, Vancouver, BC, Canada

Abstract: The progressive loss of dopaminergic neurons within the *Substantia nigra* (SN DAN) causes the motor symptoms of Parkinson's disease (PD). The cause for the high vulnerability of SN DAN is unclear, but specific ion channel activites, activity-related metabolic stress, and Ca^{2+} homeostasis are important factors. SN DAN display an autonomous pacemaker-activity, accompanied by increases in cytosolic Ca^{2+}. Cholinergic motoneurons of the dorsal motor nucleus of the vagus (DMV) display similar pacemaker-activity, but are less vulnerable in PD. In resolving this differential vulnerability, voltage-gated Ca^{2+} channels (Cav) and Ca^{2+} sensitive, voltage-gated A-type K^+ (Kv4) channels are of particular interest, as both channel types modulate the activity of DA and DMV neurons, and have been linked to PD-pathophysiology. Here, we pharmacologically analyze Cav currents and the Ca^{2+} dependency of A-type currents in SN DAN in brain slices from juvenile and adult mice, using whole-cell patch-clamp recordings with appropriate voltage-step and -ramp protocols, to dissect low and high voltage-activated Ca^{2+} currents (LVA, HVA). We analyzed wildtype (WT) and also mice overexpressing a mutant form of human α-synuclein (A53T), causing familial forms of PD (PARK1), and we compared SN DAN with DMV neurons. LVA Ca^{2+} currents in SN DAN were almost fully blocked by the T-type Cav3 inhibitor Z941 (10µM, voltage-ramps). Cav3 inhibition also affected the steady-state voltage-dependency of Cav currents, reducing window currents by ~50%, and shifting it to more positive potentials. With postnatal maturation, peak LVA currents in SN DAN became larger and shifted toward more negative potentials. However, we did not gain evidence for altered T-type currents in SN DAN form A53T mice. In contrast, in DMV neurons, T-type currents were reduced by ~50% in A53T compared to WT mice, in line with our molecular biology data. HVA currents in SN DAN were not altered with postnatal maturation, but HVA-pharmacology was more complex: L-, R- and PNQ-type blockers all inhibited SN DAN Cav currents by ~20-30%. Importantly, HVA currents of SN DAN in adult A53T mice were ~30% smaller as in WT, likely due to a respective reduction of isradipine-sensitive L-type Cav1 currents. Distinct Cav channel blockers had only subtle effects on A-type current amplitudes or kinetics in SN DAN, but their Ca^{2+} sensitivity depended mainly on intracellular stores, as Kv4 currents were reduced by ~20%

with ryanodine (10μM), and increased by ~35% with SERCA inhibition (1μM thapsigargin). We are currently further dissecting the complex roles of distinct ion channels in determining differential neuronal function and vulnerability in PD

Disclosures: **A. Gaifullina:** None. **C. Poetschke:** None. **N. Wiederspohn:** None. **N. Wattad:** None. **T. Gerada-Ganan:** None. **G. Sarigu:** None. **T.P. Snutch:** None. **J.A. Goldberg:** None. **B. Liss:** None.

Poster

361. Calcium Channels and Related Signaling

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Program #/Poster #: 361.14

Topic: B.03. Ion Channels

Support: AA016852
AA026117

Title: Ethanol Withdrawal Hyperexcitability Mediated by Protein Kinase Ce

Authors: ***H. SHAN**[1], A. KAMATH[2], D. GODWIN[3];
[1]Wake Forest Univ. Sch. Med., Winston Salem, NC; [2]wakeforest university, winston-salem, NC; [3]wakeforest school of medicine, winston-salem, NC

Abstract: Severe Alcohol Withdrawal (SAW) and detoxification produce a range of serious clinical symptoms, including intense seizures. SAW can produce kindling-like phenomena such that seizures worsen with each withdrawal. This complicates treatment and can be fatal. Benzodiazepines are the dominant treatment for seizure control, but have potential for addiction, toxicity, cross tolerance and rebound effects that can worsen long-term outcomes. Thus finding alternative mechanisms for the emergence of hyperexcitability in the neural circuitry underlying WD seizure (including thalamic and hippocampal networks) and other WD symptoms remain important research areas. Hyperexcitability is produced by an array of intrinsic and synaptic properties that are disrupted by ethanol and embedded in a supportive brain network. T-type calcium channels are functionally upregulated by multiple bouts of Chronic Intermittent Ethanol (CIE: Graef and Godwin, 2011). For our current study, standard whole cell patch recordings were made from brain slices at physiological temperatures containing the reuniens nucleus in thalamus from mice undergoing 4 bouts of CIE. Current/voltage relationships were plotted and fit with a Boltzmann function. Isolated native T currents showed a statistically significant rightward shift in voltage dependency (I/Imax) of inactivation, consistent with membrane hyperexcitability. A general PKC peptide inhibitor, delivered in the internal pipette solution, blocked the WD effect. Treatment of cells with a PKCε translocation blocking peptide in the internal pipette solution also blocked the WD shift. Cells treated with a scrambled PKCε control peptide did not significantly shift the I/V curve. T-type currents show evidence of acute tolerance to EtOH recorded near the peak of WD in reuniens relay neurons. Current clamp recordings

show enhanced excitability in the WD condition consistent with PKC-mediated gain-of-function of native T currents. Conclusion: We previously showed PKC-mediated sensitivity of T-type calcium channels to ethanol. Our prior work suggests that Cav3.2 is the target of this effect. Our current work suggests that PKCε is upregulated during CIE-mediated WD and is responsible for gain of function in native T currents.

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Poster

361. Calcium Channels and Related Signaling

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Program #/Poster #: 361.15

Topic: B.03. Ion Channels

Support: NIH MH115045

Title: $Ca_V3.3$ potentiators modulate the output of thalamic reticular neurons and increase sleep spindles in mice

Authors: *S. P. MORAN[1], E. YU[1], A. GHOSHAL[1], D. E. BAEZ-NIETO[1], L. A. WANG[1], J. R. COLEMAN, Jr.[1], N. W. HODGSON[2], Y.-L. ZHANG[1], M. FITZGERALD[1], S. JO[1], J. GALE[1], N. KURA[1], N. SHUART[1], W. MARTENIS[1], M. WEIWER[1], F. F. WAGNER[1], J. Q. PAN[1]; [1]Broad Inst. of MIT and Harvard, Cambridge, MA; [2]Boston Children's Hosp., Boston, MA

Abstract: Schizophrenia is a debilitating disorder that lacks effective treatments for the negative symptoms (e.g., social withdrawal) and cognitive disruptions (e.g., working memory deficits) in patients. Recently, large scale human genetics studies including genome wide association studies (GWAS) and exome sequencing studies have identified many replicable genomic loci for schizophrenia risk. CACNA1I was implicated in schizophrenia risk by GWAS and rare variations, and it encodes the functional core, α1 subunit, of $Ca_V3.3$ voltage-gated calcium channels. $Ca_V3.3$ channels are expressed in a subset of neurons including GABAergic neurons of the thalamic reticular nucleus (TRN) where they regulate neuronal excitability. The TRN has emerged as a crucial brain nucleus in the generation of sleep spindles, sleep dependent memory, focused attention, and cognitive flexibility, all of which are impaired in patients with schizophrenia. Our group and others have demonstrated that loss of $Ca_V3.3$ dramatically impairs TRN neuronal firing and reduces sleep spindles occurrences in mice, and reduction of sleep spindles is a highly reproducible trait of schizophrenia patients. Based on these genetic and biological evidence, we hypothesize that $Ca_V3.3$ potentiators could benefit patients with schizophrenia by rescuing sleep spindle deficits and improving sleep dependent cognitive function. Utilizing high throughput molecular pharmacology, automated patch clamp electrophysiology, ex vivo and in vivo electrophysiology, and animal behavior, we identified a set of potent $Ca_V3.3$ potentiators that selectively enhance $Ca_V3.3$ function. Furthermore, our $Ca_V3.3$ potentiators can increase sleep spindles in healthy adult mice and selectively rescue sleep

spindle deficits in genetic models of $Ca_V3.3$ hypofunction. Given the wealth of literature demonstrating a critical role of $Ca_V3.3$ function in generating sleep spindles, our demonstration of the first selective $Ca_V3.3$ potentiator may represent an exciting step towards the development of a novel class of potential therapeutics for the treatment of sleep disturbances in patients with schizophrenia.

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Poster

361. Calcium Channels and Related Signaling

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Program #/Poster #: 361.16

Topic: B.03. Ion Channels

Support: Funding: Jazz Pharmaceuticals

Title: In vitro pharmacological characterization of suvecaltamide: a potent, selective, and state-dependent modulator of voltage-gated T-type calcium channels $Ca_V3.1$, $Ca_V3.2$, and $Ca_V3.3$

Authors: *B. BRIGHAM, A. RANDALL, N. F. SHANKS, M. S. LEE;
Jazz Pharmaceuticals, Palo Alto, CA

Abstract: T-type Ca^{2+} (Ca_V3) channels are expressed in sensory and motor networks where they play key roles in setting intrinsic neuronal excitability and oscillatory activity. Defects in Ca_V3 channel function are linked to various neurological disorders. Suvecaltamide (JZP385, formerly CX-8998), a Ca_V3 modulator, reduced tremor severity and improved function in adults with moderate to severe essential tremor (ET) in a phase 2 clinical study (T-CALM, NCT03101241). Here, we detail *in vitro* properties of suvecaltamide, including its mechanism of inhibition of Ca_V3 and selectivity against other targets. Recordings were performed in HEK293 cells stably overexpressing human $Ca_V3.1$, 3.2, or 3.3 using a QPatch 48X or SyncroPatch 384PE automated patch clamp system. Relative to vehicle, suvecaltamide elicited a concentration-dependent hyperpolarizing shift in the midpoint ($V_{0.5}$) of voltage-dependent inactivation of all Ca_V3 channels ($\Delta V_{0.5inact}$ range: −3 mV at 10 nM to −28 mV at 10 μM; n≥4). In contrast, 100-1000 nM suvecaltamide had modest effects on Ca_V3 activation ($\Delta V_{0.5act}$ ≤+8 mV; n≥3). In SyncroPatch recordings, mean suvecaltamide IC_{50}s for resting (V_h=−110 mV) $Ca_V3.1$, 3.2, and 3.3 channels were >1 μM and exhibited strong state dependence (eg, IC_{50} for resting $Ca_V3.1$ was 4.3 μM vs 74 nM at $V_{0.5}$, yielding a ratio of 58). Further support for suvecaltamide's higher affinity for the inactivated state was demonstrated by slowed recovery from inactivation of all 3 Ca_V3 channel subtypes in the presence of suvecaltamide (2-8-fold shift in time constant of recovery from inactivation vs vehicle; n≥3/condition) and by use-dependent inhibition (~10-fold increase in rate

of inhibition at 1 vs 0.1 Hz stimulation). Suvecaltamide Ca_V3 selectivity was demonstrated by lack of off-target effects in a panel of 169 enzymes, receptors, ion channels, and transporters (<50% inhibition or stimulation at 10 μM except at the CB2 receptor, IC_{50}=5.3 μM). In addition, suvecaltamide had only weak inhibition of hERG (IC_{50}=21-28 μM) or $hNa_V1.5$ (IC_{50}>30 μM) current. These results collectively demonstrate that suvecaltamide is a selective, potent, and state-dependent modulator of all 3 Ca_V3 channel subtypes with markedly higher affinity for inactivated channels. At clinically relevant concentrations, suvecaltamide may selectively inhibit the form of the channel enriched under hyperexcitable conditions (eg, pathological neuronal firing) while sparing the form important for normal signaling. This may engender an optimal clinical profile. An ongoing phase 2b clinical trial (NCT05122650) will further evaluate the safety and efficacy of suvecaltamide for adults with moderate to severe ET.

Disclosures: **B. Brigham:** A. Employment/Salary (full or part-time):; Jazz Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Jazz Pharmaceuticals. **A. Randall:** A. Employment/Salary (full or part-time):; Jazz Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Jazz Pharmaceuticals. **N.F. Shanks:** A. Employment/Salary (full or part-time):; Jazz Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Jazz Pharmaceuticals. **M.S. Lee:** A. Employment/Salary (full or part-time):; Jazz Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Jazz Pharmaceuticals.

Poster

361. Calcium Channels and Related Signaling

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Program #/Poster #: 361.17

Topic: B.03. Ion Channels

Support: Funding: Jazz Pharmaceuticals

Title: Anti-tremor efficacy of suvecaltamide in a rat model of essential tremor

Authors: *N. F. SHANKS[1], M. S. LEE[1], A. RANDALL[1], S. MARKOVA[1], L. TAN[1], D. HUFFMAN[2], B. BRIGHAM[1];
[1]Jazz Pharmaceuticals, Palo Alto, CA; [2]Signal Solutions, LLC, Lexington, KY

Abstract: Low-voltage-activated Ca^{2+} channels (Ca_V3) regulate the excitability and oscillatory activity of neurons and are highly expressed in brain regions comprising a network that mediates excessive rhythmicity in essential tremor (ET). Suvecaltamide, a selective, potent, and state-dependent modulator of all 3 Ca_V3 subtypes, reduced tremor severity and improved function in a phase 2 study of participants with moderate to severe ET (T-CALM, NCT03101241). The

present studies characterize anti-tremor efficacy of suvecaltamide in harmaline-treated rats, a widely employed and clinically relevant acute model of ET. Male Sprague Dawley rats were administered a single intraperitoneal dose of harmaline and monitored continuously for the duration of the acute tremor response using a piezoelectric sensor-based home-cage system (Adapt-A-Base, Signal Solutions, KY). Harmaline dosing was optimized to maximize the number of rats demonstrating a measurable tremor response without excessive impairment. Piezoelectric measurements (9-12 Hz) were converted to power spectral signals for tremor analysis. Suvecaltamide (0.1-10 mg/kg oral) or vehicle was administered 30 minutes prior to, or 1 hour after, harmaline injection. The time course of the tremor signal was visualized by plotting tremor band contribution in 10-minute bins spanning 1 hour pre-harmaline to 5 hours post-harmaline and quantified by reporting tremor band power over predefined times including peak tremor response. Suvecaltamide's anti-tremor efficacy was compared to the only FDA-approved medication for ET, propranolol (20 mg/kg intraperitoneal or 50 mg/kg oral). When administered 30 minutes pre-harmaline, suvecaltamide dose-dependently inhibited the induction of tremor, with complete and sustainable inhibition at 10 mg/kg and partial inhibition at 1 mg/kg. Supratherapeutic concentrations of propranolol were required to transiently reduce tremor in this model. When administered 1 hour post-harmaline, suvecaltamide significantly reduced ongoing tremor in a dose-dependent manner with significant and robust reduction at doses ≥1 mg/kg and partial, transient reduction at 0.3 mg/kg. Plasma suvecaltamide concentrations associated with doses that reduced tremor were consistent with those achieved at steady state in humans at projected therapeutic doses. Oral suvecaltamide effectively reduced tremor in rats pre- or post-harmaline, and in a manner superior to propranolol, supporting the continued development of suvecaltamide for the treatment of ET. An ongoing phase 2b trial (NCT05122650) will further evaluate the safety and efficacy of suvecaltamide for adults with moderate to severe ET.

Disclosures: **N.F. Shanks:** A. Employment/Salary (full or part-time):; Jazz Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Jazz Pharmaceuticals. **M.S. Lee:** A. Employment/Salary (full or part-time):; Jazz Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Jazz Pharmaceuticals. **A. Randall:** A. Employment/Salary (full or part-time):; Jazz Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Jazz Pharmaceuticals. **S. Markova:** A. Employment/Salary (full or part-time):; Jazz Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Jazz Pharmaceuticals. **L. Tan:** A. Employment/Salary (full or part-time):; Jazz Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Jazz Pharmaceuticals. **D. Huffman:** A. Employment/Salary (full or part-time):; Signal Solutions. **B. Brigham:** A. Employment/Salary (full or part-time):; Jazz Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Jazz Pharmaceuticals.

Poster

361. Calcium Channels and Related Signaling

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 361.18

Topic: B.03. Ion Channels

Support: NIH NIDDK 12346288

Title: The energetic landscape of gating a neuronal TRP channel

Authors: *P. DECAEN;
Northwestern Univ., Chicago, IL

Abstract: PKD2 and PKD2L1 are members of the polycystin subfamily of transient receptor ion channels (TRP), and are expressed in the kidney and brain, respectively. Human variants in PKD2 are associated with autosomal dominant polycystic kidney disease (ADPKD), whereas loss of PKD2L1 function is associated with epilepsy phenotypes in mice. Polycystins are voltage-dependent, but also integrate polymodal stimuli to control channel 'gating'— a conformational change which controls flux of monovalent and calcium ions across cell membranes. Cryo-EM structural analysis has led to the proposition of distinct gates at the entry and exit of the pore module. However, results from structure-function studies of other TRP subfamilies have produced conflicting results regarding the existence of a second gate. Here we probe PKD2L1 for sites within the pore which impact the gating energetics with the goal of resolving the location of the putative gate(s). PKD2L1 presents a tenable method for integrating the biophysical regulation of polycystin because it form functional ion channels on the plasma membrane, whereas PKD2 functions in the membranes of organelles. We subjected the pore of the PKD2L1 channel a complete and unbiased mutagenic functional screen of more than 150 mutations tested using patch clamp electrophysiology. Biophysical gating parameters were collected including rates of channel opening, closing and voltage-dependence of activation and inactivation. We establish a heat-map of the entire pore domain which defines sites which control gating, and a new structural model of the channel which enumerate the essential interactions made during channel opening. Finally, we compare the impact of ADPKD-causing variants found within the pore domain in conserved sites in PKD2 and PKD2L1.

Disclosures: P. Decaen: None.

Poster

361. Calcium Channels and Related Signaling

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 361.19

Topic: B.03. Ion Channels

Title: Activation of TRPC5 Reduces Food Intake in Mice

Authors: *Y. LI, N. YIN, Y. XU;
Baylor Col. of Med., Houston, TX

Abstract: Activation of TRPC5 Reduces Food Intake in MiceThe rising prevalence of obesity has become a worldwide health concern. Obesity results from an imbalance between energy intake and energy expenditure. The brain plays a vital role in regulating energy metabolism. Transient receptor potential channel 5 (TRPC5) is a member of mammalian transient receptor potential ion channel family. TRPC5 is abundantly expressed in the central nervous system and linked to obesity development. A benzothiadiazine derivative (BTD) is a novel selective TRPC5 agonist, with > 15-fold selectivity for TRPC5 over other TRPC channels in vitro. Here, we showed that i.p. BTD (10 or 20 mg/kg) significantly reduced food intake in chow-fed and/or HFD-fed mice, while BTD at 1 or 5 mg/kg had no effect on food intake. Importantly, 10 mg/kg BTD had no effect on kaolin intake and conditioned flavor avoidance, indicating that BTD-induced anorexia was not due to nausea or emesis. Further, 10 mg/kg BTD significantly increased c-Fos expression in pro-opiomelanocortin (POMC) neurons in the hypothalamus, which may at least partly account for the anorexigenic effects. Together, these results demonstrated that BTD can reduce food intake and activate POMC neurons, identifying TRPC5 as a potential target for obesity intervention.

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Poster

361. Calcium Channels and Related Signaling

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Topic: B.03. Ion Channels

Support: NIH Grant GM 58055

Title: Effects of isoflurane on axonal endoplasmic reticulum Ca^{2+} dynamics in hippocampal neurons

Authors: *V. OSMAN[1], I. A. SPEIGEL[2], H. HEMMINGS, Jr.[1];
[1]Weill Cornell Med., Weill Cornell Med., New York, NY; [2]Weill Med. Col. of Cornell Univ., Weill Med. Col. of Cornell Univ., New York, NY

Abstract: Volatile anesthetics are essential to modern medicine, but despite their widespread clinical use their cellular and molecular mechanisms of action remain unclear. They can depress synaptic transmission by both presynaptic and postsynaptic mechanisms including reduction of activity-dependent Ca^{2+} influx into presynaptic nerve terminals. However, the principal sites of action upstream of presynaptic Ca^{2+} entry are unknown. Axonal endoplasmic reticulum (ER) controls presynaptic Ca^{2+} concentration through sequestration, and decreased ER Ca^{2+} has been linked to reductions in presynaptic Ca^{2+} influx and in synaptic vesicle (SV) exocytosis. ER Ca^{2+} efflux and influx mechanisms are essential for cellular Ca^{2+} regulation and provide possible

targets for anesthetic action. For example, specific mutations in sarcoplasmic reticulum (SR) efflux channels in skeletal muscle lead to malignant hyperthermia (MH), a potentially fatal pharmacogenetic condition. While we understand MH in skeletal muscle in reasonable detail, possible effects of MH mutations on neuron al function are unknown. In this study, we used primary cultures of rat and mouse hippocampal neurons to test isoflurane-induced changes in ER Ca^{2+} regulation using a genetically encoded fluorescent ER Ca^{2+} sensor in both wild-type and MH-susceptible mutant cells. Our results indicate that isoflurane decreases ER Ca^{2+} in neurons independent of its depression of presynaptic Ca^{2+}. Our results also demonstrate that both ER Ca^{2+} and cytosolic Ca^{2+} are significantly depressed in the presence of isoflurane in MH-susceptible neurons compared to wild-type neurons. We hypothesize that isoflurane depression of presynaptic Ca^{2+} entry and SV exocytosis involves effects on ER Ca^{2+} dynamics.

Disclosures: V. Osman: None. **I.A. Speigel:** None. **H. Hemmings:** None.

Poster

361. Calcium Channels and Related Signaling

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Topic: B.03. Ion Channels

Support: NINDS grant R01NS087033
NINDS grant 1R01NS117484

Title: Stim2 plays a key role in TLR4-mediated neuroinflammation

Authors: *H. BIRLA, F. WANG, H. HU;
Anesthesiol., Rutgers Univ., Newark, NJ

Abstract: STIM2 plays a key role in TLR4-mediated neuroinflammation
Hareram Birla[1], Fengying Wang[1], Huijuan Hu[1]*
[1]Department of Anesthesiology, Rutgers New Jersey Medical School, Newark, NJ 07103
AbstractAstrocytes are the most abundant cells in the central nervous system (CNS). Normal astrocyte activity is essential for maintaining CNS functions. However, excessive activation of these cells is a hallmark of many acute and chronic neuropathologies. In the spinal cord, astrocytes are activated under chronic pain conditions and reactive astrocytes release excessive proinflammatory cytokines, resulting in neuroinflammation and central sensitization. The Toll-like receptor 4 (TLR4) and P2X7 receptor (P2X7R) are key modulators of neuroinflammation. We have shown that activation of store-operated calcium channels (SOCs) induce cytokine production in spinal astrocytes. STIM1 and STIM2 are key components of the SOC family and mediate SOC entry (SOCE) in spinal astrocytes. However, whether STIMs contribute to P2X7R- and TLR4-mediated cytokine production is unknown, and the molecular mechanism for STIMs involvement in cytokine production remains elusive. In the present study, we show that activation of P2X7R by ATP or BzATP induced robust IL-6 production in spinal astrocytes.

Knockdown of STIM1 or STIM2 did not alter ATP- or BzATP-induced IL-6 production, suggesting STIMs are involved in P2X7R function. Interestingly, deficiency of STIM2 largely reduced TLR4-mediated IL-6, IL-1β and TNF-α production while knockdown of STIM1 had no such effect. We further observed that STIM2 protein expression was significantly upregulated in lipopolysaccharide (LPS, an agonist of TLR4)-treated astrocytes, which was blocked by NF-kB inhibition. We also found that basal calcium level and SOCE were increased in LPS-treated wild type astrocytes, but not in STIM2 knockout astrocytes. Our study reveals a novel role of STIM2 in neuroinflammation. Our findings establish a link between LTR4 and STIM2 in astrocytes and provide the basis for future assessment of SOCs in pain and other CNS disorders associated with neuroinflammation.
Keywords: TLR4, store-operated calcium channels, STIM2, astrocytes, cytokine, spinal cord

Disclosures: **H. Birla:** None. **F. Wang:** None. **H. Hu:** None.

Poster

361. Calcium Channels and Related Signaling

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Program #/Poster #: 361.22

Topic: B.03. Ion Channels

Title: Neurodegeneration-associated Fig4/PIKfyve/Vac14 complex controls PI4P subcellular distribution and calcium signaling

Authors: C. KUTCHUKIAN, V. A. LAM, M. CASAS PRAT, A. J. YAROVAYA, R. E. DICKSON, *E. J. DICKSON;
Physiol. and Membrane Biology, Sch. of Med., Univ. of California, Davis, Davis, CA

Abstract: At endosomal membranes, the phosphoinositide (PI) 5-phosphatase Fig4 forms a ternary complex together with the PI 5-kinase PIKfyve and the scaffold protein Vac14. In humans, loss-of-function mutations in the genes encoding Fig4 and Vac14 are associated with severe neurological disorders including the amyotrophic lateral sclerosis (ALS), Charcot Marie Tooth disease type 4J, and Yunis-Varón syndrome, highlighting a pivotal role for this complex in maintaining neuronal integrity. At endosomal structures, cross-talk between Fig4 and PIKfyve complexes ensures tight control of the synthesis and turnover of $PI(3,5)P_2$, the essential low-abundance PIP required for endocytosis and cargo sorting at late endosomes. Despite their divergent roles multiple studies have reported that loss-of-function of Fig4, PIKfyve, or Vac14 result in similar decreases in $PI(3,5)P_2$, suggesting a common mechanism(s) leading to neurodegeneration. An important unanswered question is whether Fig4/PIKfyve/Vac14 complex disruption impairs the cellular distribution of other PIP species. In the present study, we report that PI4P levels are significantly decreased at the trans-golgi network (TGN) in *FIG4*, *PIKFYVE* or *VAC14*-deficient HAP1 cells and in neurons treated with pharmacological inhibitors of PI 3-kinase class III and PIKfyve. We demonstrate that these changes are likely caused by reduced TGN levels of PI4P-generating enzymes PI4KIIα and PI4KIIIβ, as well as an enhanced

oxysterol-binding protein (OSBP)-mediated PI4P transfer from the TGN to the endoplasmic reticulum at membrane contact sites. Functionally, decreases in TGN PI4P result in alterations in trafficking patterns from the TGN that influence plasma membrane voltage-gated calcium channels. We determined, using super-resolution microscopy and electrophysiology techniques, that voltage-gated calcium channel distribution and activity is altered in neurons treated with PI 3-kinase class III and PIKfyve inhibitors. Upstream of changes in voltage-gated calcium channel dynamics is greater nuclear translocation of the transcription factor EB. Overall, our results suggest that the Fig4/PIKfyve/Vac14 complex tunes the molecular contents of ER-Golgi membrane contact sites to influence anterograde membrane trafficking and voltage-gated calcium channel distribution and activity. We propose that these deviant changes in lipid and calcium signaling pathways contribute to neurodegeneration in neurons with disrupted Fig4/PIKfyve/Vac14 complex function.

Disclosures: C. Kutchukian: None. **V.A. Lam:** None. **M. Casas Prat:** None. **A.J. Yarovaya:** None. **R.E. Dickson:** None. **E.J. Dickson:** None.

Poster

361. Calcium Channels and Related Signaling

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 361.23

Topic: B.03. Ion Channels

Support: NIH Grant DA025574

Title: The G protein-coupled receptor kinases, GRK2 and GRK5, modulate the nociception/orphanin FQ opioid (NOP) receptor desensitization in rat sympathetic neurons.

Authors: *M. SOLIMAN, M. FARRAG, S. MAHMOUD, L. MILLER, V. RUIZ-VELASCO; Anesthesiol., Penn State Col. of Med., Hershey, PA

Abstract: Stimulation of nociceptin/orphanin FQ peptide (NOP) opioid receptors by the endogenous ligand, nociceptin (Noc), leads to voltage-gated Ca^{2+} channel inhibition, G protein inwardly rectifying K^+ (GIRK) channel activation and negative coupling to adenylyl cyclase. G protein-coupled receptor kinase (GRK)-mediated phosphorylation of agonist-bound GPCR is a well-established mechanism of receptor desensitization. In the continued presence of the agonists, Gβγ dimers recruit GRK to the plasma membrane where GPCR are then phosphorylated by GRK. In turn, GRK recruit β-arrestins and internalization follows, which leads to a decrease in the response by the cell in the continued presence of agonist. The purpose of the present study was to identify the GRK that mediates desensitization of the Noc-mediated Ca^{2+} current inhibition in rat stellate ganglion (SG) neurons. We observed that GRK2 and GRK5 are natively expressed in SG neurons. Silencing either GRK2 or GRK5 or both employing siRNA did not overtly alter their Noc pharmacological profile. We next tested NOP receptor desensitization employing 2 recording protocols: 'high-dose', where the maximal block of Ca^{2+}

currents was measured at the end of a 10 min exposure to a high Noc concentration (3 μM); and 'low-dose', where the peak Ca^{2+} current block was measured during intermittent applications of 3 μM Noc, each preceded by the continuous exposure of an EC_{50} Noc concentration (0.03 μM). The results with the 'high dose' protocol indicated that the NOP receptors desensitized in a similar manner whether GRK2 or GRK5 were expressed or not. On the other hand, when the 'low-dose' protocol was employed, GRK2-silenced SG neurons desensitized over time but the magnitude of desensitization was not as high as that observed in scrambled siRNA control. Further, the silencing of GRK5 did not significantly alter NOP receptor desensitization when compared to scrambled siRNA control. Finally, silencing both GRK subtypes resulted in loss of NOP receptor desensitization with each intermittent Noc (3 μM) application. These results suggest that both GRK2 and GRK5 modulate the recovery of NOP receptor desensitization produced by chronic Noc exposure.

Disclosures: M. Soliman: None. **M. Farrag:** None. **S. Mahmoud:** None. **L. Miller:** None. **V. Ruiz-Velasco:** None.

Poster

361. Calcium Channels and Related Signaling

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 361.24

Topic: B.03. Ion Channels

Support: GAUK No. 410122
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Title: The role of the GDG motif of the GluN2 subunit for mechanism of NMDAR activation

Authors: *E. TOMOVIC, V. KUCHTIAK, A. BALIK, J. CERNY;
Inst. of Physiol. CAS, Inst. of Physiol. CAS, Prague, Czech Republic

Abstract: N-methyl-D-aspartate receptors (NMDARs) are a family of ionotropic glutamate receptors that mediate excitatory neurotransmission in the CNS. NMDA dependent synaptic plasticity control process of memory formation, learning and behavior. Dysfunction of NMDARs leads to the emergence of various neuropathophysiological conditions. Native NMDARs are assembled as hetero tetramers composed of two obligatory GluN1 and two GluN2A-D or GluN3A-B subunits at various combinations. The opening of the ion channel is a central feature of NMDAR function and is controlled by conformational changes of the receptor after binding of both co-agonists (glutamate/glycine) to the agonist binding domains (LBD). Activation of LBD induces conformational changes that lead to the temporary opening of the channel. Despite many NMDAR crystal or cryo-EM structures at various functional states beening solved, the process of channel opening at the molecular level is still not fully characterized. In our previous work, using molecular dynamics simulations, we proposed functional importance for several residues of NMDAR, Glu522 and Arg695 in GluN1, and Asp786 in GluN2B surrounded by two conserved

glycines (GDG motif), and we revealed intersubunit interaction between these residues in the open state of the receptor only. Therefore, we aimed to perform further biochemical and electrophysiological analysis of GDG motif in GluN2 subunits to confirm in silico data. We prepared the set of NMDAR constructs where identified amino acids were deleted or mutated to glycine in order to completely eliminate or disrupt presumed intramolecular bonds. Firstly, we assessed the functional properties of mutated subunits using electrophysiology techniques. Our results have revealed that NMDAR composed of Asp786Gly GluN2B has slower time-course of MK-801 block with the average Tau_w 2990ms compared to average Tau_w 897ms of wild type NMDAR. The deletion of GDG motif in GluN2A and GluN2B subunits leads to lower affinity to glutamate and also altered GDG motif in GluN2A affects the inhibitory effect of GluN2A specific LBD negative modulator TCN-201 with average inhibition 35% compared to inhibition 78% in wild type. In addition, we performed the immunocytochemical staining of the surface-expression of receptor in the HEK293 cells where presence of altered GDG motif decreased significantly the surface expression of NMDAR. These results showed the effect of GluN2 GDG motif for NMDAR assembly and localization. Our analysis supports in silico data for proposed functionally important intrasubunit interaction of Asp786 GluN2B in the open state of the NMDA receptor.

Disclosures: **E. Tomovic:** None. **V. Kuchtiak:** None. **A. Balik:** None. **J. Cerny:** None.

Poster

361. Calcium Channels and Related Signaling

Location: SDCC Halls B-H

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Gift in memory of Elsie Louise Torrance Higgs (Muinntir Bana-Ghaisgeach)

Title: Inhibition of glutamate-carboxypeptidase-II in dorsolateral prefrontal cortex: Potential therapeutic target for neuroinflammatory cognitive disorders

Authors: *S. YANG[1], D. DATTA[2], E. K.-Y. WOO[3], A. DUQUE[4], Y. M. MOROZOV[4], J. I. ARELLANO[1], B. S. SLUSHER[5], M. WANG[6], A. F. ARNSTEN[7];
[1]Yale Univ., Yale Univ., New Haven, CT; [2]Yale Univ., Yale Univ., Branford, CT; [3]Yale Univ. Interdepartmental Neurosci. Program, New Haven, CT; [4]Yale Univ. Sch. Med., Yale Univ. Sch. Med., New Haven, CT; [5]Johns Hopkins Drug Discovery, Johns Hopkins Drug Discovery, Baltimore, MD; [6]Yale Univ. Sch. of Med., Yale Univ. Sch. of Med., New Haven, CT; [7]Yale Med. Sch., Yale Med. Sch., New Haven, CT

Abstract: Glutamate carboxypeptidase-II (GCPII) expression in brain is increased by inflammation, e.g. by COVID19 infection, where it reduces NAAG stimulation of metabotropic glutamate receptor type 3 (mGluR3). GCPII-mGluR3 signaling is increasingly linked to higher cognition, as genetic alterations that weaken mGluR3 or increase GCPII signaling are associated with impaired cognition in humans. Recent evidence from macaque dorsolateral prefrontal cortex (dlPFC) shows that mGluR3 are expressed on dendritic spines, where they regulate cAMP-PKA opening of potassium (K^+) channels to enhance neuronal firing during working memory. However, little is known about GCPII expression and function in the primate dlPFC, despite its relevance to inflammatory disorders. The present study used multiple label immunofluorescence and immunoelectron microscopy to localize GCPII in aging macaque dlPFC, and examined the effects of GCPII inhibition on dlPFC neuronal physiology and working memory function. GCPII was observed in astrocytes as expected, but also on neurons, including extensive expression in dendritic spines. Recordings in dlPFC from aged monkeys performing a working memory task found that iontophoresis of the GCPII inhibitors 2-MPPA or 2PMPA markedly increased working memory-related neuronal firing and spatial tuning, enhancing neural representations. These beneficial effects were reversed by an mGluR2/3 antagonist, or by a cAMP-PKA activator, consistent with mGluR3 inhibition of cAMP-PKA-K^+ channel signaling. Systemic administration of the brain penetrant inhibitor, 2-MPPA, significantly improved working memory performance without apparent side effects, with largest effects in the oldest monkeys. Taken together, these data endorse GCPII inhibition as a potential strategy for treating cognitive disorders associated with aging and/or neuroinflammation.

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Poster

362. Signaling Mechanisms in Long-Term Plasticity I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 362.01

Topic: B.05. Synaptic Plasticity

Support: NSF IOS 1557474
Whitehall Foundation

Title: Dopaminergic modulation of excitability and electrical synapses in the thalamic reticular nucleus

Authors: *M. J. VAUGHN[1], J. S. HAAS[2];
[2]Dept. of Biol. Sci., [1]Lehigh Univ., Bethlehem, PA

Abstract: The thalamic reticular nucleus (TRN) is a thin shell of electrically coupled inhibitory GABAergic neurons that regulate afferent sensory relay of the thalamus. The TRN receives

dopaminergic innervation from the midbrain, and it expresses high concentrations of D_1 and D_4 receptors. Dopamine release in the TRN could add a reward valence to thalamic processing of salient sensory surround signals. Although modulation of presynaptic inputs to TRN has been described, the direct effect of dopamine on TRN neurons and its electrical synapses is largely unknown and is key to understanding modulatory control of thalamocortical processing. Dopamine has been frequently shown to modulate electrical synapse strength in other systems, including retinal amacrine cells and the goldfish mixed synapse onto Mauthner cells. To characterize how dopamine affects neuronal excitability and electrical synapses in the TRN, we patched pairs of TRN neurons and injected them with 500-ms current pulses to measure resting membrane potential, input resistance, threshold, spiking frequency, and coupling conductance. Measurements were taken before and after bath application of dopamine, D_1 agonist, or D_4 agonist. Our results show that bath application of dopamine did not consistently modulate excitability or electrical synapse strength. However, agonization with specific dopamine receptor agonists revealed modulatory effects based on receptor subtype. D_1 agonization robustly increased the excitability of TRN neurons without affecting the conductance of electrical synapses. Activation of D_1 receptors enhanced excitability by increasing input resistance, depolarization of resting membrane potential, and increasing tonic spike rate. D_4 agonization also increased excitability through depolarization of resting membrane potential and input resistance increases, but it lowered tonic spike rate by decreasing spiking gain. Further, D_4 but not D_1 receptor activation depressed electrical synapses. Inhibition of PKA also blocked the D_4 receptor effects, indicating that the PKA pathway is necessary for D_4 receptors to depress electrical synapses and alter spiking properties. Together, our results suggest that coactivation of D_1 and D_4 receptors may interfere with each other due to opposing signaling cascades; in instances where D1 and D4 receptors are separately engaged, there is substantial potential for dopaminergic inputs to alter excitability and connectivity in the TRN. We further hypothesize that dopaminergic inputs modulate inhibitory signals sent to thalamus by TRN neurons, providing an avenue for emotional salience to affect cortical attention to the sensory surround.

Disclosures: **M.J. Vaughn:** None. **J.S. Haas:** None.

Poster

362. Signaling Mechanisms in Long-Term Plasticity I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 362.02

Topic: B.05. Synaptic Plasticity

Support: NIA / 5R01AG067713-02
NINDS / 5R01NS118786-02

Title: Distinct synaptic pools of DAPK1 differentially regulate activity-dependent synaptic accumulation of CaMKII

Authors: *J. TULLIS[1], K. BAYER[2];
[1]CU Anschutz Pharmacol., Univ. of Colorado Denver, Aurora, CO; [2]Pharmacol., Univ. of Colorado Anschutz Med. Campus, Denver, CO

Abstract: The complex synaptic organization of molecules is critical for regulating the synaptic plasticity processes that underlie learning and memory. Anchoring of the Ca^{2+}/calmodulin (CaM)-dependent protein kinase II (CaMKII) to the NMDA-receptor subunit GluN2B is a tightly regulated process that is required for long-term potentiation (LTP) of synaptic strength. In contrast, during long-term depression (LTD), this interaction is specifically suppressed via the death-associated protein kinase 1 (DAPK1) competitively binding to GluN2B. This competitive GluN2B binding is thought to mediate the synaptic targeting of DAPK1, however, DAPK1 also contains a binding site for F-actin, the cytoskeletal scaffold that is basally enriched in the dendritic spines that form the post-synaptic compartments of excitatory synapses. Notably, this dendritic spine F-actin is disassembled after both LTP and LTD stimuli, although only transiently after LTP stimuli. We decided to investigate the contribution of F-actin on the postsynaptic targeting of DAPK1 and CaMKII to dendritic spine synapses in hippocampal neurons, both basally and after chemical stimuli to induce LTP or LTD (cLTP or cLTD). Utilizing fluorescence microscopy to measure and track the subcellular localization of both DAPK1 and CaMKII in dissociated hippocampal cultures, we find DAPK1 is highly enriched at synapses via F-actin under basal conditions. By itself, the F-actin binding was not sufficient to suppress synaptic CaMKII accumulation. However, the basal F-actin binding was required for an LTD-induced transition of DAPK1 to a different, likely GluN2B-bound state that was no longer dependent on F-actin for synaptic targeting and that was essential for suppressing synaptic CaMKII accumulation. Thus, our results reveal two distinct sub-synaptic pools of DAPK1 and their distinct functions in regulating the differential synaptic accumulation of CaMKII during LTP versus LTD.

Disclosures: J. Tullis: None. **K. Bayer:** None.

Poster

362. Signaling Mechanisms in Long-Term Plasticity I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 362.03

Topic: B.05. Synaptic Plasticity

Support: CONACyT grant 250870
CONACyT grant FOINS 474
DGAPA-PAPIIT-UNAM grant IN212919

Title: Simultaneous activation of the ventral tegmental area and basolateral amygdaloid nucleus projections into the Insular cortex induces a slow-onset long-term potentiation in the insular cortex

Authors: *L. F. RODRIGUEZ-DURAN[1], D. L. LÓPEZ-IBARRA[1], F. BERMÚDEZ-RATTONI[1], M. L. ESCOBAR[2];
[1]Inst. de Fisiología Celular, UNAM, CDMX, Mexico; [2]UNAM, Fac Psicologia, Mexico City, Mexico

Abstract: Catecholaminergic and glutamatergic systems in the Insular cortex (IC) are related to reward expectation and decision making. Also, both catecholaminergic and glutamatergic projection to the IC participate in the maintenance of rewarding contextual memory. The ventral tegmental area (VTA), a primary catecholaminergic source that is involved in the reward circuit, projects to forebrain structures as the IC. Likewise, basolateral amigdaloid nucleus (Bla) projects glutamatergic fibers to the IC, and this projection has been involved in the acquisition and maintenance of several behavioral tasks. Bla-IC projection also expresses a well-known form of synaptic plasticity namely long-term potentiation (LTP), a widely proposed mechanism of memory storage induced by a wide variety of stimulation protocols. It is important to note that both VTA catecholaminergic and Bla glutamatergic neurons send their projections to the agranular region of anterior IC, stressing the importance of this area for integrating motivational and emotional signals. Recent studies have shown that optogenetic stimulation can induce different forms of synaptic plasticity, including LTP. Optogenetic LTP (oLTP) has been induced by direct monosynaptic stimulation of fibers expressing modified channel rhodopsin. Also, oLTP can be induced via the activation of pre-synaptic terminals coupled with post-synaptic activity. However, whether simultaneous optogenetic activation of catecholaminergic and glutamatergic projection to the neocortex can induce oLTP remains unexplored. In this work, we activate optogenetically the terminal fibers of Bla-IC projection in concomitance with the terminal fibers of VTA-IC projection. We found that simultaneous activation of the mentioned pathways induces a slow-onset oLTP in the Bla-IC projection. Furthermore, we found that oLTP in the IC is dependent on glutamatergic NMDA receptors and dopaminergic D1/D5 receptors, showing that the combination of these neurotransmitters can induce synaptic plasticity in the neocortex. This data provides evidence of catecholamines and glutamate synergic actions to induce LTP and suggests that those mechanisms are involved in reward-related learning and memory.

Disclosures: L.F. Rodriguez-Duran: None. **D.L. López-Ibarra:** None. **F. Bermúdez-Rattoni:** None. **M.L. Escobar:** None.

Poster

362. Signaling Mechanisms in Long-Term Plasticity I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 362.04

Topic: B.05. Synaptic Plasticity

Support: Korea NRF grant 2016R1A2B2016533

Title: Differential expression of IP_3 receptors underlies layer-specific cholinergic modulation of long-term synaptic plasticity in rat visual cortex

Authors: K.-H. CHO[1], K. JOO[1], ***D.-J. RHIE**[2];
[1]Dept. Physiol., [2]Dept. Physiol., Catholic Neurosci. Inst., Coll. Med., Catholic Univ. Korea, Seoul, Korea, Republic of

Abstract: Cholinergic modulation of synaptic plasticity is important for learning, memory and cortical information processing in the cortex. Since cortical inputs from upstream and downstream structures terminate layer-specifically, layer-specific modulation of synaptic plasticity might underlie pathway-specific modulation of information processing. We have demonstrated that cholinergic stimulation induced long-term synaptic plasticity dendritic location-dependently in layer 2/3 pyramidal neurons (L2/3 PyNs) of the primary visual cortex (V1) (Cho et al, 2012). However, underlying cellular/molecular mechanisms are unknown. To investigate mechanisms responsible for dendritic location-dependent cholinergic modulation of long-term synaptic plasticity, we hypothesized that one of calcium release machinery is missing in distal apical dendrites, located in layer 1 (L1), of L2/3 PyNs. Initially we investigated calcium release in basal and distal apical dendrites with intracellular application of the inositol trisphosphate (IP_3) analogue 3F-IP_3 (0.1 mM) in the same previous preparation. Calcium release was evoked by focal stimulation at nearby dendrites (~10 μm). Calcium release and long-term potentiation (LTP) was evoked in basal dendrites (n = 5), but not in distal apical dendrites (n = 3~4), similar to cholinergic stimulation. Immunohistochemical staining showed no immunoreactivity for IP_3 receptor type 1 (IP_3R1) in distal apical dendrites of L2/3 PyNs whereas fine basal dendrites exhibited strong immunoreactivity. Distal apical as well as basal dendrites showed dense immunoreactivity for muscarinic acetylcholine receptors type 1. Electron microscopy revealed well-developed cistern of smooth endoplasmic reticulum and spine apparatus in distal apical dendrites located in L1 and in perisomatic dendrites located in L2/3 of the visual cortex from 3 to 5-week-old rats (n = 4). Quantitative analysis of immunopositive dendritic length for IP_3R1 in L1 and L2/3 showed no difference in apical/basal length ratio (~0.2, n = 3~6) in 100-μm square area between V1 and the agranular insular cortex, the lateral part of the orbitofrontal cortex. These findings indicate that the dendritic location-dependent differential expression of IP_3Rs is responsible for layer-specific cholinergic induction of LTP resulting from dendritic calcium release, between L1 and perisomatic area in L2/3 PyNs of the cortex. Therefore, the layer-specific expression of dendritic IP_3Rs might differentially regulate bottom-up and top-down inputs to cortical PyNs depending on brain states.

Disclosures: K. Cho: None. **K. Joo:** None. **D. Rhie:** None.

Poster

362. Signaling Mechanisms in Long-Term Plasticity I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 362.05

Topic: B.05. Synaptic Plasticity

Support: CIHR#154276

Title: Prime Time: The enhancement of long-term potentiation by corticosterone and BDNF

Authors: *J. THACKER[1], L. BETTIO[2], A. ABBASIAN[1], L. RALPH[1], L. KOEK[1], A. E. SMITH[3], J. GEORGIOU[1], B. R. CHRISTIE[2], G. L. COLLINGRIDGE[1];
[1]Lunenfeld-Tanenbaum Res. Inst., Toronto, ON, Canada; [2]Univ. of Victoria, Victoria, BC, Canada; [3]Allied Hlth. and Human Performance, Univ. of South Australia, Adelaide, Australia

Abstract: The facilitation and enhanced persistence of long-term potentiation (LTP) is an objective for many therapeutic approaches of brain disorders. LTP is a synaptic phenomenon critical for learning and memory and can be characterized by its many mechanistically distinct forms. Notably, whether LTP is independent of *de novo* protein synthesis (LTP1) or requires it (LTP2) can determine the longevity of synaptic plasticity. Recently, our lab has shown how the activation of protein kinase A (PKA) via the regulation of calcium-permeable AMPA receptors (CP-AMPARs) is both necessary and sufficient for LTP2, but not LTP1 (Park et al., 2021). Interestingly, we have previously shown that both PKA and CP-AMPARs can be engaged by exercise (Thacker et al., 2019) or stress, however the mechanism has not been fully realized. One hypothesis is that exercise recruits both glucocorticoid and neurotrophic signaling pathways simultaneously that converge and mediate PKA activity to increase the propensity for LTP2. Herein we tested this hypothesis by co-applying the glucocorticoid corticosterone (CORT, 200nM) with exogenous brain-derived neurotrophic factor (BDNF, 20ng/mL) for 30 minutes prior to the induction of compressed theta burst stimulation (cTBS; 3 trains of 5 pulses at 100Hz with inter-train interval of 10s) to see if we could invoke LTP2 when otherwise the stimulation patterning would have resulted in LTP1. Field electrophysiology recordings were conducted in acute hippocampal slices from young Sprague-Dawley rats (P35-45). Our data reveal that when compared to controls (44 ± 3%, n = 13), BDNF alone (83 ± 6%, n = 15, $p<0.05$) significantly enhanced LTP whereas CORT alone (45 ± 6%, n = 18, $p>0.05$) did not. Moreover, we observed that CORT+BDNF significantly enhanced LTP (116 ± 12%, n = 15) compared to control and this longer-lasting increase was greater than that of BDNF alone ($p<0.05$). We are currently determining whether the enhanced synaptic plasticity is related to a shift from LTP1 to LTP2 (i.e., dependent on protein synthesis) and whether PKA is necessary for the observed changes in both Sprague-Dawley rats and mice. References: Park, P., Georgiou, J., Sanderson, T. M., Ko, K. H., Kang, H., Kim, J. I., ... & Collingridge, G. L. (2021). PKA drives an increase in AMPA receptor unitary conductance during LTP in the hippocampus. *Nature communications*, *12*(1), 1-15. Thacker, J. S., Xu, Y., Tang, C., Tupling, A. R., Staines, W. R., & Mielke, J. G. (2019). A single session of aerobic exercise mediates plasticity-related phosphorylation in both the rat motor cortex and hippocampus. *Neuroscience*, *412*, 160-174.

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Poster

362. Signaling Mechanisms in Long-Term Plasticity I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 362.06

Topic: B.05. Synaptic Plasticity

Title: Developing Photo-Inducible, Isozyme-Specific PKC Inhibitor System to Study Learning and Memory

Authors: *G. OZ[1], L. A. COLGAN[1], Y. HAYANO[1], H. NISHIZONO[2], R. YASUDA[3]; [1]Max Planck Florida Inst. for Neurosci., Jupiter, FL; [2]Kanazawa Med. Univ. Med. Res. Inst., Kanazawa, Japan; [3]Max Planck Florida Inst., Jupiter, FL

Abstract: Accumulating evidence suggests that the Protein Kinase C (PKC) family of isozymes are critically involved in memory acquisition and maintenance, in addition to their involvement in general housekeeping functions of cells. Additionally, PKCs are implicated in many diseases including six of the top ten leading causes of death in America: cardiovascular and respiratory diseases, cancer, stroke, diabetes, and Alzheimer's disease (AD). However, the high structural homology between the 12 PKC isozymes has inhibited the delineation of each isozyme's role in these diverse functions. Once a better understanding of the specific isozymes is achieved, the PKC family will be a notable therapeutic target for these diseases. **This project aims to overcome this limitation by developing a light-inducible, reversible and isozyme selective inhibitory system for PKC that will enable us to pinpoint their roles in learning and memory**. In order to achieve this goal, we have designed, optimized and validated an optogenetic PKC inhibitor for PKCα, (optoPKCIα) as well as PKCδ (optoPKCδ). We further tested the involvement of PKCα during hippocampal synaptic plasticity by using our novel optoPKCIα. This generalizable approach can be applied to specifically control the function of other PKCs in a millisecond-temporal precision. Hence, our selective inhibitory system will overcome the limitations to dissect the divergent roles of PKC isozymes in cells and in the pathophysiology of diseases associated with PKC dysfunction. Together, these isozyme-specific inhibitors form a powerful toolkit that can be used to further dissect to causal roles of the PKC family in numerous disorders and identify key candidates for targeted therapies.

Disclosures: G. Oz: None. **L.A. Colgan:** None. **Y. Hayano:** None. **H. Nishizono:** None. **R. Yasuda:** None.

Poster

362. Signaling Mechanisms in Long-Term Plasticity I

Location: SDCC Halls B-H

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Program #/Poster #: 362.07

Topic: B.05. Synaptic Plasticity

Support: NIH R01-MH117964
NIH R21-MH117788
University of Iowa Hawkeye Intellectual and Developmental Disability Research Center P50 HD 103556

Title: Chemogenetic elevation of cAMP in the hippocampus produces resilience against the effect of sleep deprivation on long-term synaptic plasticity.

Authors: E. N. WALSH[1,2,3], M. SHETTY[1,2], K. DIBA[4,5], T. ABEL[1,2,3];
[1]Dept. of Neurosci. and Pharmacol., [2]Iowa Neurosci. Institute, Carver Col. of Med., [3]Interdisciplinary Grad. Program in Neurosci., Univ. of Iowa, Iowa City, IA; [4]Dept. of Anesthesiol., [5]Neurosci. Grad. Program, Univ. of Michigan, Ann Arbor, MI

Abstract: Sleep facilitates memory storage and even brief periods of sleep loss lead to impairments in memory, particularly for memories that are hippocampus dependent. In previous studies, we have shown that the deficit in memory seen after sleep loss is accompanied by deficits in hippocampal synaptic plasticity. Our previous work has also found that sleep deprivation is associated with reduced levels of cyclic adenosine monophosphate (cAMP) in the hippocampus, and that the reduction of cAMP mediates the diminished memory performance observed in sleep deprived animals. Based on these findings, we hypothesized that cAMP acts as a mediator for not only the cognitive deficits caused by sleep deprivation, but also the observed deficits in synaptic plasticity. By expressing the *Drosophila Melanogaster* G-protein coupled octopamine receptor (DmOctβ1R) in hippocampal neurons and selectively activating the receptors by systemically injecting the ligand octopamine, we were able to increase cAMP levels in those neurons during a five-consecutive hour sleep deprivation period. Immediately following sleep deprivation, hippocampal slices were prepared for electrophysiological recordings. Our results show that chemogenetic enhancement of cAMP during the period of sleep deprivation prevents deficits in a persistent form of long-term potentiation (LTP) that is induced at the Schaffer collateral synapses in the hippocampal CA1 region. We also found that elevating cAMP levels only in the early or later half of sleep deprivation successfully prevented LTP deficits. These findings reveal that cAMP-dependent signaling pathways are key mediators in the impact of sleep deprivation at the synaptic level. Targeting these pathways will help us better understand the consequences of sleep deprivation and identify areas of intervention. Future experiments to help address this goal will incorporate manipulations of sleep/wake circuitry to understand the difference between internally driven wakefulness and external drivers of sleep loss, and will examine intra-hippocampal circuits to understand how the plasticity and cAMP deficits emerge.

Disclosures: **E.N. Walsh:** None. **M. Shetty:** None. **K. Diba:** None. **T. Abel:** None.

Poster

362. Signaling Mechanisms in Long-Term Plasticity I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 362.08

Topic: B.05. Synaptic Plasticity

Support: R01 NS036715
MOST-109-2311-B-001-006-MY2

Title: Ica69 regulates hippocampal ltp and learning and memory

Authors: *S.-L. CHIU[1,2], C.-M. CHEN[2], R. L. HUGANIR[2];
[1]Inst. of Cell. and Organismic Biol., Academia Sinica, Taipei, Taiwan; [2]Neurosci., Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: LTP, or long-term potentiation, is one major cellular mechanism for learning and memory. Activity-dependent increases in synaptic AMPA receptors are important for enhanced synaptic efficacy during LTP. Here we reported a novel function of a secretory trafficking protein, ICA69, in AMPA receptor trafficking, synaptic plasticity and animal cognition. ICA69 is first identified as a diabetics-associated protein well characterized for its function in the biogenesis of secretory vesicles and trafficking of insulin from ER, Golgi to post-Golgi in pancreatic beta cells. In the brain, ICA69 can be found in the AMPA receptor protein complex through its interaction with PICK1, which binds directly to GluA2 or 3 AMPA receptor subunits. We discovered that ICA69 determines the subcellular localization and stability of PICK1, which in turn regulates AMPA receptor function. Biochemical analysis of postsynaptic density proteins from hippocampi of Ica1 knockout (mice lacking ICA69 protein) and their wild-type littermates showed comparable AMPA receptor protein levels. Moreover, electrophysiological recording and morphological analysis of CA1 pyramidal neurons from Ica1 knockout also showed normal AMPAR-mediated currents and dendrite architecture, indicating that ICA69 does not regulate synaptic AMPA receptor function and neuron morphology at the basal state. However, Ica1 knockout mice showed a selective impairment in NMDA receptor-dependent LTP but not LTD at Schaffer collateral to CA1 synapses, which is correlated with behavior deficits in tests of spatial and associative learning and memory. Together, we identified a crucial and selective role of ICA69 in activity-dependent AMPA receptor delivery, linking ICA69-mediated synaptic strengthening to animal cognition.

Disclosures: **S. Chiu:** None. **C. Chen:** None. **R.L. Huganir:** None.

Poster

362. Signaling Mechanisms in Long-Term Plasticity I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 362.09

Topic: B.05. Synaptic Plasticity

Support: Natural Sciences and Engineering Council of Canada (NSERC) Grant RGPIN-2016-05538
Canadian Institutes of Health Research (CIHR) Grant FRN: 162179

Title: Non-ionotropic NMDA receptor signaling reverses long-term potentiation in the spinal cord dorsal horn resulting in the attenuation of pain hypersensitivity

Authors: *D. RODRIGUEZ[1], A. D'SOUZA[1], H. ZHANG[1], M. ZAIN[1], S. FUNG[1], L. BENNETT[1], R. BONIN[1,2];

[1]Pharmaceut. Sci., Univ. of Toronto, Toronto, ON, Canada; [2]Univ. of Toronto Ctr. for the Study of Pain, Toronto, ON, Canada

Abstract: Pathological pain is associated with changes to the synaptic strength of nociceptive pathways located in the spinal cord dorsal horn. During long-term potentiation (LTP), strong activation of presynaptic sensory afferents generates an overall increase in the excitability of postsynaptic dorsal horn neurons, which results in the amplification of nociceptive signals relayed to the brain and a corresponding increase in sensitivity to painful stimuli (i.e. hyperalgesia). Synaptic plasticity is highly dependent on NMDA receptor activity; recent investigations have linked this receptor with non-ionotropic (NI-NMDA) signaling mechanisms that favour dendritic spine shrinkage and long-term depression (LTD). In this study, we test the hypothesis that NI-NMDA activity mediates synaptic depotentiation in sensitized spinal pain pathways and promotes the reversal of hyperalgesia. For ex vivo experiments, C fiber-evoked postsynaptic field potentials (fPSPs) were recorded in acute spinal cord explants from adult mice. Dorsal roots containing sensory afferents were stimulated using a bipolar electrode, and a recording electrode was inserted into the superficial dorsal horn to measure postsynaptic responses. For behavioural experiments, hyperalgesia was induced via intraplantar injections of capsaicin or complete freund's adjuvant (CFA); Von Frey assays were then used to measure mechanical sensitivity in the injected paw. We found that NI-NMDA signaling can be engaged using glycine-site antagonists, which significantly decreased the overall magnitude of dorsal horn LTP. Importantly, this reversal of hyperexcitability translated into significant decreases in paw mechanical sensitivity following capsaicin- or CFA-induced hyperalgesia. In agreement with structural LTP studies conducted in the brain, NI-NMDA signaling in the spinal cord is also dependent on protein phosphatase-1 and p38 MAP kinase activity. Our results identify NI-NMDA signaling as a novel target for the treatment of pathological pain. Furthermore, the ability to reverse LTP in an activity-dependent manner may have important implications beyond nociceptive processing, for example in the modulation of hippocampal LTP which is thought to be crucial for memory formation and learning.

Disclosures: **D. Rodriguez:** None. **A. D'Souza:** None. **H. Zhang:** None. **M. Zain:** None. **S. Fung:** None. **L. Bennett:** None. **R. Bonin:** None.

Poster

362. Signaling Mechanisms in Long-Term Plasticity I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 362.10

Topic: B.05. Synaptic Plasticity

Support: NIMH Grant 126613-01
NIH Grant 120300-02

Title: Pattern detection in the TGFβ cascade controls the induction of long-term synaptic plasticity

Authors: *P. MIRANDA, N. KUKUSHKIN, A. A. MIRISIS, M. SCHREIBMAN, T. J. CAREW;
Neural Sci., New York Univ., New York, NY

Abstract: While typically viewed as hormone-like molecules operating with little temporal resolution, growth factors can also play key roles in orchestrating the timing of molecular events required to form a long-term memory (LTM). In a two-trial training paradigm that induces LTM for sensitization in the marine mollusk *Aplysia californica*, growth factor TGFβ is required during Trial 2 but not Trial 1 (Kopec et al, 2015). This finding suggests a temporally specific inter-trial regulation of TGFβ's signaling cascade, either upstream or downstream of TGFβ release. Since TGFβ is necessary and sufficient to promote LTM, we hypothesized that it contributes to the inter-trial interactions that determine the temporally restricted requirement of repeated trial learning. However, the precise mechanisms that determine the differential requirement for TGFβ during two trial training remain unknown. To examine how TGFβ signaling cascade is involved in synaptic plasticity, we utilized electrophysiological recordings with sensory to motor neuron co-cultures. Experiments using an analog two-trial training with serotonin (5HT) pulses demonstrated that proteolytic activation of the biologically inert TGFβ pro-ligand by the BMP-1/Tolloid metalloprotease must occur specifically during Trial 2 to establish long term synaptic facilitation (LTF). BMP-1 application paired with a single 5HT pulse induces LTF, whereas neither a single 5HT pulse, nor BMP-1 alone, induces LTF. Inhibition of endogenous metalloprotease activity with the zinc chelator Ca●EDTA blocks the induction of LTF. This block is subsequently rescued by an application of excess zinc ions, which are required for proteolytic actions of BMP-1. Consistent with these findings, we found that by using a culture-based bioassay, TGFβ signaling is differentially engaged by two-trial training. Specifically, the rate of TGFβ signaling is unchanged after Trial 1, but is significantly increased after Trial 2. Collectively, these data support a model in which the interaction between the two training trials involves temporally distinct molecular steps that engage effective TGFβ signaling: first, the release of the TGFβ pro-ligand from SNs, and second, its activation by proteolytic cleavage. This model suggests a unique role for GF signaling in which the TGFβ cascade provides a molecular platform for pattern detection essential for the induction of LTM.

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Poster

362. Signaling Mechanisms in Long-Term Plasticity I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 362.11

Topic: B.05. Synaptic Plasticity

Support: R01 A1 Plasticity 59313840
Virus-Like Intercellular Communication in the Nervous System (R01 Transformative) 59316254

Title: Irsp53 facilitates Arc capsid assembly and release during long-term potentiation

Authors: *A. RAVENS[1], K. R. SULLIVAN[1], J. D. SHEPHERD[2];
[1]UNIVERSITY OF UTAH, Salt Lake City, UT; [2]Neurobio., Univ. of Utah, Salt Lake City, UT

Abstract: The immediate early gene *Arc* is critical for long-term synaptic plasticity and memory formation. Arc has structural homology to retroviral Gag capsid proteins. We recently showed that Arc proteins oligomerize to form virus-like capsids that package RNA and are released from cells in extracellular vesicles (EVs). However, the mechanisms of Arc capsid assembly and release are unclear. Intriguingly, the I-BAR-containing protein IRSp53 was recently shown to regulate HIV-1 Gag capsid assembly and release. IRSp53 is an abundant synaptic protein that is implicated in dendritic spine formation. Given the structural similarities between Arc and HIV-1, we hypothesized that IRSp53 may facilitate Arc capsid assembly and release. We find that IRSp53 binds directly with Arc and this interaction facilitates recombinant Arc capsid assembly. Overexpression of IRSp53 specifically enhances Arc release into EVs, and this effect is dependent on IRSp53's SH3 domain, the site that recruits and binds many actin cytoskeleton effectors, including WAVE2, Dynamin1, and N-WASP. Arc is required for various forms of long-term depression (LTD), but not long-term potentiation (LTP). However, Arc expression is induced by LTP, and we hypothesized that LTP induction enhances Arc release. We find that dendritic IRSp53 is upregulated during LTP, but not LTD. Using imaging of live neurons, we find that Arc and IRSp53 colocalize and traffic anterograde from soma to dendrites, sometimes followed by loss of signal that may be release events. These results help elucidate the molecular mechanisms that facilitate Arc capsid assembly and release, identifying a potentially new secretory pathway in neurons.

Disclosures: A. Ravens: None. **K.R. Sullivan:** None. **J.D. Shepherd:** None.

Poster

362. Signaling Mechanisms in Long-Term Plasticity I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 362.12

Topic: B.05. Synaptic Plasticity

Support: NIH R01 MH117964

Title: Selective impact of PDE4A5 on different forms of long-lasting long-term potentiation

Authors: *S. TADINADA[1], U. MUKHERJEE[2], E. WALSH[3], T. ABEL[4];
[1]Neurosci. and Pharmacol., Univ. of Iowa, Iowa city, IA; [3]Neurosci. Program, [4]Neurosci. and Pharmacol., [2]Univ. of Iowa, Iowa City, IA

Abstract: Acute sleep deprivation for 5h impairs memory consolidation and long-lasting forms of long-term potentiation (L-LTP), which can be rescued by chemogenetic activation of cAMP/PKA signaling or by blockade of PDE4 phosphodiesterases using rolipram. The long

isoform of the PDE4A subfamily, PDE4A5, is increased following acute sleep deprivation and its overexpression is sufficient to phenocopy the effects of acute sleep deprivation (SD) on memory consolidation. This long isoform consists of a unique N-terminus, upstream conserved regions 1 (UCR1) and 2 (UCR2) and a highly conserved catalytic domain and C-terminus. Here, we investigate whether PDE4A5 overexpression phenocopies the L-LTP deficits seen with acute SD. We found that hippocampal overexpression of PDE4A5 resulted in deficits in L-LTP induced by theta burst stimulation (15 bursts, 4 pulses/burst at 100Hz), but not L-LTP induced by repeated tetanic stimulation (spaced 4-train or massed 4-train patterns of stimulation). In addition, this deficit in L-LTP induced by TBS is selective to overexpression of full length PDE4A5, but not the N-terminal truncated form, PDE4A5Δ4, or a PDE4A isoform that localizes in the membrane, PDE4A1. Further investigation of the localization of these PDE4A variants in cell culture revealed that PDE4A5 localizes uniformly throughout the neurons, but the truncated form, PDE4A5Δ4 localizes primarily to the cell body, suggesting a key role for the unique N-terminus in subcellular localization. This pattern specific effect of PDE4A5 on L-LTP might therefore be in part due to its localization such that, its presence in neuronal processes is detrimental to L-LTP induced by TBS, but not to repeated tetanic stimulation. These results suggest that cAMP/PKA signaling in neuronal processes might be critical to some, but not all forms of L-LTP induced by different patterns of stimulation.

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Poster

362. Signaling Mechanisms in Long-Term Plasticity I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 362.13

Topic: B.05. Synaptic Plasticity

Support: NIMH T32 Grant 5T32MH016880-41

Title: Endocannabinoid exposure effects on molecular signaling and synaptic plasticity in the hippocampus

Authors: *A. MAYBERRY[1], C. HOEFFER, Jr.[1], M. M. HUNTSMAN[3], L. IMMINK[2], C. BORSKI[1], R. A. MILSTEAD[1];
[1]Integrative Physiol., [2]Psychology and Neurosci., Univ. of Colorado, Boulder, CO; [3]Pharmaceut. Sci., Univ. of Colorado, Anschutz, Aurora, CO

Abstract: The Cannabinoid family of receptors (CBR's) includes two types of receptors: CB1 and CB2. CB1 receptors are more prominently found in neuronal cells and brain tissue, with CB2 receptors first characterized in immune cells and associated tissues and subsequently identified in multiple regions and cell types of the central nervous system (CNS). The role of CBRs has been researched in various regions and tissues of the body, including the brain. Studies have shown that CBR-regulated signal transduction inhibits the cyclic AMP/PKA pathway in the

hippocampus and stimulates cAMP in the globus pallidus. Current research proposes that the difference in effect is likely isoform specific expression of adenylyl cyclase in different regions of the CNS. Research shows that cannabinoids can activate p42/p44 mitogen-activated protein kinases (MAPK), also known as extracellular signal-regulated kinase 1 and 2 (ERK1 and ERK2) in the brain. It is hypothesized that this activation requires the recruitment of phosphatidylinositol-3-kinase (PI3K) and protein kinase B (PKB/AKT) to phosphorylate and activate the MAPK pathway. Evidence for this interaction is shown in studies where multiple PI3K inhibitors blocked CB1 receptor-mediated signaling in MAPK. Research was done on the interaction of CBRs and the PI3K/AKT pathway showing increased phosphorylation of the AKT pathway in the presence of Delta9-tetrahydrocannabionol (THC), a known CB1 and CB2 agonist. These different pathway regulations have been shown to impact neuronal remodeling and synaptic plasticity. After exposing the hippocampus to endocannabinoid agonist, Win 55, 212 (Win), preliminary western blot data indicates sex differences and complex responses in the expression of AKT and its isoforms. Additionally, this early data indicates that blocking eCB signaling impairs Akt activation, having a direct impact on the presence of each AKT isoform. This work aims to study mechanisms of interaction between cannabinoids in the hippocampus, including molecular pathway signaling related explicitly to different isoforms of multiple proteins, ascertain the effects of varying endocannabinoid concentrations, and exposure times and determine if there are signaling differences between the sexes. Additionally, further characterization of endocannabinoid effects using field electrophysiology (ephys) to determine how Win and AEA impact synaptic plasticity, precisely long term-potentiation (LTP), a persistent strengthening of synapses leading to a long-lasting increase in signal transmission between neurons, and long-term depression (LTD), a continued reduction in neuronal signaling of a circuit.

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Poster

362. Signaling Mechanisms in Long-Term Plasticity I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 362.14

Topic: B.05. Synaptic Plasticity

Title: Lithium differentially affects synaptic plasticity in a dose-dependent manner

Authors: *H. WARK, L. K. BEKAR;
Univ. of Saskatchewan, Saskatoon, SK, Canada

Abstract: Background: Lithium carbonate (LiCO) is a key treatment option for the prevention of mood episodes associated with bipolar disorder (BD). Unfortunately, patients often discontinue LiCO treatment because of the negative impact on cognition. However, research on the impact LiCO has on psychomotor speed and memory is unclear, as some studies report

lithium affects cognitive functioning, while others do not. Lithium orotate (LiOr) is an alternative treatment suggested to possess superior uptake properties compared to LiCO, which may reduce dosing requirements and lessen cognitive side effects. As lithium has been shown to have a dose-dependent effect, we hypothesize that lithium chloride (LiCl; dissociation kinetics same as LiCO) will differentially affect synaptic plasticity in a dose-dependent manner while LiOr will have a similar effect at lower concentrations.
Methods: Dose-dependent LiCl and LiOr effects on synaptic plasticity were assessed in the hippocampal Schaffer collateral-CA1 synapse in male C57BL/6 mouse slices. LTP was induced by theta burst stimulations (8 bursts of 4 high frequency pulses at 5 Hz repeated 3 times, 60 seconds apart) at 32°C. LTD was induced by 1 Hz stimulation for 15 minutes at room temperature. We used typical therapeutic lithium concentrations between 0.4-1.2 mM to assess lithium effects on cognition.
Results: Lower concentrations of LiCl increased LTP while higher concentrations decreased LTP compared to the control. LiOr had a similar dose-dependent effect on LTP, but had a more robust effect at lower concentrations. We observed 0.6 mM LiCl has the largest effect on LTP, while a lower concentration of 0.4 mM LiOr caused a similar increase in LTP. We observed a similar effect between higher LiOr and LiCl concentrations, as 1 mM and 1.2 mM both decreased LTP compared to controls. In contrast, 0.4 mM-1.2 mM of LiCl and LiOr blocked LTD.
Conclusion: Therapeutic lithium concentrations demonstrate a dose-dependent response on synaptic plasticity. This differential effect could explain the contrasting findings on the effects lithium has on cognition as small changes in lithium concentration drastically change the synaptic response. This study has the potential to influence clinical lithium application and may lessen the negative impact lithium treatment has on cognition.

Disclosures: H. Wark: None. **L.K. Bekar:** None.

Poster

362. Signaling Mechanisms in Long-Term Plasticity I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 362.15

Topic: B.05. Synaptic Plasticity

Support: Academy of Finland (330776, 336376)
University of Oslo Convergence Environment (4MENT)
Research Council of Norway (248828)

Title: Exploration of the effects of schizophrenia-associated genes on cortical plasticity: a computational modelling study.

Authors: *T. MÄKI-MARTTUNEN[1], K. BLACKWELL[2], J. VERHELLEN[3], G. T. EINEVOLL[4], M.-L. LINNE[1], S. DJUROVIC[3], A. A. SHADRIN[3], T. ELVSÅSHAGEN[5], O. A. ANDREASSEN[5];

[1]Fac. of Med. and Hlth. Technol., Tampere Univ., Tampere, Finland; [2]George Mason Univ., Fairfax, VA; [3]Univ. of Oslo, Oslo, Norway; [4]Norwegian Univ. Life Sci., Aas, Norway; [5]Oslo Univ. Hosp., Oslo, Norway

Abstract: Symptoms and electrophysiological phenotypes of schizophrenia implicate altered synaptic plasticity as a potential cellular-level disease mechanism. Supporting this hypothesis, genes encoding intracellular signaling and membrane proteins associated with plasticity, including ion channels and neurotransmitter receptors, have been consistently identified as risk genes in the recent genome-wide association studies (GWAS). Here, we applied a recent biochemically detailed model of cortical synaptic plasticity (Mäki-Marttunen et al. *Elife* 9 (2020): e55714) to explore this hypothesis. This mass action based, computational model describes the signaling networks connecting neuromodulatory receptors with phosphorylation of AMPA receptors and the consequent insertion to or removal from the spine membrane. We concentrated on the genes identified in the most recent GWAS of schizophrenia (Trubetskoy et al. *Nature* 604.7906 (2022): 502-508). We analysed the effects of alterations in the model parameters corresponding the functions of proteins encoded by these genes. We found that while long-term potentiation and depression (including spike-timing dependent plasticity) in the neocortex remained unaltered by some of these genes, they were significantly altered by others. Mild alterations (±20%) of expression levels of the sczhizophrenia-associated genes could lead to strengthened or weakened plasticity, and importantly, specific combinations of these variants led to crucial impairments of induction and maintenance of long-term plasticity. We discuss the implications of these predictions on electrophysiological phenotypes of schizophrenia, and in particular, mismatch negativity and plasticity of visual evoked potentials. Our results suggest polygenic mechanisms behind altered synaptic plasticity in schizophrenia that may underlie the often complex endophenotypes and symptoms. **Acknowledgements:** UNINETT Sigma2 resources (project NN9529K) and CSC (project 2003397) were used for simulations.

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Poster

362. Signaling Mechanisms in Long-Term Plasticity I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 362.16

Topic: B.05. Synaptic Plasticity

Support: NIH T32 GM007377
NIH T32 MH112507
NIH R01 NS06736

Title: Activity-dependent regulation of Cdc42 by the RhoGEF Ephexin5 in dendritic spines

Authors: *S. PETSHOW, K. ZITO;
UC Davis, Univ. of California, Davis, Davis, CA

Abstract: The activity-dependent plasticity of dendritic spines and their associated synapses is a necessary component of learning and memory. Spine structural plasticity requires the local rearrangement of the actin cytoskeleton, which is regulated by the Rho GTPases RhoA, Cdc42, and Rac1 and their activators, the RhoGEFs. Ephexin5 (E5) is one such RhoGEF shown to be important for regulating spiny synapse development; however, the precise role of Ephexin5 and in the regulation of spine plasticity is unknown. While E5 has been shown to activate RhoA in neuronal tissue, evidence from somatic cells show that E5 also activates Cdc42 and Rac1. Notably, RhoA and Cdc42/Rac1 have been shown to play opposing roles in regulating dendritic spine structure, with RhoA activation inducing spine collapse and Cdc42/Rac1 activation inducing spine outgrowth and stabilization. Therefore, uncovering the full range of neuronal GTPase substrates of E5 is critical for understanding its role in spine plasticity. It is our hypothesis that E5 undergoes a neuronal activity-dependent shift in substrate specificity from RhoA to Rac1/Cdc42, relevant to its role in regulating spine plasticity. To determine the substrates of E5 in neuronal tissue, we isolated active Rho GTPases from whole brains lysates from E5 knockout (E5 KO) and wildtype (WT) mice. We found that both active RhoA and active Cdc42 levels were lower in the E5KO compared to WT. To determine the substrates of E5 during activity-dependent dendritic spine plasticity, we utilized 2-photon glutamate uncaging in tandem with fluorescence lifetime imaging (FLIM) of genetically-encoded FRET biosensors to measure Rho GTPase activity in live E5 KO or WT hippocampal CA1 neurons. Using FRET-FLIM, we were unable to detect changes in baseline activity levels of RhoA or Cdc42 in E5KO neurons compared to WT. However, in response to glutamatergic stimulation, we found that E5 KO neurons displayed lower Cdc42 activation compared to WT, while RhoA activation was unaltered. Our results support a model in which E5 regulates the local activation of Cdc42 in dendritic spines in response to glutamatergic signaling.

Disclosures: S. Petshow: None. **K. Zito:** None.

Poster

362. Signaling Mechanisms in Long-Term Plasticity I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 362.17

Topic: B.05. Synaptic Plasticity

Support: NIH Grant R15DA038092

Title: Effects of the Ketogenic Diet on Hippocampal CA1 Long Term Potentiation in Young Rodents

Authors: J. R. CHRISTENSEN[1], M. P. DEW[2], E. SAITO[3], A. EVERETT[2], J. WEIGHT[2], N. VALENTINE[3], C. KEMBERLING[3], B. BIKMAN[3], ***J. G. EDWARDS**[3];
[1]Biophysics, [2]Neurosci., [3]Cell Biol. and Physiol., Brigham Young Univ., Provo, UT

Abstract: The Ketogenic diet (KD) gained notoriety over the last few decades, originally for its potential to treat epilepsy. Though recently used as a weight loss aid, its effects on the neurological system are not well understood. We examined the cognitive impact of the KD on behavior and synaptic plasticity, employing CA1 hippocampal LTP as a measure in Sprague-Dawley rats and C57BL/6 mice. Two treatment groups were employed including a 3-4 week high lipid diet to increase ketone bodies *in vivo*, or bathing hippocampal slices in ketone beta-hydroxybutyrate (BHB)-enriched artificial cerebrospinal fluids (ACSF) to control the concentration of ketones. To ensure scientific rigor of experiments researchers were blinded as to which treatment group they were analyzing. First, we examined the impact on theta-burst induced CA1 LTP of ketone exposure to cut brain slices. In 3-8 week old female rats, we note that those exposed to 7.5 mM BHB with 3.5 mM glucose for > 2 hours demonstrated significantly ($p<0.05$) increased LTP (188 ± 10%; n=13) compared to controls of 0 mM BHB and 11 mM glucose (150 ± 8%; n=13). These experiments require replication in male rats. In contrast, in preliminary studies of slices from 2-5 week old mice, we have not observed a difference in response between slices exposed to 7.5 mM BHB and 2.5 mM glucose (n=6) compared to controls (n=7), though this data is currently underpowered and not yet able to examine sex effect. Mice given a 3-4 week KD lipid diet only reach ~2mM levels of blood ketones on this diet, less than what can be attained in humans. In behavioral Morris water maze experiments of these mice we noted no significant ($p>0.5$) difference in time to platform or time in correct quadrant comparing mice treated with the high-fat diet chow (n=11) to control chow (n=12). While our electrophysiology data on mice remain to be completed, our data suggest that a higher concentration of BHB than we could induce *in vivo* in rodents via diet had an effect on plasticity in brain slices alone, but no effect on memory behavior in concentrations attained *in vivo*. Future studies are now examining a more stringent diet to increase *in vivo* ketones, a longer diet period, sex differences, and studies on mature rodents. Overall, our data suggest examining ketogenic diets for impact on neurological function such as LTP and memory behavior warrants further investigation.

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Poster

362. Signaling Mechanisms in Long-Term Plasticity I

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 362.18

Topic: B.05. Synaptic Plasticity

Support: NIH R01 MH067284

Title: Pairing-dependent plasticity in a dissected fly brain is input-specific and requires synaptic CaMKII enrichment and nighttime sleep

Authors: *M. ADEL, N. CHEN, Y. ZHANG, M. REED, C. QUASNEY, L. GRIFFITH; Brandeis Univ., Waltham, MA

Abstract: In *Drosophila, in vivo* functional imaging studies revealed that associative memory formation is coupled to a cascade of neural plasticity events in distinct compartments of the mushroom body (MB). In-depth investigation of the circuit dynamics, however, will require an *ex vivo* model that faithfully mirrors these events to allow direct manipulations of circuit elements that are inaccessible in the intact fly. The current *ex vivo* models have been able to reproduce the fundamental plasticity of aversive short-term memory, a potentiation of the MB intrinsic neurons (Kenyon cells; KCs) responses after artificial learning *ex vivo*. However, this potentiation showed different localization and encoding properties from those reported *in vivo* and failed to generate the previously reported suppression plasticity in the mushroom body output neurons (MBONs). Here, we develop an *ex vivo* model using the female *Drosophila* brain that recapitulates behaviorally evoked plasticity in the KCs and MBONs. We demonstrate that this plasticity accurately localizes to the MB α'3 compartment and is encoded by a coincidence between KCs activation and dopaminergic input. The formed plasticity is input-specific, requiring pairing of the conditioned stimulus (CS) and unconditioned stimulus (US) pathways; hence we name it pairing-dependent plasticity (PDP). PDP formation requires an intact *CaMKII* gene and is blocked by previous-night sleep deprivation but is rescued by rebound sleep. In conclusion, we show that our *ex vivo* preparation recapitulates behavioral and imaging results from intact animals and can provide new insights into mechanisms of memory formation at the level of molecules, circuits, and brain state.

Disclosures: M. Adel: None. **N. Chen:** None. **Y. Zhang:** None. **M. Reed:** None. **C. Quasney:** None. **L. Griffith:** None.

Poster

362. Signaling Mechanisms in Long-Term Plasticity I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 362.19

Topic: B.05. Synaptic Plasticity

Title: The Impact of Repetitive Mild Traumatic Brain Injury (rmTBI) on Synaptic Strength, Neuroinflammation, and Plasticity: A Pilot Study

Authors: *S. S. ALDALIL[1], S. RAVI[2], D. BARROSO[2], T. EVERRETT[3], J. MCINTYRE[3], J. F. ABISAMBRA[2];
[1]Dept. of Biol. and the Neurosci. Program at Sewanee: The Univ. of the South, The Univ. of the South, Sewanee, TN; [2]Neurosci. & Ctr. for Translational Res. in Neurodegenerative Dis., [3]Neurosci., Univ. of Florida, Gainesville, FL

Abstract: Traumatic brain injury (TBI) results from violent external forces that cause damage to the brain. TBI accounts for 2.5 million emergency department visits, hospitalizations, and deaths in the U.S. with children aged 0-4 years, and older adults aged ≥75 years being the groups most likely to have a TBI-related ED visit (Thurman et al., 1999.) A major impact of TBI on neuronal function is impairment in learning and memory, and the mechanisms leading to long term learning and memory deficits following TBI remain largely unknown. Long-term potentiation (LTP) is a measure of neuronal plasticity, and it plays a major role in learning and memory. Open- head injury models, such as fluid percussion and controlled cortical impact, induce focal tissue destruction and lack head movement which decreases their resemblance to the majority of human impact acceleration injuries that are chiefly characterized by diffuse axonal injury (DAI) (Courtney and Courtney, 2015.) On the other hand, Closed-Head Impact Model of Engineered Rotational Acceleration (CHIMERA) is a non-invasive TBI model where the injury is mainly achieved through inducing DAI (Wellington et al., 2017.) Given that plasticity depends greatly on synaptic strength, and that a major feature of mild repetitive traumatic brain injury (mrTBI) is DAI, we hypothesized that CHIMERA-induced mrTBI reduces synaptic strength and LTP. In this sham-controlled study, C57BL/6 mice (n=7, both sexes, aged 7-8 months) received mrTBI (two consecutive injuries, 24h apart.) Field excitatory post-synaptic potentials were measured in the CA1 region of the hippocampus to evaluate baseline amplitude and LTP. Confirmatory immunohistochemistry was performed to assess white matter gliosis.The microglial marker Iba-1 was increased in the optic tract of injured mice group whereas the astroglial marker GFAP was increased in both the optic tract and corpus callosum regions compared to sham. There was a significant reduction in synaptic strength in the injured mice compared to sham. Moreover, while not conclusive, some disruptions in LTP occurrence in the injured group compared to the sham control group were observed. These preliminary data suggest that mrTBI induced by CHIMERA contributes to white matter inflammation, reduces synaptic strength, and facilitates LTP changes in the CA1 region of the hippocampus.

Disclosures: S.S. Aldalil: None. **S. Ravi:** None. **D. Barroso:** None. **T. Everrett:** None. **J. McIntyre:** None. **J.F. Abisambra:** None.

Poster

363. Intrinsic Properties and Electrical Synapses

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 363.01

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: Convergent Technology R&D Program for Human Augmentation through the National Research Foundation of Korea (NRF) funded by Ministry of Science and ICT (2019M3C1B8090842)
Brain Korea 21 FOUR Project

Title: Thalamocortical input regulates intrinsic plasticity of excitatory stellate cells in the layer 4 barrel cortex of adult mice.

Authors: *M. JEONG[1], S. CHUNG[2];
[1]Dept. of Physiol., Grad. Sch. of Med. Science, Brain Korea 21 Project, Yonsei Univ. Col. of Med., Seoul, Korea, Republic of; [2]Dept. of Physiol., Brain Korea 21 Project, Yonsei Univ. Col. of Med., Seoul, Korea, Republic of

Abstract: Recent work has revealed that thalamocortical (TC) synapses can be plastic even after the end of the critical period. Recently, we developed a unilateral infraorbital nerve lesioning (IO) mouse model in which synaptic plasticity of the spared TC input is restored after the critical period. However, it remains to be elucidated whether intrinsic properties of postsynaptic neurons receiving TC synapses are also changed in IO mice. Therefore, we investigated the intrinsic plasticity of excitatory stellate cells in the spared layer 4 barrel cortex in IO mice using the TC brain slice patch-clamp method. 4~8 week old male mice were used for ION denervation and sham operation. 500ms-length current steps were injected to excitatory stellate cells in the spared barrel cortex layer 4 to evoke AP in current-clamp mode. The AP firing frequency and other AP parameters were statistically analyzed to compare IO mice with Sham-controls. The firing frequency of layer 4 excitatory stellate cells increased significantly in the late period after the surgery (PO 19- 21; late PO) of IO mice compared with sham controls. The increased firing frequency mainly comes from decreased AP threshold in the late PO mice. These results suggest increased neuronal excitabilities in layer 4 excitatory stellate cells after ION denervation. A lower AP threshold makes excitatory stellate cells generate other APs more easily. It is speculated that voltage-gated sodium channels in layer 4 excitatory stellate cells would be altered by TC synaptic plasticity reopening. This study demonstrated that intrinsic firing properties of TC recipient neurons are also changeable even after the critical period.

Disclosures: M. Jeong: None. **S. Chung:** None.

Poster

363. Intrinsic Properties and Electrical Synapses

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 363.02

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NIH Grant K08NS118114
NIH Grant R01NS100016

Title: Characterization of ectopic action potentials in parvalbumin-expressing inhibitory neurons of the neocortex

Authors: *Y. ZHANG[1,2], S. SAPANTZI[2], B. B. THEYEL[3];
[1]NIH, Bethesda, MD; [2]Dept. of Neurosci., [3]Psychiatry and Human Behavior, Brown Univ., Providence, RI

Abstract: The canonical action potential originates from the axon initial segment and propagates orthodromically, towards distal axon terminals, and antidromically into the soma and dendrites.

Nevertheless, it has been documented for decades that action potentials sometimes originate from distal regions of the axons and their terminals, and propagate antidromically through the initial segment and into the soma (*Brain Res Rev,* 21:42, 1995). These are known as ectopic action potentials. For example, acetylcholine-releasing neurons in the striatum are able to generate ectopic action potentials that play a role in dopamine release (*Science,* 375:1378). In the cortex under non-pathological conditions, previous studies have demonstrated ectopic action potentials in hippocampal Neuropeptide Y-expressing neurons and are occasionally seen in parvalbumin-expressing interneurons (PV+ cells) (*Nat Neurosci.* 14:200, 2011). Our group has determined that a large portion (96%) of PV+ cells in the mouse orbitofrontal cortex fire ectopically after several hundred somatically evoked action potentials. However, little is known about the mechanism, function, and origin of these ectopic action potentials in the cortex. To elucidate the mechanisms behind these ectopic action potentials, which likely initiate in or near presynaptic terminals, we use whole-cell recordings and pharmacological interventions in acutely prepared slices of mouse orbitofrontal cortex. Our data show that the thresholds for ectopic initiation appear to decrease with the application of the mGluR1 antagonist (LY67385), voltage gated potassium channel blockers (4-AP or tetraethylammonium), and an HCN channel antagonist (Ivabradine). On the other hand, the number of evoked ectopic action potentials appears to decrease with the application of a P/Q-type (Cav2.1) calcium channel blocker (ω-agatoxin-IVa). These preliminary results suggest that ectopic action potentials in PV+ cells either depend on or are modulated by P/Q-type calcium channels, mGluR1, voltage-gated potassium channels, and HCN channels. Given the known roles of PV+ cells in sensory processing and seizure susceptibility, further investigations of these spontaneous, sustained, inhibitory ectopic action potentials that arise when PV+ interneurons are sufficiently excited may be useful in better understanding their functional roles in cortical network dynamics.

Disclosures: Y. Zhang: None. **S. Sapantzi:** None. **B.B. Theyel:** None.

Poster

363. Intrinsic Properties and Electrical Synapses

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 363.03

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: BBSRC (BB/P019854/1)
Newcastle University Faculty Fellowship

Title: In vivo differences in intracellular pH and chloride of cortical interneurons

Authors: *L. ALBERIO[1], R. T. GRAHAM[1], C. MACKENZIE-GRAY SCOTT[1], L. SAIEVA[1], A. MARSHALL[1], G. RATTO[2], A. J. TREVELYAN[1];
[1]Biosci. Inst., Newcastle Univ., Newcastle upon Tyne, United Kingdom; [2]Inst. Nanoscienze CNR, Inst. Nanoscienze CNR, Pisa, Italy

Abstract: Intracellular pH and chloride levels play an important role in determining neuronal excitability. In particular, the activity of most neuronal receptors is highly sensitive to deviations even within the physiological pH range (7.0-7.4). Furthermore, neuronal intracellular pH and chloride both affect fast synaptic inhibition through GABA-A receptors, since these are permeable to both Cl- and bicarbonate ions. For this reason, changes in pH and $[Cl^-]_i$ have a very powerful effect on network excitability. However, it is not known how pH and Cl^- regulation differs between neuronal classes. This question can now be addressed thanks to the development of ClopHensor (Arosio et al., 2010), a genetically encoded sensor, and its optimisation for *in vivo* 2-photon microscopy (Sulis Sato et al., 2017). We produced a modified ClopHensor, with an improved linker sequence, packaged into an AAV vector for cell-specific gene delivery (cre/lox system), and used this to target different subsets of cortical neurons in the somatosensory cortex (S1) of anaesthetised mice. We initially investigated $[Cl^-]_i$ in cortical pyramidal cells, at different times of the day, finding a large increase in $[Cl^-]_i$ during the more active phase of their circadian cycle (night, ZT17). This change is equivalent to ~10mV shift in E_{Cl}, and markedly altered both network excitability and cortical processing (Pracucci et al., 2021, biorxiv, undergoing revision). We next examined pH and $[Cl^-]_i$ levels *in vivo* in the two largest subpopulations of cortical interneurons, using conditional expression driven by Parvalbumin- (PV), and Somatostatin- (SST) promoters, respectively.
We will present population statistics of pH and $[Cl^-]_i$ in PV and SST interneurons *in vivo*, obtained using 2-photon microscopy. Our measures show marked differences between all three populations of cortical neurons in both their pH and $[Cl^-]_i$ values, suggesting the presence of independent regulation mechanisms in these cell classes and possible differences in the strength of their inhibitory activity.

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Poster

363. Intrinsic Properties and Electrical Synapses

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 363.04

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NIH NINDS R01 NS119977
Burroughs Welcome Fund Career Award for Medical Scientists to E.M.G.

Title: Ndnf-in dysfunction in a mouse model of dravet syndrome

Authors: *S. R. LIEBERGALL[1,2], E. M. GOLDBERG[4,1,3];
[1]Dept. of Neurosci., [2]Med. Scientist Training Program, [3]Dept. of Neurol., Univ. of Pennsylvania Perelman Sch. of Med., Philadelphia, PA; [4]Div. of Neurology, Div. of Pediatrics, Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: Neurodevelopmental disorders, such as autism spectrum disorder (ASD), schizophrenia, and childhood epilepsy, have been linked to dysfunction of forebrain GABAergic inhibitory interneurons (INs). Dravet Syndrome (DS) is a neurodevelopmental disorder characterized by severe epilepsy and features of ASD due to variants in *SCN1A* encoding the voltage-gated sodium channel α subunit Nav1.1. DS pathology is attributed to IN dysfunction, given that cerebral cortex INs preferentially rely on Nav1.1 for action potential generation and propagation. INs exhibit a broad diversity of electrophysiological, anatomical, and molecular properties; understanding the contribution of different classes of INs to microcircuit function in normal brain, and dysfunction in the setting of pathology, is important for elucidating the mechanisms of neurodevelopmental disorders. Three of the major subtypes of GABAergic INs, namely those expressing parvalbumin, somatostatin, and vasoactive intestinal peptide, show impaired action potential generation in an *Scn1a+/-* mouse model of DS. Here, we attempt to determine if a fourth major subtype of IN - those expressing Neuron-Derived Neurotrophic Factor (Ndnf) - are also dysfunctional in DS. We performed current clamp recordings of Ndnf-INs in layer 1 primary somatosensory cortex under fluorescent guidance in acute brain slices prepared from Ndnf-Cre.*Scn1a+/-* mice and age-matched littermate Ndnf-Cre.*Scn1a+/+* controls. We found that Ndnf-INs display abnormalities in sodium channel-dependent properties of individual action potentials and of repetitive firing in *Scn1a+/-* mice relative to wild type, but do not show major differences in passive membrane properties which are not directly dependent on sodium channel function. In summary, Ndnf-INs are a recently identified and understudied subclass of INs whose intrinsic firing is impaired in the setting of heterozygous loss of function of *Scn1a*, suggesting that Ndnf-INs also rely on Nav1.1, are dysfunctional in DS, and may contribute to DS pathophysiology.

Disclosures: S.R. Liebergall: None. **E.M. Goldberg:** None.

Poster

363. Intrinsic Properties and Electrical Synapses

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 363.05

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: Department of Veterans Affairs Merit Review Award 1I01BX002565

Title: The axon initial segment is structurally and functionally altered in the EAE model of multiple sclerosis with an increased vulnerability of type A layer 5 neocortical pyramidal neurons

Authors: *A. HARRIS[1], S. BROOKINS[2], J. L. DUPREE[1], K. M. JACOBS[1];
[1]Virginia Commonwealth Univ., Richmond, VA; [2]VCU, Richmond, VA

Abstract: The experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis gives rise to a diversity of inflammation-associated pathobiological consequences, including a

reduction of neurons that positively stain for the axon initial segment (AIS) scaffolding protein ankyrin-G (aG), crucial for dense compartmentalization of sodium channels at the AIS. To investigate the possibility of AIS perturbation, we recorded high-resolution action potentials from layer 5 pyramidal neurons that were filled with biocytin and subsequently immuno-stained for aG and Nav1.6 sodium channels within EAE mice and saline-injected controls. Layer 5 pyramidal neurons were parsed into type A (extratelencephalic; N = 33/41 EAE/control) and type B (intratelencephalic; N = 34/25 EAE/control) sub-types, given the myriad physiological differences between these cardinal subtypes. Results were evaluated with t-tests for EAE vs control, or for percentages with z-tests. Strikingly, within the EAE condition, we observed a significant decrease in the percentage of aG-positive neurons relative to controls only for type A neurons (Z test, $p = 0.025$ for type A and $p = 0.826$ for type B). While Nav1.6 was present in the same percentage of neurons in both EAE and control, in the aG negative type A neurons, there was a significant shortening of the Nav1.6 staining in EAE compared to control ($p=0.008$). The length of the aG staining was not altered within EAE compared to control (in type A, $p = 0.818$ or type B, $p=0.138$). Using a second derivative analysis of the action potential, the voltage acceleration at the AIS was measured in isolation from that at the soma. In control type A neurons there was a significant correlation between the peak acceleration at the AIS and the length of aG and Nav1.6 staining ($r = 0.43$ and 0.41). These correlations were lost in EAE type A neurons ($r=-0.19$ for aG and 0.06 for Nav1.6), suggesting that the AIS is under significant structural remodeling. The time from action potential threshold to peak acceleration at the soma was increased in EAE compared to control only for type A neurons ($p = 0.044$), suggesting a greater spatial separation between AIS and somatic activation points. Surprisingly, the peak voltage acceleration at the AIS and soma was significantly increased in EAE compared to controls for both type A and type B neurons ($p = 0.001$ and 0.001). Together these results suggest a dissociation between aG staining and the presence of functional sodium channels and indicate altered sodium channel function in the absence of Nav1.6-staining length changes. These effects on the AIS may contribute to abnormal temporal integration within the neocortical network in the MS condition.

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Poster

363. Intrinsic Properties and Electrical Synapses

Location: SDCC Halls B-H

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Program #/Poster #: 363.06

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: American Epilepsy Society fellowship 630186
NIH R01NS064025
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Title: Circadian regulation of dentate gyrus excitability mediated by G-protein signaling

Authors: *J. GONZALEZ[1], H. LEE[1], A. M. VINCENT[1], A. L. HILL[1], L. K. GOODE[2], G. D. KING[3], K. L. GAMBLE[2], J. I. WADICHE[1], L. OVERSTREET-WADICHE[1];
[1]Neurobio. and McKnight Brain Inst., [2]Psychiatry and Behavioral Neurobio., Univ. of Alabama At Birmingham, Birmingham, AL; [3]Biol., Creighton Univ., Omaha, NE

Abstract: The central circadian regulator within the suprachiasmatic nucleus transmits time of day information by a diurnal spiking rhythm that is driven by intrinsic activity of molecular clock genes controlling membrane excitability. Most brain regions, including the hippocampus, harbor similar intrinsic circadian transcriptional machinery but whether these molecular programs generate oscillations of membrane properties is largely unexplored. We prepared horizontal hippocampal slices from two to five month-old male and female mice during the light phase (Zeitgeber Time, ZT 5.5 or 11.5 where ZT 12 refers to lights off) and performed recordings between projected ZT 8-11 (referred to as “Light phase”) or ZT 14-17 (“Dark phase”). Perforant path recruitment of granule cell spiking was robustly different across diurnal phase, with lower excitability during the Light phase. The intrinsic excitability of dentate granule neurons exhibited a 24-hour oscillation, where the peak of granule cell excitability occurred at ZT 16 and the trough at ZT 4. Diurnal changes in excitability were mediated by G-protein regulated mechanisms, with constitutive activation of G-protein coupled inwardly rectifying potassium (GIRK) channels and suppression of a Na^+ leak channel during the Light phase. To address whether diurnal regulation of granule cell excitability relies on a cell-autonomous transcriptional oscillation, we generated SCN-independent disruption of the molecular clock by conditional *Bmal1* ablation, targeting granule cells using *Pomc-Cre* with visualization by the Ai14 reporter. Disruption of the circadian transcriptional machinery enhanced excitability selectively during the Light phase via disruption of G-protein signaling, as the constitutive GIRK conductance was suppressed and a Na^+ leak conductance was activated. Additionally, we evaluated whether the enhanced excitability of *Bmal1* conditional KO granule cells during the Light phase translates to an increase in the size of a memory engram *in vivo*. Analysis of *c-fos* activation following fear conditioning showed an increase in the engram recruitment with no change contextual memory. In conclusion, these results reveal that circadian transcriptional machinery regulates intrinsic excitability, providing new insight into the role of the local molecular clock in the control of neuronal excitability.

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Poster

363. Intrinsic Properties and Electrical Synapses

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Program #/Poster #: 363.07

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: Charles H. Revson Foundation Postdoctoral Fellowship
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AARF-21-852397

Title: Brief synaptic inhibition persistently interrupts firing of fast-spiking interneurons

Authors: *S. CHAMBERLAND, E. R. NEBET, M. VALERO, M. HANANI, R. EGGER, S. LARSEN, K. EYRING, G. BUZSAKI, R. W. TSIEN;
Neurosci. Inst., New York Univ. Sch. of Med., New York City, NY

Abstract: Neurons perform input-output operations that integrate synaptic inputs with intrinsic electrical properties, operations generally constrained by the brevity of synaptic events. Here we report that sustained firing of CA1 hippocampal fast-spiking parvalbumin-expressing interneurons (PV-INs) can be persistently interrupted for up to several hundred milliseconds following brief $GABA_AR$-mediated inhibition *in vitro* and *in vivo*. A single presynaptic neuron could interrupt PV-INs firing, occasionally with a single action potential (AP), and reliably with AP bursts. Experiments and computational modeling revealed that the persistent interruption of firing maintains neurons in a depolarized, quiescent state through a cell-autonomous mechanism. Strikingly, interrupted PV-INs are highly responsive to Schaffer collateral inputs. The persistent interruption of firing provides a disinhibitory circuit mechanism favoring spike generation in CA1 pyramidal cells. Overall, our results demonstrate that neuronal silencing can far outlast brief synaptic inhibition owing to well-tuned interplay between neurotransmitter release and postsynaptic membrane dynamics, a phenomenon impacting microcircuit function.

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Poster

363. Intrinsic Properties and Electrical Synapses

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 363.08

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NIH R01 NS112500
American Epilepsy Society Postdoctoral Fellowship
Dravet Syndrome Postdoctoral Fellowship

Title: Enhanced input/output functions in the hippocampus of Scn1b knockout mice

Authors: *J. H. CHANCEY, M. A. HOWARD;
Univ. of Texas At Austin, Univ. of Texas At Austin, Austin, TX

Abstract: Neuronal information processing relies on the interplay between synaptic properties, dendritic intrinsic properties that amplify or suppress synaptic signals, and firing properties that produce neuronal output. Disrupting this interplay can result in epilepsy and other neurological deficits. Mutations in the *SCN1B* gene have been linked to severe epileptic encephalopathies, including Dravet syndrome (DS). *Scn1b* knockout (KO) mice model many symptoms of DS, including early onset spontaneous and febrile seizures, wide-ranging neurological deficits, motor impairments, and a high mortality rate. *SCN1B* encodes the protein β1, which regulates several ion channels that control neuronal excitability, and has roles in cell adhesion, neurite outgrowth, and gene regulation. We used whole-cell electrophysiology and 2-photon calcium imaging of CA1 pyramidal cells (PCs) in acute hippocampal slices from male and female *Scn1b* KO and wild-type (WT) littermates. We measured synaptic currents and postsynaptic potentials via stimulation of Schaffer collateral (SC) axons, dendritic active properties via calcium imaging of backpropagating action potentials, and somatic intrinsic properties with current injections. We found changes in synaptic, dendritic, and somatic physiology in *Scn1b* KO pyramidal cells. While both excitatory and inhibitory synaptic currents were smaller in KO PCs compared to WT, hyper-responsive dendrites and somata amplified synaptic signals, resulting in an increased occurrence of simple and complex spiking in KO neurons in response patterned synaptic stimulation. This inflated input/output relationship is a fundamental change in cellular information processing in the hippocampus, likely underlying the seizures and associated cognitive defects in *SCN1B*-linked DS. Our results suggest that understanding the mechanisms of neural dysfunction with loss of β1 requires examination not only of individual aspects of cell physiology but their interactions.

Disclosures: J.H. Chancey: None. **M.A. Howard:** None.

Poster

363. Intrinsic Properties and Electrical Synapses

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 363.09

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: 5R01MH115188-05

Title: 14-3-3 protein knockout increases excitability of hippocampal ca1 pyramidal neurons in mice

Authors: *J. B. LOGUE[1], J. ZHANG[2], Y. WU[1], Y. ZHOU[1];
[1]Florida State Univ., Florida State Univ., Tallahassee, FL; [2]Florida State Univ. Col. of Med., Tallahassee, FL

Abstract: 14-3-3 proteins are a family of regulatory proteins that are expressed abundantly in the brain and enriched at synaptic junctions. Several neurological and psychiatric disorders have been linked to 14-3-3 protein dysfunctions. Our lab has previously shown that 14-3-3 functional knockout (FKO) mice have reduced expression of synaptic proteins such as NMDA receptor subunits, reductions in hippocampal long-term potentiation, and present desynchronization in theta waves between the hippocampus and prefrontal cortex. Additionally, these 14-3-3 FKO mice exhibit behaviors that correspond with the symptoms of psychiatric disorders such as schizophrenia. Recently, we identified an increase in activity of hippocampal pyramidal neurons that express the 14-3-3 FKO by analyzing cFos expression as an indicator of neuronal activation. In this work, we are using slice electrophysiology and whole cell patch clamp to evaluate the impact of 14-3-3 inhibition on electrophysiological properties of hippocampal CA1 pyramidal neurons. To assess 14-3-3 FKO induced excitability changes, we measure differences in the 14-3-3 FKO mouse and their wild-type control using voltage follower, current clamp, and voltage clamp. We use voltage follower to assess the basal firing rate between neurons, apply synaptic blockers to assess the intrinsic firing of these neurons, and utilize current clamp to determine rheobase and propensity to fire in response to stimulation. Our results show a significant increase in firing rate and an increased responsivity to current injection in the neurons from 14-3-3 FKO mice. We are currently using voltage clamp to identify changes in inward and/or outward current in these neurons, as well as to assess potential alterations in synaptic input. We expect this study to provide critical insights linking the dysfunction of these regulatory 14-3-3 proteins to the altered neuronal and synaptic activity that underlie the neural circuitry and behavioral aberrations exhibited in these 14-3-3 FKO mice.

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Poster

363. Intrinsic Properties and Electrical Synapses

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Program #/Poster #: 363.10

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NIA R01AG054180

Title: Cell type specific alterations of Alzheimer's disease mutations on intrinsic firing properties within the hippocampus of genetically diverse mice

Authors: *M. BERCHULSKI[1], J. FUNKE[1], N. HADAD[1], K. O'CONNELL[1], C. C. KACZOROWSKI[2];
[2]Genomics, [1]The Jackson Lab., Bar Harbor, ME

Abstract: Cognitive resilience to Alzheimer’s disease (AD) is a phenomenon whereby individuals with known AD mutations or high levels of pathology exhibit better than predicted cognitive performance. Identification of the factors governing resilience may lead to novel

therapeutic interventions to treat AD. Analysis of hippocampal transcriptional profiles from susceptible and resilient AD-BXD strains indicates that transcriptomic changes in excitatory neurons in the CA1 and Dentate Gyrus (DG) are associated with resilience, we now seek to examine physiological changes that may occur along with these transcriptomic changes. We adapted the Patch-Seq method (Cadwell et al., 2017) to investigate cell type-specific transcriptional and electrophysiological changes associated with AD using the AD-BXD genetic reference panel (Neuner et al., 2019). Whole-cell patch clamp was used to evaluate intrinsic excitability of neurons within the DG and CA1 of 14 month old mice with cognitive deficits compared to age matched controls; their cellular contents, including the nucleus, were collected after each recording to characterize the transcriptome. Comparison of the intrinsic properties of DG and CA1 neurons of hippocampal slices from 5XFAD mice and their non-transgenic (Ntg) counterparts revealed that DG granule cells from 5XFAD mice have a lower input resistance and an increased sag ratio in response to hyperpolarizing current injections as well as a faster action potential rise-time. Pyramidal neurons from the CA1 of 5xFAD mice exhibit more depolarized action potential thresholds, an increased current threshold, a shorter decay time, a significant increase in the medium afterhyperpolarization (mAHP) following a burst of action potentials, and an increased sag ratio compared to Ntg controls. While these intrinsic properties are proxy measures for neuronal excitability, we did not observe a significant effect of genotype on firing rate in response to 1s and 15s depolarizing current steps. Our findings newly demonstrate disease related changes to intrinsic firing properties of DG granule cells and CA1 pyramidal cells at 14 months of age indicating that these neurons may be hypoexcitable. Future work will add a younger age group to explore age related changes as well as add additional AD-BXD strains characterized as resilient or susceptible to discover changes in intrinsic properties that could underlie resilience. We will also use an integrated analysis of transcriptomic and electrophysiological data to identify relevant functional targets for validation.

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Poster

363. Intrinsic Properties and Electrical Synapses

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 363.11

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Title: Excitability is reduced in a membrane-potential-dependent manner in a genetic mouse model of Angelman Syndrome

Authors: *A. E. PRATT, C. MALLOY, J.-H. HU, D. A. HOFFMAN;
Eunice Kennedy Shriver Natl. Inst. of Child Hlth. an, Bethesda, MD

Abstract: Angelman Syndrome (AS) is a rare neurodevelopmental disorder that causes delayed development and a predisposition to seizures, among other neurological phenotypes. Loss-of-

function mutations in the maternal allele of Ube3a, the gene that encodes for E3A ubiquitin ligase, is known to be the cause of this condition. By utilizing a heterozygous knockout (KO) model for Ube3a, which is a common model of Angelman Syndrome, we have characterized the excitability of mice lacking hippocampal Ube3a expression via whole-cell patch clamp recordings. Our results show that Ube3a-KO mice exhibit reduced excitability in pyramidal neurons in the CA1 region of the hippocampus. Specifically, we observed a reduced firing frequency in KO mice that was primarily due to increased initial inter-spike interval and latency to fire. These differences were only observed when resting membrane potential was held at a hyperpolarized condition (-70 mV). Input resistance was also significantly reduced in the KO group and whole-cell capacitance was significantly increased. Additionally, our group is conducting extracellular field recordings to determine how plasticity might be affected along Schaffer Collateral inputs to the CA1 in this KO model. Future work aims to elucidate the mechanisms behind altered excitability in this model and to identify the direct target of Ube3a that contributes to these differences.

Disclosures: **A.E. Pratt:** None. **C. Malloy:** None. **J. Hu:** None. **D.A. Hoffman:** None.

Poster

363. Intrinsic Properties and Electrical Synapses

Location: SDCC Halls B-H

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Program #/Poster #: 363.12

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

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Swiss National Supercomputing Centre (CSCS) under projects ID ich011 and ich002

Title: A computational model of the early derailment of firing properties in CA1 pyramidal neurons of the ventral hippocampus in Tg2576 AD mice.

Authors: *E. GIACALONE[1], E. SPOLETI[2], P. KRASHIA[3,4], L. LA BARBERA[2,4], A. NOBILI[2,4], C. LUPASCU[1], F. KELLER[3], M. MIGLIORE[1], M. RENZI[5], M. D'AMELIO[3,4]; [1]Inst. of Biophysics, Natl. Res. Council, CNR, Palermo, Italy; [2]Fac. of Sci. and Technologies for Humans and Envrn., [3]Fac. of Med. and Surgery, Univ. Campus Bio-Medico, Rome, Italy; [4]Dept. of Exptl. Neurosciences, IRCCS Santa Lucia Fndn., Rome, Italy; [5]Dept. of Physiol. and Pharmacol., Sapienza Univ., Rome, Italy

Abstract: Alzheimer's Disease (AD) is a form of progressive dementia characterized by a gradual neurodegeneration causing a decline in cognitive and non-cognitive functions. For more than 25 years, AD research has been grounded on the so-called amyloid hypothesis as the main cause of the disease. Nevertheless, its involvement on brain circuit alterations and cellular derailments in very early stages is still under intense exploration. It is essential to understand the key mechanisms of early cellular alteration caused by the AD. In the last years, several studies have been focused on the role of dopamine within the hippocampal circuitry during AD progression, especially as an early hallmark of the disease. In line with this notion, the study of the Tg2576 mouse model of AD has already revealed functional and behavioral AD-related deficits in the dorsal hippocampus correlating with the precocious degeneration of dopaminergic system. In this poster, we show a computational model that is able to support experimental findings, and suggest experimentally testable predictions, on the cellular mechanisms underlying the early modifications in firing properties and the impaired excitability experimentally observed in ventral CA1 pyramidal neurons of Tg2576 mice. The model suggests that the early derailments of firing properties observed and the neuronal alterations found could depend on dysfunctional sodium and potassium conductances, leading to anticipated depolarization-block of action potential firing. In particular, we found that both a shift of sodium channel activation kinetics and the alteration of specific potassium conductances (K_{DR} and K_{Ca}) can underlie the altered firing behavior. These results propose that the early perturbation in the expression and in functional involvement of these channels can contribute significantly in modulating AP firing behavior of Tg2576 ventral CA1 pyramidal neurons.

Disclosures: **E. Giacalone:** None. **E. Spoleti:** None. **P. Krashia:** None. **L. La Barbera:** None. **A. Nobili:** None. **C. Lupascu:** None. **F. Keller:** None. **M. Migliore:** None. **M. Renzi:** None. **M. D'Amelio:** None.

Poster

363. Intrinsic Properties and Electrical Synapses

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Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

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Association France Alzheimer

Title: The Alterations of CA1 Pyramidal Neurons excitability in an APPPS1 Model of Alzheimer's Disease

Authors: *P. VITALE[1], A. SALGUEIRO-PEREIRA[2], C. LUPASCU[1], R. MIGLIORE[1], M. MIGLIORE[1], H. MARIE[2];
[1]Inst. of Biophysics, Italian Natl. Res. Council, Palermo, Italy; [2]Inst. de Pharmacologie Moleculaire et Celluaire (IPMC), Valbonne, France

Abstract: Increasing accumulation of the β-amyloid in the brain is one of the factors underlying the decline of cognitive functions in Alzheimer's disease (AD). Due to its role in memory processing, the hippocampus is a key brain region in both aging and amyloidopathy progression. Investigating specific mechanisms related to the electrophysiological properties changes during the AD progression, might help in identifying the source of deviant behaviors and in the development of more effective treatments. We performed a detailed analysis of electrophysiological somatic traces recorded as a function of increasing current injections, from mice hippocampus CA1 pyramidal neurons, comparing their features at different ages under control and AD conditions (Radde et al. 2006 https://doi.org/10.1038/sj.embor.7400784). The three ages selected are representative of different stages of the disease: 1) at 1 month the brain should be not affected by the disease at all, 2) at 3-4 months, the mice are still asymptomatic but the β-Amyloid begins to accumulate, 3) at 9-10 months the brain functions are strongly affected, and the β-Amiloid's plaques extension makes the mice strongly symptomatic. Using the feature extraction tool of the EBRAINS Cellular level modeling workflows (https://ebrains.eu/service/cls-interactive/), we decided to extract 14 features, representatives of the firing behavior of the electrophysiological recordings, and perform a two-by-two statistical comparison between the genotypes. We observed strong alterations in membrane time constant and action potential width and weak alterations in firing behavior. Furthermore, consistently with an important role systematically suggested for the non-specific current I_h in determining these changes, we found amyloidopathy-dependent alterations in its kinetic. Using the Run Fitter tool available on NEURON, we implemented a new model to consider the changes in this channel kinetics under AD condition. Finally, using an automatic classification analysis we were also able to distinguish, with a good degree of accuracy, the age of the animal from which the recording was carried out and if the cell was affected by AD. This joint approach of experimental recordings, statistic, and computational modeling, allowed new insight into the excitability alterations caused by this widely diffused neurodegenerative disease. These results could be useful for both modelers, who can use them for investigating AD effects in computational models, and experimentalists, who can improve their analyses of recordings to better recognize the AD progression with age. (Vitale et al. 2021 https://doi.org/10.3389/fnagi.2021.668948)

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Poster

363. Intrinsic Properties and Electrical Synapses

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Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

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EU Joint Program–Neurodegenerative Disease Research (JPND) project CIRCPROT (jointly funded by BMBF, MIUR, ANR, and EU Horizon 2020 grant agreement no. 643417)

Title: Hippocampal Place Cell's Computational model

Authors: *C. MAZZARA, M. MIGLIORE;
IBF, CNR, Palermo, Italy

Abstract: Hippocampal place cells play a pivotal role in spatial navigation. To shed some light on the dendritic integration mechanisms that can underlie the formation of a Place Cell, in this work we used a morphological and biophysical detailed computational model of a CA1 pyramidal neuron that shows how this neuron can turn into a place cell. Few computational models, using Place Cells firing according to the geometric position of cues around an animal, have been published to study a variety of issues involving spatial navigation (e.g. (Zipser, 1986; Sharp, 1991; Burgess et al., 1994; O'Keefe and Burgess, 1996; Hartley et al., 2000). However, in all of them, Place Cells have been implemented with simplified or artificial multilayer neural networks consisting of abstract point neurons, and the actual process of Place Cells formation was given for granted. The results support in vivo and in vitro findings (Bittner et al., 2015) suggesting that this process critically depends on a strong and transient depolarizing plateau potential, propagating along the whole neuron and conjunctively interacting with those synaptic inputs most activated during the navigation. The model shows the conditions under which a CA1 pyramidal cell can self-tune into a Place Cell coding for specific cue location(s), following a reward signal activated during an environment exploration. The model is robust in that, in accordance with the experimental data, it creates a Place Cell that within the same spatial context remains stable during different trials (different trajectories). This is the first computational model capable of simulating the creation of a Place Cell and it can be used directly in large-scale networks of the hippocampus, to study cognitive functions and dysfunctions at a higher level of integration.

Disclosures: C. mazzara: None. **M. Migliore:** None.

Poster

363. Intrinsic Properties and Electrical Synapses

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Title: Test simulations and validation of the Adaptive GLIF model for hippocampal CA1 neurons Test simulations and validation of the Adaptive GLIF model for hippocampal CA1 neurons Test simulations and validation of the Adaptive GLIF model for hippocampal CA1 neurons

Authors: *C. LUPASCU[1], E. SPERA[2], V. DE FALCO[3,4], A. IUORIO[5], M. MIGLIORE[6], A. MARASCO[7];
[1]Natl. Res. Council, Inst. of Biophysics, Natl. Res. Council, Inst. of Biophysics, Palermo, Italy; [2]IBF, CNR, Palermo, Italy; [3]Scuola Superiore Meridionale, Napoli, Italy; [4]Inst. Nazionale di Fisica Nucleare, Napoli, Italy; [5]Univ. of Vienna, Univ. of Vienna, Vienna, Austria; [6]Natl. Res. Council, Natl. Res. Council, Palermo, Italy; [7]Univ. of Naples Federico II, Univ. of Naples Federico II, Napoli, Italy

Abstract: An adaptive generalized leaky integrate-and-fire (A-GLIF) model for hippocampal CA1 neurons and interneurons, including more realistic reset rules for the membrane potential and intrinsic currents at each spike event was implemented with the aim to reproduce a rich set of electrophysiological behaviours, with a particular focus on the spike times as a function of the current injection. A custom procedure for parameters optimization was carried out using the geneticalgorithm() python library. To determine the A-GLIF model parameters for each cell, we first extracted from the somatic experimental traces the resting potential EL, the reset potential Vr, and the threshold potential Vth, in addition to all spike times at all constant currents. Although, in general, parameters like the rheobase current Ith, the membrane capacitance Cm, and the membrane time constant τm, can also be inferred from the experimental traces, or fixed according to the literature, we have preferred to treat them as fitting parameters, together with all the other model parameters, K, kadap and the initial condition for Idep and Iadap. To test and validate our model we considered a set of somatic voltage traces recorded from 84 cells: 58 pyramidal and 26 interneurons, obtained from in vitro rat hippocampal CA1 slices, in response to somatic constant current injections, from 200pA to 1000pA with a step of 200pA. The 314 traces from pyramidal neurons were all classified as continuous accommodating cells (cAC); for interneurons, 54 traces were classified as cAC, 72 traces as bursting cells (bAC), and 62 traces as continuous non-accommodating cells (cNAC). To test the ability of our A-GLIF implementation to capture the spike time patterns observed under experimental protocols using current steps of different amplitudes, we used as a reference the traces generated by a realistic hippocampal CA1

pyramidal neuron model. We first optimized the A-GLIF model to reproduce the traces obtained with NEURON under a constant stimulation of 400, 600, 800 and 1000 pA. Then, to validate the model, we tested it on constant currents different from those used to optimize the model parameters (i.e. 500 and 700 pA) and on a set of piece-wise constant current injections and synaptic inputs.
In conclusion, by analyzing the experimental firing properties, we were able to find a scheme to quantitatively characterize and predict the firing behaviours of hippocampal pyramidal neurons and interneurons, in response to any stimulation protocol.
This can be fundamental when dealing with very large-scale networks including multiple brain areas/regions.

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Poster

363. Intrinsic Properties and Electrical Synapses

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Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

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Title: The physiological variability of channel properties in neurons across different animal species: the case of hippocampal CA1 pyramidal neurons in mice and rats

Authors: *F. LIBRIZZI[1], P. VITALE[1], M. PEZZOLI[2], Y. SHI[3], A. ROMANI[3], M. MIGLIORE[1], R. MIGLIORE[1];
[1]Italian Natl. Res. Council, Inst. of Biophysics, Palermo, Italy; [2]Ecole Polytechnique Fèdèrale de Lausanne, Lab. of Neural Microcircuitry, Geneve, Switzerland; [3]Ecole Polytechnique Federale de Lausanne, Blue Brain Project, Geneve, Switzerland

Abstract: The basic function of any neuron in a network is the transformation of multiple spatiotemporal synaptic input patterns into single, properly tuned, output spikes; these in turn may act as an input for other neurons in the network. Neurons must perform this function in a wide range of different physiological conditions, which can change even drastically during the neuron lifespan. As a consequence, every neuron must be characterized by a considerable robustness against the variability of the overall conditions. Furthermore, it must be able to compensate for spurious signals or even for the effects of pathological conditions, which may

lead to abnormal inputs. The mechanisms through which this robustness is achieved, and how they are implemented in different animal species are not completely understood. Several experimental and theoretical findings suggest the involvement of significant degeneracies and/or correlations, in the values of ionic channels conductance of neurons, since this may allow to maintain a proper input-output transfer even within thoroughly different conditions. A powerful strategy for the investigation of these processes consists in the construction and in the analysis of morphologically and biophysically detailed data-driven computational neuron models, to which suitable electrophysiological properties, ionic peak conductances as well as passive properties, are attributed, in order to catch the main experimental features observed for the same cells (Van Geit et al. 2016, https://doi.org/10.3389/fninf.2016.00017). This bottom-up approach has been recently exploited in the case of rat CA1 pyramidal neurons (Migliore et al. 2018, https://doi.org/10.1371/journal.pcbi.1006423), indicating the presence of two distinct set of conductances: one relatively stable across all cell models, responsible for the main firing properties, and the other bringing about a significant degeneracy. Here we analyze the electrophysiological features of mouse CA1 pyramidal neurons and apply the same unified data-driven simulation workflow for the implementation of detailed computational single cell models. The results of the analysis of the experimental features and of the cell parameters were systematically compared across the two species, also in relation with their morphological differences. The comparison revealed an analogous degeneracy in the space of parameters values, with different cross-correlation pattern and a characteristically different I_h current in the two species.

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Poster

363. Intrinsic Properties and Electrical Synapses

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Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

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EU Joint Program–Neurodegenerative Disease Research (JPND) project CIRCPROT (jointly funded by BMBF, MIUR, ANR, and EU Horizon 2020 grant agreement no. 643417)

Title: Stimulus-induced increase of the depolarization baseline in mouse hippocampal CA1 pyramidal neurons

Authors: *R. MIGLIORE[1], D. BIANCHI[2], P. VITALE[3], M. GARAD[4], P. A. POUSINHA[6], H. MARIE[7], V. LESSMANN[5], M. MIGLIORE[8];
[1]NATIONAL RESEARCH COUNCIL, Italian Natl. Res. Council, PALERMO, Italy; [2]Inst. of Biophysics, Natl. Res. Council, Palermo, Italy; [3]cnr, CNR, palermo, Italy; [5]Otto-von-Guericke Univ., [4]Otto-von-Guericke Univ., Magdeburg, Germany; [6]CNRS IPMC UMR 7275, CNRS IPMC UMR 7275, Valbonne, France; [7]Inst. de Pharmacologie Moleculaire et Celluaire (IPMC), Inst. de Pharmacologie Moleculaire et Celluaire (IPMC), Valbonne, France; [8]Natl. Res. Council, Natl. Res. Council, Palermo, Italy

Abstract: In this work we analyze stimulus-induced dynamic increase of the depolarization baseline (DBL, defined as the minimum value of the membrane potential between action potentials) charactering the firing activity of mouse hippocampal CA1 pyramidal neuron recorded during standard current-clamp experiments. This dynamic cannot be reproduced exploiting conventional Hodgkin-Huxley ionic channels or any current computational model. Here we address this issue by building an effective model of the overall effect that is characterized by an input-dependent change in the membrane ionic permeability (which could transiently and significantly reduce the ionic chemical gradient across the membrane of a cell through a progressive shift of the ion reversal potentials) together with an input-dependent shift in the activation/inactivation kinetic of its ionic channels [1]. We tested this implementation using the recordings of two independent laboratories as a reference. Our results show that the model is able to reproduce the experimental firing patterns and the correct trend for the DBL level as a function of the input current. We investigate, in addition, what would happen in a CA1 pyramidal cell during a bursting excitatory synaptic activity eliciting up- and down-states, similar to those observed during spatial exploration or expected from a Theta-burst LTP induction protocol. We found that the lack of DBL in a neuron model, subjected to a Theta-burst synaptic activation protocol, will essentially filter out the signal component in the θ range from the response and will amplify oscillations in the low γ range. Such findings suggest that the DBL mechanism can significantly affect mouse hippocampus model operations during synaptic activity.1.Bianchi, D., Migliore, R., Vitale, P., Garad, M., Pousinha, P., Marie, H., Lessmann, V., Migliore, M., Membrane electrical properties of mouse hippocampal CA1 pyramidal neurons during strong inputs (2022) Biophysical Journal 121, 644-657 https://doi.org/10.1016/j.bpj.2022.01.002.

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Poster

363. Intrinsic Properties and Electrical Synapses

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Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

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Title: Theta gamma phase amplitude coupling in a CA1 circuit model

Authors: *A. P. PONZI, M. MIGLIORE;
Inst. of Biophysics, CNR, Inst. of Biophysics, CNR, Palermo, Italy

Abstract: Theta gamma phase amplitude coupling (TGPAC) is known to occur in the hippocampus. In previous work we found that TGPAC emerged in a CA1 microcircuit model constructed using detailed multi-compartment cell models with reconstructed 3D dendritic morphologies optimized to match the electro-physiological properties of oriens-lacunosum moleculare cells (OLM), parvalbumin basket cells (PVBC) and pyramidal cells (PYR) and connected in appropriate dendritic domains using facilitating and suppressing synapses with strength and probability matching experimental findings. Here we investigate the frequency dependence and properties of TGPAC in more detail in a larger circuit. We find TGPAC and the theta and gamma frequencies depend on an interaction of specific OLM ion channel properties, in particular calcium activated potassium channels, I-h channel and K-D channels, with network feedback properties and can be modified in experimentally testable ways by changes to the relevant ion channel parameters. We find theta frequency can be varied between 3 and 10 Hz and slow gamma frequency between 20 and 60 Hz. We also investigate how the network responds under various types of external driving input which may occur in cognitive and behavioural tasks, such as spatial alternation. We find PYR cell spiking separates into distinct dynamical activity clusters locked to both theta and gamma rhythms which can inhibit each other via the interneurons and activate at different epochs in different task environments. We also find that both theta and gamma power and theta and gamma frequencies covary in good agreement with empirical observations. We compare our findings with results from the literature in cognitive tasks.

Disclosures: **A.P. Ponzi:** None. **M. Migliore:** None.

Poster

363. Intrinsic Properties and Electrical Synapses

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Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: EU Grant agreement No. 650003 (Human Brain Project SGA3
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Title: An adaptive GLIF model for hippocampal CA1 pyramidal neurons and interneurons

Authors: *A. MARASCO[1], E. SPERA[2], V. DE FALCO[4], A. IUORIO[5], C. A. LUPASCU[3], M. MIGLIORE[6];
[1]Univ. of Naples Federico II, Univ. of Naples Federico II, Napoli, Italy; [2]Inst. of Biophysics, [3]CNR, Palermo, Italy; [4]Scuola Superiore Meridionale, Naples, Italy; [5]Univ. of Vienna, Univ. of Vienna, Vienna, Austria; [6]Natl. Res. Council, Natl. Res. Council, Palermo, Italy

Abstract: Hippocampal CA1 pyramidal neurons and interneurons exhibit complex and highly variable firing dynamics, including adapting, non-adapting and bursting, that play a key role in modulating the dynamics of the network to which they belong. These patterns can be successfully described by morphologically and biophysically realistic conductance-based models [1], but they cannot be easily reproduced by point neuron models. This is an important issue, because very full-scale networks aiming at modeling multiple brain regions must be implemented using simplified neurons due to the current technical limitations of supercomputer systems. A class of models which achieve a reasonable compromise between model complexity, biological plausibility, and computational efficiency is given by *generalized leaky integrate-and fire* (GLIF) models [2,3]. However, GLIF model introduced in [2] well describe cerebellar firing patterns but cannot adequately capture firing properties (spike times and number) under somatic current injection of hippocampal neurons, whereas the models proposed in [3] require a special set of experimental protocols that cannot be routinely carried out in a laboratory. For these reasons, we have developed an *adaptive generalized leaky integrate-and-fire* (A-GLIF) model for CA1 pyramidal neurons and interneurons in which the nonlinear nature of the firing dynamics was successfully reproduced by linear ordinary equations as in [2] but equipped with nonlinear and more realistic update conditions at spike event, which strictly depends on the external stimulation current. An ad-hoc nondimensionalization procedure, allowed to perform a rigorous analysis of the equilibria stability as well as the monotonicity properties of the membrane potential as a function of the model's parameters. Furthermore, it was possible to determine general constraints on the model parameters to reduce the computational cost of the optimization procedure based on somatic spike times in response to a set of constant currents injections. Moreover, additional mathematical constraints deduced via the experimental firing properties of hippocampal neurons and interneurons (e.g., firing block) allow to quantitatively reproduce and predict the firing behaviours in response to any stimulation protocol using constant currents (including amplitudes that were not used for the optimization), piecewise constant currents, and synaptic inputs. In this framework, we were also able to propose a procedure to create infinite copies of neurons with firing properties statistically indistinguishable from experiments.[1] Migliore et al 2018 [2] Geminiani et al 2018[3] Teeter et al 2018

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Poster

363. Intrinsic Properties and Electrical Synapses

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Program #/Poster #: 363.20

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Title: The intrinsic responsive properties of glutamatergic and GABAergic deep cerebellar nuclear neurons engaged by repetitive stimuli support different encoding and signalling roles.

Authors: *C. M. PEDROARENA;
Systems Neurophysiol., CIN-HIH Univ. of Tübingen, Tubingen, Germany

Abstract: The intrinsic responsive properties of neurons, passive and active together with synaptic properties are critical in setting the diverse spike output that characterizes different neuron types, how they encode information and how they influence their targets. The deep cerebellar nuclei (DCN), the cerebellar output stage, are populated by glutamatergic (Glu) and GABAergic (Gaba) neurons and both neuron types receive the cerebellar cortex output via the inhibitory Purkinje cell (PC) axons. PCs are spontaneously active in awake animals at relatively high frequency (10-100 Hz). To answer how the intrinsic properties of Glu and Gaba neurons contribute to responses to repetitive PC inputs, here using slices from (P21 to P60) wild type and GAD-GFP mice of both sexes maintained at 36 degrees, and WCP recordings in current clamp mode, we investigated the effect of repetitive brief (5 ms), small, hyperpolarizing current pulses delivered at 30 to 70 Hz for hundreds of seconds. Putative Glu neurons responded to the stimulation with a decrease in their spontaneous firing rate that was maximal at the beginning of the pulse delivery but adapted in the course of tens of seconds, e.g. using pulse delivery at 52 Hz the decrease in firing rate adapted to 19 % of the initial change in rate (n= 7). In contrast, putative Gaba neurons showed sustained decreases in their spontaneous firing rate adapting little, e.g. to 79 % of the initial change in rate using 52 Hz stimulation frequency (n= 10). Furthermore, at steady state Gabaergic cells showed lower firing rates with higher stimulation frequencies, while no correlation was detected for Glu cells. Next, as pauses in PC discharge associated to specific behaviours are considered relevant, after neurons reached steady state were introduced pauses lasting 0.05 to 2 s in the pulse stimulation. Pauses resulted in increases in firing rate, which in Glu neurons were above pre-stimulation rate (e.g. 56 % higher than pre-stimulation baseline for pauses introduced during 52 Hz stimulation, n=7), and returned slowly to baseline with a time constant of 10 s (SEM=1.1 s, n=7). In contrast, Gaba neurons responded to stimulation pauses with increases close to the original baseline rate, (8% higher for 52 Hz stimulation (n=10). Thus, the differential DCN Glu vs. Gaba neurons responsive properties, together with previously found slow short-term synaptic depression of PC-DCN Glu neurons synapses, suggest DCN Glu neurons, in contrast to Gaba ones, are unlikely to respond to protracted changes in PC rates with sustained changes in their firing rate. However, the intrinsic properties of both types of neurons seem adequate to signal pauses in PC discharge as short as 50 ms.

Disclosures: C.M. Pedroarena: None.

Poster

363. Intrinsic Properties and Electrical Synapses

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 363.21

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: Vivas AFAR # 66-8790
Vivas MIRA # 62-5709
Weill Neurohub

Title: Aging alters the ion channel composition and excitability of postganglionic sympathetic neurons

Authors: *L. DE LA CRUZ, C. MORENO, O. VIVAS;
Univ. of Washington, Univ. of Washington, Seattle, WA

Abstract: The overactivity of sympathetic nerves is a hallmark of aging. The predominant view in the field is that this overactivity is caused by alterations in the central sympathetic nuclei localized in the brain. We challenged this view and evaluated if the intrinsic electrical properties of postganglionic sympathetic neurons, localized outside the central nervous system, are altered with age. We used the superior cervical ganglion as a model for sympathetic ganglia, and compared the electrophysiological properties of isolated neurons from young adult (12 weeks), middle-aged (64 weeks), and old (115 weeks) mice. We measured the membrane potential and ionic currents by current- and voltage-clamp 12 h after isolation. We observed that 40% of middle-aged and 60% of old neurons fire spontaneously, while only 3% of young neurons fire action potentials. The spontaneous firing in middle-aged and old neurons was associated with a more depolarizing resting potential, with no difference in the input resistance. We then evaluated the evoked activity by stimulating with increasing current injections. Supporting the idea that old neurons are more excitable, both middle-aged and old neurons fired more action potentials with each current injection. Also, they needed less current injection to elicit at least one action potential. Furthermore, we tested the hypothesis that an age-driven alteration in ionic currents produced this increase in excitability. Given the well-recognized role of KCNQ2/3 channels in controlling the resting membrane potential and firing properties of sympathetic neurons, we compared KCNQ2/3 current density at different ages. We found that this current decreased with age. Interestingly, sodium currents did not change, suggesting that not all ion channels are altered in the same manner by age. Finally, we evaluated whether rapamycin, a treatment that is known to increase lifespan and healthspan, can reverse the age-related alterations of postganglionic sympathetic neurons from middle-aged mice. We found that rapamycin treatment reversed some of the functional characteristics of sympathetic excitability, including changes in KCNQ2/3 current density, supporting the idea that a general phenomenon related with aging leads to the dysfunction of the sympathetic postganglionic neurons. In conclusion, we propose that aging alters a specific set of ion channels that renders postganglionic sympathetic neurons hyperexcitable. This work is supported by Vivas AFAR # 66-8790 and Vivas MIRA # 62-5709. Lizbeth de la Cruz is a Weill Neurohub postdoctoral fellow.

Disclosures: **L. de la Cruz:** None. **C. Moreno:** None. **O. Vivas:** None.

Poster

363. Intrinsic Properties and Electrical Synapses

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 363.22

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NSF IOS 1755098
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Alfried Krupp Senior Fellowship

Title: Modulation of electrical synaptic transmission by the hyperpolarization-activated inward current I_h

Authors: *W. STEIN[1,3], M. DEMAEGD[4], L. Y. BRAUN[1], A. G. VIDAL-GADEA[1], A. L. HARRIS[2], C. STAEDELE[5];
[1]Sch. of Biol. Sci., [2]Dept. of Physics, Illinois State Univ., Normal, IL; [3]Alfried Krupp Kolleg, Greifswald, Germany; [4]Ctr. for Neural Sci., New York Univ., New York, NY; [5]Inst. for Neuro- and Sensory Physiol., Univ. Med. Ctr. Göttingen, Göttingen, Germany

Abstract: Electrical synapses are ubiquitous throughout the nervous system and have been described in many vertebrate and invertebrate systems. Current flow at electrical synapses is subject to alterations by a host of abiotic factors and nervous system-intrinsic modulators. These alterations can either be mediated through direct actions on the gap junction proteins, or indirectly through interactions with other electrical processes in neurons, such as voltage-gated ion channels. However, electrical synapses and voltage-gated ionic currents are often studied independently from one another, and our understanding of such interactions, and their consequences for the electrical behavior of the synapse, is still in its infancy. We used a voltage-dependent electrical synapse between a descending modulatory projection neuron (MCN1) and a motor neuron (LG) in the crustacean stomatogastric ganglion to study the interaction of the hyperpolarization-activated inward current (I_h) and electrical synaptic transmission. We found that I_h is critical to the function of the electrical synapse. When we blocked I_h with CsCl, the apparent voltage dependence of the electrical synapse shifted by 18.7 mV to more hyperpolarized voltages. This placed the dynamic range of the electrical synapse outside of the range of voltages used by the LG motor neuron (-60.2 mV - -44.9 mV). Dual electrode current- and voltage-clamp recordings show that this voltage shift was not due to a change in the properties of the gap junction itself. Instead, it was the result of a sustained effect of I_h on the presynaptic MCN1 axon terminal membrane potential. I_h-induced depolarization of the axon terminal membrane potential increased the electrical postsynaptic potentials and currents. With I_h

present, the axon terminal resting membrane potential depolarized, shifting the dynamic range of the electrical synapse towards the functional range of the motor neuron. Thus, we demonstrate that the function of an electrical synapse is critically influenced by a voltage-dependent ionic current (I_h).

Disclosures: W. Stein: None. **M. DeMaegd:** None. **L.Y. Braun:** None. **A.G. Vidal-Gadea:** None. **A.L. Harris:** None. **C. Staedele:** None.

Poster

363. Intrinsic Properties and Electrical Synapses

Location: SDCC Halls B-H

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Program #/Poster #: 363.23

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

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European Research Council Advanced Grant (TOGETHER)
Knut och Alice Wallenbergs Stiftelse
Internal funds from Stockholm University

Title: Connexin subunit mosaicism as a potential mechanism for rectification in electrical coupling between tuberoinfundibular dopamine (TIDA) neurons in the rat.

Authors: *A. MANTZAFOU, C. BROBERGER;
Stockholm Univ., Stockholm, Sweden

Abstract: Electrical synapses formed by gap junctions are central to the operation of many networks in the mammalian CNS. Gap junctions allow for the synchronization of network activity, the bidirectional exchange of charge and metabolites such as cAMP, and, as recently shown (Stagkourakis et al., 2018, eLife), electrical coupling can be a determining factor of oscillation frequency in rhythmically active networks. This latter effect in turn can determine the behavioural outcome of network activity. In the rat hypothalamic tuberoinfundibular dopamine (TIDA) neurons, strong electrical interneuronal coupling ultimately impacts the parental phenotype of this species (Stagkourakis et al., 2020, Cell). TIDA neurons control pituitary release of prolactin, from the anterior pituitary, a hormone that has a powerful effect on parental care in both females and males.
Notably, however, while bidirectional, TIDA-TIDA electrical coupling exhibits substantial asymmetry, such that the coupling coefficient between two coupled cells is different depending on the direction of current, and correlates to which cell leads the other in action potential discharge during depolarized UP states (Stagkourakis and Broberger, unpublished). Directional rectification has been described in several gap junction-connected systems. While asymmetry can result from differences in the intrinsic electrical properties of coupled cells, an alternative (not mutually exclusive) explanation may be the molecular composition of gap junction hemichannels. Here, we applied RNAscope™ *in situ* hybridization to determine the expression

patterns of the main gap junction forming- proteins in mammals, the connexin (Cx) family, in TIDA neurons. Experiments were performed on coronal sections of P21-28 male rat brain. The majority of TIDA neurons (tyrosine hydroxylase-positive in the dorsomedial arcuate nucleus) expressed Connexin-36 (Cx36) (93%, 84 cells from 8 animals), the main Cx isoform of neuronal cell types in rodents. Interestingly, a subset of cells (24%) showed colocalization with Cx43 mRNA, an isoform traditionally thought not to be expressed in neurons. Moreover, a typical pattern of a few TIDA (2-5) adjacent to a single Cx43-positive one was observed, suggesting the possibility that small asymmetrical TIDA clusters exist.
These results support the idea of an extensively coupled network maintaining network-wide oscillations, with Cx36 as the primary junctional protein and sites of mosaic synapses adding functional asymmetry. Electrophysiological studies are underway to determine the functional implications of this connectivity architecture on the cell and network level.

Disclosures: **A. Mantzafou:** None. **C. Broberger:** None.

Poster

363. Intrinsic Properties and Electrical Synapses

Location: SDCC Halls B-H

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Program #/Poster #: 363.24

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NICHD F32HD102182
NINDS R01NS105758

Title: Neurobeachin regulates the asymmetric subcellular distribution of electrical synapse proteins

Authors: *E. MARTIN, J. C. MICHEL, J. S. KISSINGER, A. C. MILLER;
Univ. of Oregon, Eugene, OR

Abstract: Brain development relies upon neurons making precisely organized and highly specialized synaptic connections. These synapses are diverse with varying molecular compositions determining their differential form and function. Like chemical synapses, electrical synapses are constructed from an asymmetric assortment of cell adhesion, scaffolding, and regulatory molecules which compose their pre- and postsynaptic compartments, yet little is known about how these molecules localize to their specific subcellular locations. In addition, electrical and chemical synapses are thought to be biochemically distinct, however, the autism- and epilepsy-associated gene Neurobeachin is required for both electrical and chemical synaptogenesis. Previous studies indicate Neurobeachin controls chemical synapse receptor trafficking, but it is unknown how it regulates electrical synapse formation. Here we investigated the relationships between Neurobeachin, the neuronal gap-junction-channel forming Connexins, and the electrical synapse scaffold ZO1. Using the zebrafish Mauthner electrical synapses, we find that Neurobeachin localizes to the electrical synapse independent of ZO1 and Connexins.

We demonstrate that Neurobeachin functions postsynaptically where it is required for the robust localization of ZO1 and Connexins. We show that Neurobeachin binds ZO1 but not Connexin proteins. Finally, we find that Neurobeachin is required to restrict postsynaptic electrical synapse proteins to dendritic synapses. At nearby chemical synapses, Neurobeachin is required for receptor localization, suggesting Neurobeachin regulates specific subcellular targeting of electrical and chemical synapse proteins to the dendrite. Together, this work provides a cell biological mechanism to explain the molecularly asymmetric development of the electrical synapse and reveals a new frontier in the shared biochemistries between electrical and chemical synapses.

Disclosures: **E. Martin:** None. **J.C. Michel:** None. **J.S. Kissinger:** None. **A.C. Miller:** None.

Poster

363. Intrinsic Properties and Electrical Synapses

Location: SDCC Halls B-H

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Program #/Poster #: 363.25

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Title: Colocalization of connexin and synaptopodin at the axon initial segment in adult mammalian projection neurons

Authors: *J. M. JANSSEN[1,2], **G. FUSS**[2], F. PELLETIER[3], C. LETERRIER[3], M. ENGELHARDT[1,2];
[1]Inst. of Anat. and Cell Biology, Med. Faculty, Johannes Kepler Univ., Linz, Austria; [2]Inst. of Neuroanatomy, Mannheim Ctr. for Translational Neurosci. (MCTN), Med. Fac. Mannheim, Heidelberg Univ., Mannheim, Germany; [3]Aix Marseille Université, CNRS, INP UMR7051, NeuroCyto, Marseille, France

Abstract: The axon initial segment (AIS) is an important microdomain for action potential generation which is involved in modulating neuronal activity when network states change. There is physiological evidence that electrical synapses connecting to the AIS could serve as a fundamental circuit element for neuronal communication and that direct electrical coupling between axonal domains would be crucial to certain forms of high-frequency network oscillations.
A recent study showed clusters of connexin, the building blocks of electrical synapses, at pyramidal AIS, however, their subcellular composition, potential contact points and developmental profile remain unknown. Investigating the subcellular composition of M1 pyramidal neuron AIS we not only found that connexin puncta indeed appear at the majority of these AIS, but they also colocalize with synaptopodin, an actin-binding protein essential for the formation of the cisternal organelle, an intra-axonal Ca2+ store.
Using multi-channel immunofluorescence, confocal microscopy and 3D reconstruction, we show that 86% of all pyramidal neurons investigated exhibit connexin43 (Cx43) expression at the AIS in direct proximity to synaptopodin-positive structures. Cx43 is known to be expressed by

astrocytes which indicates potential glia/neuron interactions at the AIS. To further investigate the subcellular distribution of connexins, protein expansion microscopy (ProExM) and 3D-PAINT superresolution microscopy were used to visualize nanoscale structural interactions between synaptopodin-positive cisternal organelles and axonal structures immunoreactive for Cx43, Cx36 and Cx45.
Our future directive will be to determine whether the observed connexin staining refers to actual gap junctions and to understand their functional role within neuronal assemblies and the axonal microdomain.

Disclosures: **J.M. Janssen:** None. **G. Fuss:** None. **F. Pelletier:** None. **C. Leterrier:** None. **M. Engelhardt:** None.

Poster

364. Epilepsy: Seizure Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 364.01

Topic: B.08. Epilepsy

Support: NIH Grant R37 NS119012
NIH Grant R01NS120945
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the UVA Brain Institute

Title: Glycolysis regulates neuronal excitability via lactate receptor HCA_1R

Authors: *D. SKWARZYNSKA[1], H. SUN[1], J. WILLIAMSON[1], I. KASPRZAK[1], J. KAPUR[2]; [1]Univ. of Virginia, Charlottesville, VA; [2]Dept Neurol., Univ. Virginai Hlth. Sci. Ctr., Charlottesvle, VA

Abstract: Neuronal excitability and brain metabolism are linked via factors such as adenosine and ATP. We demonstrate that glycolytic by-product, lactate rapidly reduces neuronal excitability during metabolic stress via its receptor, hydroxycarboxylic acid receptor type 1 (HCA_1R). Epileptic seizures increase glycolytic rate. We investigated the real-time fluctuations in extracellular lactate concentration *in vivo* during brief (<20sec) and prolonged (>5min) seizures using a lactate probe with EEG monitoring. We induced prolonged and kindled seizures in HCA1R KO and littermate wild-type (WT) mice to study the role of HCA_1R during metabolic stress. We investigated the effect of activation of HCA_1R on CA1 principal neurons *in vitro* by using GCaMP7. Active and passive neuronal membrane properties from CA1 neurons were assessed using patch-clamp electrophysiology. Spontaneous excitatory postsynaptic currents (sEPSCs) were recorded in a voltage clamp. Evoked excitatory postsynaptic currents (eEPSCs) were recorded in response to stimulation of Schaffer collateral axons. Extracellular lactate concentration rose quickly during brief and prolonged seizures reaching mM concentration rage (0.777mM, n=6, p=0.02; 0.957mM, n=5, p=0.03, *t-test*, respectively), which is sufficient to

activate HCA_1R. HCA_1R KO mice were more susceptible to developing kindled (WT n=7; KO n=6; p=0.02, Fisher's test) and prolonged seizures (WT n=16; KO n=9; p=0.02, Fisher's test). Moreover, HCA_1R KO mice developed longer (n=9; p=0.03, Kaplan-Meier survival comparison) and more severe seizures (n=6; p<0.0001, 2-tailed Mann-Whitney test) than WT mice. In GCaMP7 imaging, lactate decreased excitability of CA1 neurons (n=4 p<0.0001, One-Way ANOVA). HCA_1R agonist, 3Cl-HBA, reduced the firing of CA1 neurons in HCA_1R WT but not in KO mice (n=4, p<0.0001, *t-test*). In patch-clamp recordings, both lactate (n=9 control; n=5 lactate; p=0.04, unpaired *t-test*) and 3CL-HBA (n=12 cells; p=0.04, *t-test*) hyperpolarized membrane potential of CA1 neurons. HCA_1R activation reduced the sEPSC frequency (WT n=6 cells, p=0.04; KO n=7 cells, p=0.84, *t-test*) and altered the paired-pulse ratio of eEPSCs in HCA1R WT but not in KO mice, suggesting that it reduces glutamate release from the presynaptic terminals. Overall, our studies demonstrate that excessive neuronal activity accelerates glycolysis to generate lactate, which translocates to the extracellular space to slow neuronal firing and inhibit excitatory transmission via HCA_1R. These studies may identify novel anticonvulsant target and seizure termination mechanisms.

Disclosures: D. Skwarzynska: None. **H. Sun:** None. **J. Williamson:** None. **I. Kasprzak:** None. **J. Kapur:** None.

Poster

364. Epilepsy: Seizure Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 364.02

Topic: B.08. Epilepsy

Support: UNAM Grant FM/DI/096/2019

Title: Insulin effect on epileptiform activity in prefrontal cortex.

Authors: *J. RAMÍREZ-SÁNCHEZ, A. MONDRAGON GARCIA, N. VILLALOBOS VÁSQUEZ, J. GARDUÑO TORRES, S. HERNÁNDEZ LÓPEZ;
Fisiología, Univ. Nacional Autónoma De México, CDMX, Mexico

Abstract: Accumulating evidence indicates that insulin-mediated signaling in the brain plays an important role regulating neuronal functions. Alterations of insulin signaling are associated with the development of neurological disorders including Alzheimer's disease and Parkinson's disease. Also, hyperglycaemia and insulin resistance have been associated with seizure activity and brain injury. In a recent work, we found that insulin increased inhibitory $GABA_A$-mediated tonic currents in prefrontal cortex (PFC) possibly by promoting the trafficking of extrasynaptic $GABA_A$ receptors from the cytoplasm to the cell membrane. In this work, we used local field potential recordings and calcium imaging to investigate the effect of insulin on seizure-like activity in PFC slices. Seizure-like events (SLEs) were induced by perfusing the slices with magnesium free ACSF containing the proconvulsive compound 4-aminopyridine (4-AP). We

found that insulin decreased the frequency, the amplitude, and the duration of the (SLEs) as well as the synchronic activity of PFC neurons evoked by 4-AP. These insulin effects were mediated by the PI3K/Akt signaling pathway and blocked by gaboxadol (THIP), a δ $GABA_A$ receptor agonist. Our results suggest that insulin reduces the neuronal excitability by an increase of GABAergic tonic currents. These actions could help to explain the alterations of cognitive processes associated with changes in insulin signaling.

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Poster

364. Epilepsy: Seizure Mechanisms

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Program #/Poster #: 364.03

Topic: B.08. Epilepsy

Support: NINDS Grant NS102609-01A1

Title: Layer- and cell type- specific cortical circuit deficits underlying the absence epilepsy

Authors: *S. SONG[1], A. MAHESHWARI[1], J. L. NOEBELS[1], X. JIANG[2];
[1]Baylor Col. of Med., [2]Neurosci., Baylor Col. of Med., Houston, TX

Abstract: Absence seizure is a special type of childhood epilepsy featured by generalized spike-wave (SW) EEG. While earlier studies attribute the origin of SW discharges of absence seizure to the thalamus, there is now growing evidence supporting the somatosensory cortex (S1) as its focal onset zone. To provide new insights into the minimal circuit elements necessary for the induction of SW discharge, we take advantage of a monogenic mouse model of absence epilepsy, stargazer (stg), to examine what circuit defects are developed in the S1 as a result of mutations in Cacng2, a gene encoding an AMPA receptor trafficking protein stargazin. We performed a comprehensive circuit mapping using multi-cell patch recordings that examined all the major cell types in the S1 from stg mice. Our pan-circuit mapping pinpointed a dormant state of deep layer PV+ interneurons in S1 as a result of the disruption of excitatory drive to these interneurons in stg. This layer- and cell type-specific defect is consistent with the restriction of Cacng2 expression to deep layer PV+ interneurons, and thus is primary and occurs independently of seizure stages. In contrast, the connectivity changes of SST+ interneurons in stg were secondary and occurred only when seizures manifested in this model. These layer- and cell type-specific, seizure-stage-related disruptions of the cortical circuit as a result of single-gene mutations not only provide new insight on circuit mechanisms underlying absence seizure, but also help to direct the field toward the development of circuit-based interventions for absence epilepsy and its related comorbidities.

Disclosures: S. Song: None. **A. Maheshwari:** None. **J.L. Noebels:** None. **X. Jiang:** None.

Poster

364. Epilepsy: Seizure Mechanisms

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Program #/Poster #: 364.04

Topic: B.08. Epilepsy

Support: NIH RO1 NS103090

Title: Aberrant Parvalbumin-Positive Interneuron Activity in SCN8A Epileptic Encephalopathy

Authors: *R. MIRALLES, A. BOSCIA, M. K. PATEL;
Univ. of Virginia Neurosci. Program, Charlottesville, VA

Abstract: *SCN8A* epileptic encephalopathy (EE) is a severe epilepsy syndrome resulting from *de novo* gain-of-function mutations in the voltage-gated sodium channel $Na_v1.6$, encoded by the gene *SCN8A*. The sodium channel $Na_v1.6$ is expressed in both excitatory and inhibitory neurons, and the function of inhibitory interneurons is critical to constrain activity of excitatory neurons. Inhibitory interneuron dysfunction has been linked to various genetic epilepsy syndromes, indicating that their characterization is vital in *SCN8A* EE. Parvalbumin-positive (PV) interneurons, which express $Na_v1.6$ and are critical for balancing network excitability, have yet to be studied in the context of *SCN8A* EE. To assess the role of PV interneurons within *SCN8A* EE, we used two patient-derived mouse models of *SCN8A* epileptic encephalopathy, *Scn8a*$^{D/+}$, where the *SCN8A* mutation N1768D is expressed globally, and *Scn8a*$^{W/+}$-PV, where the *SCN8A* mutation R1872W is selectively expressed in PV interneurons. We performed whole-cell patch clamp electrophysiology experiments to assess membrane properties and cell excitability of PV interneurons in wild-type (WT), *Scn8a*$^{D/+}$, and *Scn8a*$^{W/+}$-PV mice. Our results indicate that PV interneurons in *Scn8a*$^{D/+}$ and *Scn8a*$^{W/+}$-PV mice are initially hyperexcitable. Despite initial hyperexcitability, PV interneurons in *Scn8a*$^{D/+}$ and *Scn8a*$^{W/+}$-PV mice experience premature depolarization block, a state of action potential failure, leading to potential overall inhibitory hypoexcitability in these mouse models of *SCN8A* EE. Furthermore, we assessed spontaneous seizure susceptibility in *Scn8a*$^{W/+}$-PV mice via simultaneous video and EEG recording. Expression of the *SCN8A* mutation R1872W solely in PV interneurons led to the development of spontaneous seizures in the *Scn8a*$^{W/+}$-PV mice. Together, our data indicate that failure of PV interneuron spiking via depolarization block may elicit a disruption within the inhibitory network in *SCN8A* EE, leading to potential unchecked excitation. Further understanding the contributions of PV interneurons in *SCN8A* EE is critical to recognizing the physiological consequences of increased $Na_v1.6$ activity, particularly when developing mechanistically-based treatments.

Disclosures: R. Miralles: None. **A. Boscia:** None. **M.K. Patel:** None.

Poster

364. Epilepsy: Seizure Mechanisms

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Program #/Poster #: 364.05

Topic: B.08. Epilepsy

Title: Dysfunctional GABAergic inhibition in genetic absence epilepsy; underlying mechanisms for seizure generation.

Authors: *B. LEITCH[1], S. PANTHI[1], M. HASSAN[1], N. LYONS[1], D. PEIRIS[1], A. SU[2], N. ADOTEVI[3];
[1]Univ. of Otago, Dunedin, New Zealand; [2]Univ. of Auckland, Auckland, New Zealand; [3]Univ. of Virginia, Charlottesville, VA

Abstract: Childhood absence epilepsy is a generalized non-convulsive epilepsy with a multifactorial genetic aetiology. The hallmark spike-wave discharges seen in EEGs during absence seizures arise from disturbances within the cortico-thalamo-cortical (CTC) network. However, the cellular and molecular mechanisms underlying seizure generation in patients from different genetic backgrounds are not fully understood. Current antiepileptic drugs fail to suppress seizures or induce intolerable side-effects in over 30% patients. Hence, there is a need to decipher causative mechanisms for more patient specific treatment strategies. We previously demonstrated that absence seizures are associated with dysfunctional feedforward-inhibition (FFI) within CTC microcircuits in the stargazer mouse model of absence epilepsy. The aim of the current study was to identify GABAergic neurochemical changes within the CTC network. High performance liquid chromatography, quantitative western blotting and immunocytochemistry were used to investigate the expression levels of GABA, its synthesizing enzymes (GAD65 & 67) and transport proteins (GAT1 & 3) in epileptic stargazer compared to non-epileptic littermates. Global expression levels of GABA and GAD65 were increased in the somatosensory cortex but reduced in the thalamus, whereas glutamate levels were unchanged. Interestingly, GABA was reduced in parvalbumin positive FFI interneuron terminals in both the cortex and thalamus. The increase in total GABA levels in the cortex was not due to an increase in branching of inhibitory neurons during development; and thus proportionally more GABAergic terminals within the neuropil of the epileptic mouse. Collectively, these data indicate that loss of FFI is associated with reduced presynaptic GABA expression in FFI microcircuits. However, global expression levels of GABA exhibit regional specific changes; levels of GABA and GAD65 are increased specifically in the somatosensory cortex. This may represent a compensatory upregulation of GABA in other interneurons or extracellularly in response to altered excitatory/inhibitory balance.

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Poster

364. Epilepsy: Seizure Mechanisms

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Topic: B.08. Epilepsy

Support: NIH R01 NS112500

Title: Cerebellar Purkinje cell firing deficits in the Scn1b knockout mouse model of the epileptic encephalopathy Dravet syndrome

Authors: F. I. GUILLEN[1], *M. A. HOWARD[2];
[1]Univ. of Texas At Austin, Dept. of Neurosci., Austin, TX; [2]UNIVERSITY OF TEXAS AT AUSTIN, UNIVERSITY OF TEXAS AT AUSTIN, Austin, TX

Abstract: β subunits, often called auxiliary subunits, play key roles in gating, trafficking, anchoring, and plasticity of voltage-gated ion channels. Despite not containing the pore-forming aspect of the ion channel, β subunits are necessary for normal synaptic integration and generation of action potentials. β1, encoded by *SCN1B,* is most known for interacting with $Na_v1.1$. β1 also modulates other voltage-gated Na+ and K+ channels, and possibly other ion channels, and has ion channel-independent roles in development. *SCN1B* mutations are linked to the GEFS+ (generalized epilepsy with febrile seizures plus) spectrum of epileptic encephalopathies. Severe GEFS+ syndromes, such as Dravet syndrome (DS), are characterized by medically intractable seizures and cognitive, social and/or sensorimotor dysfunction. DS patients also tend to have severe movement disorders, low muscle tone, abnormal gait, and incoordination (ataxia), even when seizures are medically controlled. The cerebellum is a major controller of movement and cognition, is involved in some epilepsy disorders, and shows high expression of *SCN1B* in both Purkinje and granule cells, but its role in DS and GEFS+ is not known. Our goal is to determine the role of β1 in cerebellar Purkinje cells (PCs). Here, we used whole-cell current clamp recordings of PCs in acute cerebellar slices from male and female *Scn1b* knockout (KO) and wild-type (WT) littermate mice, aged P15-19. We found that *Scn1b* KO PCs have decreased excitability compared to WT. While spontaneous firing rates were similar between WT and KO PCs, KO PCs fired fewer action potentials with increasing depolarizing current injections, both from resting membrane potential, and when starting from hyperpolarized membrane potentials to eliminate spontaneous activity. Our findings of PC hypoexcitability are similar to reported decreased firing in *Scn1b* null cerebellar granule cells, and are in contrast to our and other's findings of hyperexcitability in *Scn1b* mutant hippocampal and cortical pyramidal neurons. Thus, β1 plays an important role in PC excitability and can modulate neuronal excitability in either direction. PC hypoexcitability may be an important mechanism underlying motor and cognitive deficits, and potentially seizures, in DS and GEFS+.

Disclosures: F.I. Guillen: None. **M.A. Howard:** None.

Poster

364. Epilepsy: Seizure Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 364.07

Topic: B.08. Epilepsy

Support: NIH/NINDS Grant F31NS125955
NIH/NINDS Grant R01NS095842

Title: The effect of amygdala kindling and seizures on dorsal raphe nucleus 5-HT neuron activity and CO_2-induced arousal in mice

Authors: *K. G. JOYAL, M. A. WEBER, G. F. BUCHANAN;
Neurol., Univ. of Iowa, Iowa City, IA

Abstract: Sudden unexpected death in epilepsy (SUDEP) is the leading cause of death in patients with refractory epilepsy. While the exact etiology of SUDEP is unknown, dysregulation of the serotonin (5-HT) system has been linked to these untimely deaths. 5-HT plays a critical role in sleep-wake regulation and arousal. Impaired postictal arousal occurs after seizures and is considered a risk factor for SUDEP. Most cases of SUDEP occur at night with the victim found prone in bed. This implies that the victim was unable to arouse, despite the increasing carbon dioxide (CO_2) levels which would normally trigger a protective arousal reflex. This reflex is mediated by 5-HT neurons in the dorsal raphe nucleus (DRN). We hypothesized that seizures and epilepsy itself dysregulate activity of DRN 5-HT neurons and contributes to loss of postictal arousal and SUDEP risk. Adult (8-12 wks) male and female *Pet1-cre* mice were injected with an AAV vector allowing Cre-dependent expression of a calcium indicator (pAAV.Syn.Flex.GCaMP6s.WPRE.SV40) into the DRN [-4.6, ML: 0.0, DV: -1.8] with a borosilicate optical cannula implanted into the same region. The animals were also instrumented for EEG/EMG recording and implanted with a bipolar stimulating/recording electrode into the basolateral amygdala [AP: -1.3 mm; ML: -2.8 mm; DV: -4.7 mm]. Following recovery, animals underwent afterdischarge threshold determination and amygdala kindling (80-380 mA; 1 s train of 1 msec biphasic square waves; 60 Hz; 2x/day). Animals were then exposed to 7% CO_2 or room air at 0 s, 30 s and 100 s following the termination of a kindled seizure while fiber photometry was used to measure DRN 5-HT neuron activation. Despite previous literature suggesting a decrease in DRN 5-HT neuron activity during a seizure, we saw a large increase in DRN 5-HT activation immediately upon seizure induction that quickly fell back to baseline at the start of post-ictal generalized EEG suppression. When the animal was exposed to CO_2 following a seizure, there was an oscillatory pattern of DRN 5-HT neuron activation beginning 120-150 s following seizure termination, regardless of the timing of the CO_2 exposure. These results suggest that a spike in DRN 5-HT activity during a seizure leads to a refractory period whereby these chemosensitive neurons are not able to respond to a CO_2 stimulus. This may the first step in uncovering the neuronal circuitry involved in suppressing the hypercapnic arousal response following seizures and implicates a potential therapeutic target for those at highest risk for SUDEP.

Disclosures: K.G. Joyal: None. **M.A. Weber:** None. **G.F. Buchanan:** None.

Poster

364. Epilepsy: Seizure Mechanisms

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Topic: B.08. Epilepsy

Support: The Regents of the University of California A19-3376-S006, A22-2853-S006

Title: Human de novo mutations in ppp2r5d increase neuronal excitability

Authors: *A. MAYER[1], A. GANGULY[2], R. TADAVARTY[2], K. FOLEY[1], S. STRACK[3], H. XIA[2];
[1]Neurosci., [2]Pharmacol. and Physiol., Univ. of Rochester, Rochester, NY; [3]Neurosci. and Pharmacol., Univ. of Iowa, Iowa City, IA

Abstract: Human *de novo* mutations in the gene encoding PPP2R5D, a regulatory subunit of protein phosphatase 2A (PP2A), results in PPP2R5D-related intellectual disability. While all human patients experience intellectual developmental delay, a subset develop epilepsy; however, the cellular mechanisms underlying epileptic activity are unknown. PP2A is a serine/threonine phosphatase responsible for approximately half of all serine/threonine dephosphorylation events in mammalian cells. PP2A holoenzyme is composed of a scaffolding “A” subunit, a regulatory “B” subunit, and a catalytic “C” subunit. While there are few A and C subunits, there are many B subunits to regulate PP2A physiological function. Understanding the cellular processes PPP2R5D targets PP2A to, and determining how mutations in PPP2R5D alter the holoenzyme function is critical for elucidating the cellular mechanisms underlying epilepsy in patients. Heterozygous knock-in mouse models ($PPP2R5D^{E198K/+}$ and $PPP2R5D^{E420K/+}$) P28 to P35 were used to study the effects of PPP2R5D mutations on neuronal properties. We first used electrophysiological recordings of CA1 pyramidal neurons to assess neuronal excitability. A hallmark of epilepsy models is increased neuronal excitation versus inhibition. Consistent with this, stimulation of Shaffer-Collaterals resulted in a significant increase in the excitation to inhibition ratio in the CA1 pyramidal neurons of $PPP2R5D^{E420K/+}$ mice compared to WT littermates (n=3, *t*-test). We used current clamp experiments to measure action potential (AP) firing directly. Experiments revealed an increase in AP firing frequency and a decrease in AP firing threshold in $PPP2R5D^{E198K/+}$ and $PPP2R5D^{E420K/+}$ mice (n=2, ANOVA, *t*-test). Repeating experiments with AMPA, NMDA, and $GABA_A$ receptor antagonists (NBQX, APV, and picrotoxin (PTX), respectively) reduced the excitability difference between WT and mutant neurons; however, mutant neurons still had enhance excitability (n=3, ANOVA). The change in excitability with postsynaptic receptor antagonists suggests mutations in PPP2R5D alter synaptic transmission. To investigate changes in excitatory synaptic transmission, miniature excitatory postsynaptic potentials (mEPSCs) were recorded from CA1 pyramidal neurons. There was no change in mEPSC amplitude or frequency suggesting PPP2R5D mutations do not alter glutamatergic synaptic transmission, but rather have an effect on GABAergic transmission. Not mutual exclusively, APV, NBQX and PTX did not completely reduce the enhanced AP firing in mutant mouse models, suggesting PPP2R5D mutations could affect channels important for intrinsic membrane excitability.

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Poster

364. Epilepsy: Seizure Mechanisms

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Program #/Poster #: 364.09

Topic: B.08. Epilepsy

Support: EC Graphene Flagship 881603

Title: Application of Graphene micro-transistor arrays to investigate the interactions between infraslow oscillations, DC potential shifts, and higher frequency activity in rodent models of seizures and epilepsy.

Authors: *R. C. WYKES[1,3], D. RATHORE[2], N. K. CODADU[1], A. BONACIINI-CALI[4], E. MASIVIDAL-CODINA[4], A. SMITH[5], Y. TIMOFEEVA[6], E. FERNADEZ[7], K. E. VOLYNSKI[1], J. A. GARRIDO[4], A. GUIMERÀ-BRUNET[7];
[1]Exptl. Epilepsy, [2]Dept. of Clin. and Exptl. Epilepsy, UCL Queen Square Inst. of Neurol., London, United Kingdom; [3]Nanomedicine Lab., Univ. of Manchester, Manchester, United Kingdom; [4]Catalan Inst. of Nanoscience and Nanotechnology (ICN2), Barcelona, Spain; [5]Dept. of Computer Sci., [6]Univ. of Warwick, Coventry, United Kingdom; [7]Inst. de Microelectrònica de Barcelona, IMB-CNM (CSIC), Barcelona, Spain

Abstract: There is a need to examine brain signals outside traditional EEG bands (0.3-80Hz), as these regimes contain useful electrographic biomarkers for neurological disorders, in particular epilepsy. These include high gamma (80-200Hz), ripples and high frequency oscillations (HFOs) (200-500Hz); as well as infraslow oscillations (ISO < 0.1Hz) and ultraslow potential shifts (UPS). ISO and UPS have remained poorly explored. This is due to technical difficulties electrographically recording such slow potentials which require DC-coupled amplifiers and highly stable electrodes. However, UPS include clinically relevant events including pre-seizure DC-shifts (1-3mV), and large (tens of millivolt) spreading depolarisations (SD) which have been reported to either trigger seizures, terminate seizures, or contribute to Sudden Unexplained Death in Epilepsy (SUDEP).To overcome the technical limitations of metal-based micro-electrodes, we use either epicortical or intracortical arrays of graphene micro-transistor arrays (gSGFETs), capable of wide bandwidth, high fidelity DC-coupled recordings, to investigate the contribution of ISO and UPS to seizure dynamics in awake brain.Chronic implantation of gSGFET probes into the somatosensory cortex of a rat model of absence epilepsy revealed that spontaneous spike-and-wave discharges (5-9Hz) were phase-coupled to an infraslow oscillation (ISO <0.1Hz) most prominent in the superficial layers, suggesting that these ISOs open susceptibility windows for seizure initiation.In both chemoconvulant-induced seizures, and spontaneous seizures from rodent models of chronic epilepsy, we can record both pre and post seizure DC shifts. We report a layer-specific localisation of ‘active’ DC shifts preceding seizures. Active DC shifts provide an

electrophysiological biomarker for seizure onset zone localisation and they can further provide a quantifiable parameter to investigate the mechanisms underlying seizure initiation. These signals co-localised with areas of the brain where pHFO's were detected using the same gSGFET array. Around 30% (model dependent) of seizures were followed by a post-ictal SD. To gain further insights into the spatiotemporal interactions between seizures and SDs we use transparent gSGFET arrays in combination with mesoscopic calcium imaging. These experiments indicate a complex pattern, both spatially and temporally in respect to areas of the brain transitioning to seizure and/or SD.We believe that application of gSGFET arrays to epilepsy research will advance our understanding of how slow brain signals influence seizure dynamics.

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Poster

364. Epilepsy: Seizure Mechanisms

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Program #/Poster #: 364.10

Topic: B.08. Epilepsy

Support: CNPq
CAPES
FAPEG
FAPESP

Title: Vascular effects of intermittent apnea in rats with epilepsy induced by electrical amygdala kindling model

Authors: C. QUINTINO[1], P. P. GHAZALE[1], A. B. DE SÁ[1], K. P. GOMES[1], P. P. P. BRAGA[1], E. P. MENDES[1], C. H. DE CASTRO[1], ***D. COLUGNATI**[1], F. SCORZA[2], A. P. PANSANI[1]; [1]Univ. Federal De Goiás, Goiânia, Brazil; [2]Sao Paulo Federal Univ., Sao Paulo, Brazil

Abstract: Epilepsy is the most common chronic neurological disease. Sudden and unexpected death in epilepsy (SUDEP) is the leading cause of death related to epilepsy and has multifactorial causes. Studies show that obstructive apnea is a risk factor for SUDEP. So, we aimed to evaluate the effect of intermittent apnea (IA) on vascular function of rats with epilepsy induced by the electrical amygdala kindling model. Wistar rats were distributed into four groups: Ct (without epilepsy and apnea); IA (without epilepsy and with apnea); Ep (with epilepsy and without apnea) and Ep+IA (whit epilepsy and apnea). To induce epilepsy was used the amygdala kindling model. After total kindling (3 successive generalized tonic-clonic seizures (GTCS)), 5 additional GTCS were induced in the groups with epilepsy. In group Ep+IA 30 minutes after the seizures, the animals were submitted to IA, by inflation and deflation of a tracheal balloon. The IA group was pared to EP+IA The apnea protocol consisted of 30 occlusions/hour with 10 seconds of

duration for 8 hours. That protocol ware repeated for 5 days. After that, the rats were euthanized, the thoracic aorta dissected, and the aortic vascular reactivity tests were performed. The maximal response (Emax) to acetylcholine was decreased in both IA (73,44±2,80%) and Ep (74,49±2,65%) compared to the Ct group (8610±2,83). The $EC50_{log}$ was increased in the IA (-7,38 ±0,12mol/L) and Ep+IA (-7,03±0,12mol/L) groups compared to Ct (-8,06±0,16mol/L) and Ep (-7,81±0,14mol/L) groups. When tested with sodium nitroprusside (SNP) (aortic rings without endothelium), we observed higher relaxation (Emax) in the group IA (94,63±2,15%) compared to the Ct group (87,57±2,18 %) and decreased the $EC50_{log}$ (Ct = -7,92±0,08mol/L; IA = -8,69±0,09mol/L). Also, the $EC50_{log}$ in the EP group (-9,21±0,13mol/L) was decreased compared to Ct group and EP+IA (-8,66±0,08 mol/L). In the contraction test with endothelium using phenylephrine (PHN), the Emax of the group Ep+IA (109,70±6,00%) was decreased compared to the Ep group (143,70±8,04). And without endothelium Emax was decreased in the Ep+IA (117,90±4,21%) group compared to Ep (148,00±3,86%) and Ct group (146,20±4,67%). In addition, the $EC50_{log}$ was reduced only in the Ep+IA group (-6,66±0,08mol/L). Our results suggest that both epilepsy and obstructive apnea can cause vascular alterations. Furthermore, when combined our results showed different responses that are worth further investigation.

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Poster

364. Epilepsy: Seizure Mechanisms

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Topic: B.08. Epilepsy

Support: NIGMS P20GM109089

Title: Seizure forecasting in the intra-hippocampal kainic acid mouse model

Authors: D. GREGG[1], I. LANE[2], C. LISGARAS[3], H. E. SCHARFMAN[4], *S. A. MCKENZIE[1];
[1]Neurosciences, UNM HSC, Albuquerque, NM; [2]Dept. of Biomed. Engin., UNM, Albuquerque, NM; [3]Saul R. Korey Dept. of Neurology, Lab. of Developmental Epilepsy, Albert Einstein Col. of Med., Bronx, NY; [4]Dementia Res., NYU and Nathan Kline Inst., Orangeburg, NY

Abstract: Responsive neural stimulation (RNS) is effective in controlling pharmacoresistant seizures which occur in about a third of all patients with epilepsy. The mechanism of action underlying the therapeutic value of RNS is not well understood, as the overall patient outcome correlates poorly with acute seizure termination and improves over time. To gain a better understanding of how RNS treats focal epilepsy, and to develop responsive algorithms that are anticipatory rather than reactive, pre-clinical focal epilepsy models are needed. The intra-

hippocampal kainic acid (IHKA) mouse model produces subjects that experience spontaneous convulsions. Recently, a large database (N = 5 mice; 413 seizures, 1512 hrs) of daily 24-hour continuous video/EEG recordings was reported (Lisgaras and Scharfman, 2022). Here, this database was used to identify a seizure vulnerable state and to develop algorithms to forecast spontaneous seizures in this mouse model. The local field potentials were taken from four skull screws and the state space at each moment in time (1s window) was defined by >300 features including: the wavelet power spectra, phase-amplitude coupling, and cross-channel coherence. We observed several changes in the hour prior to a seizure: delta power increased, hippocampal and cortical low frequency coherence decreased (< 5Hz), and delta/gamma phase amplitude coupling increased. In the minutes before a seizure, gamma power was low while delta power was high. Seizures arose from a narrow range of brain states, however, this state space is also represented during moments when seizures are not imminent. This combination of results suggests that a classifier should show low false positives (high specificity) and high false negatives (low sensitivity) in detecting whether a seizure will occur in the near future. Because seizures are rare, we used the RUSBoost algorithm to build a set of weak classifiers to predict discretized time until a seizure. Indeed, we found high specificity (> 0.9) and low sensitivity (~ 0.5) for predicting whether a seizure would occur within the hour. Sensitivity and specificity were high (>0.9) for classifying times as non-proximal to a seizure (>1 hour away) and for times in which the seizure was imminent (<10s away). Models trained on data two weeks post-IHKA generalized poorly to later sessions, while models trained on later sessions showed significantly better inter-session cross-validation, perhaps reflecting changes in the seizure phenotype with disease progression. These results set the stage for future work in which neural data is classified in real-time and used for closed-loop stimulation to prevent seizures before they begin.

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Poster

364. Epilepsy: Seizure Mechanisms

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Topic: B.08. Epilepsy

Support: NIH/NINDS R37NS10090
James G. Hirsch, MD endowed student Fellowship

Title: Juxtacellular recordings of single neurons in a mouse model of Spike Wave Discharges

Authors: *W. KHAN[1], M. VALCARCE-ASPEGREN[1], L.-A. SIEU[1], X. ZHENG[1], S. LIU[1], S. MCGILL[1], S. CHOPRA[2], C. P. MCCAFFERTY[3], H. BLUMENFELD[1];
[1]Dept. of Neurol., [2]Yale Sch. of Med., New Haven, CT; [3]Dept. of Anat. and Neurosci., Univ. Col. Cork, Cork, Ireland

Abstract: Spike and Wave Discharges (SWD) are commonly associated with absence seizures which are generalized onset seizures that occur most commonly in children. Work done in rats has shown that there are differences in the activity of neurons in response to SWDs and these can be categorized into four main categories (sustained decrease, sustained increase, onset peak and no change). Our goal in this project is to elucidate the mechanism and significance of these differences. 6-11 weeks old C3H/HeJ were implanted under anesthesia with tripolar intracranial EEG electrodes in the frontal and parietal cortex with a ground electrode mid-cerebellum. EEG was recorded from the awake head fixed mice following recovery along with single unit activity (SUA) from the right barrel cortex through a burr hole created during electrode implantation. SUA was obtained with a glass electrode containing a bleached silver wire, in a solution of neurobiotin, dissolved in saline. The electrode was lowered into the barrel cortex until neuronal activity was seen. A MultiClamp 700B was used to record voltage and signals were digitized at a sampling rate of 20kHz. 600pA of current passed at 2Hz was used to label the neurons with neurobiotin. In total, SUA was obtained from 27 neurons and their activities could be categorized into seizure synchronized and seizure nonsynchronized activities with most falling in the former category. There were also variations in the activities of the neurons synchronized to the seizure that could be broadly classified into 4 categories. These categories are sustained decrease (neuronal firing rate is below baseline throughout seizure), sustained increase (neuronal firing rate is above baseline throughout seizure), onset peak (neuronal firing rate is above baseline only at the beginning of the seizure) and no change (neuronal firing rate is at baseline). These differences in the activities of neurons during absence seizures are not species specific. Neurons in both rats and mice can possess one of 4 different types of activities during an absence seizure (sustained increase, sustained decrease, onset peak and no change), suggesting that there is a characteristic neuronal response to absence seizures that is conserved across species and could serve as the basis to understand the neuronal response to absence seizures in humans.

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Poster

364. Epilepsy: Seizure Mechanisms

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Topic: B.08. Epilepsy

Support: NIH/NINDS R37NS100901

Title: Sensory stimulation can interrupt seizures in a rodent model of absence epilepsy

Authors: *X. ZHENG[1], C. MCCAFFERTY[1,2], R. TUNG[1], B. F. GRUENBAUM[1], H. BLUMENFELD[1];
[1]Sch. of Med., Yale Univ., New Haven, CT; [2]Univ. Col. Cork, Cork, Ireland

Abstract: Absence seizures are characterized by behavioral arrest, apparent loss of consciousness, and an electroencephalographic (EEG) pattern of spike-wave discharges (SWDs). Previous studies suggest that absence seizures can sometimes be interrupted by external sensory stimuli, but the underlying properties of seizures which allow for their interruption and restoration of consciousness remain unquantified. Therefore, the aim of this study was to investigate the behavioral and electrophysiological qualities of the interrupted and uninterrupted SWDs, based on a validated rodent model of absence epilepsy. In this project, Genetic Absence Epilepsy Rats from Strasbourg (GAERS) were conditioned to respond to an auditory stimulus of moderate intensity by licking at a reward port, and fronto-parietal EEG was recorded from these animals. We found that the number of SWDs terminated within 1s of the presentation of an auditory stimulus was disproportionately high, even after removing all brief seizures (duration < 3s) from the analysis. Of 427 recorded absence seizures, 46.8% ended within 1 second of the presentation of an auditory stimulus. We therefore classified this group of seizures to be "interrupted". We processed and compared EEG power in the wave band (5-9 Hz), in the spike band (15-100 Hz), and at low frequencies indicative of arousal state (0-4 Hz) around the time of seizure start, seizure end and stimulus onset in 200 interrupted and 227 uninterrupted seizures. We did not find any statistically significant differences. Additionally, the lick rates of the animals prior to seizure start did not demonstrate significant behavioral differences between interrupted and uninterrupted seizures. Our finding suggests that absence seizures in the rodent model can be interrupted by external sensory stimuli during SWDs. Based on these preliminary results, the SWD EEG characteristics as well as the pre- and post-seizure arousal levels, as indicated by lick rate and EEG band power, of the interrupted and uninterrupted seizures do not show significant differences statistically. The neural mediators of seizure interruptability and the potential of using sensory stimulation as a therapeutic method should be investigated further in additional studies.

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Poster

364. Epilepsy: Seizure Mechanisms

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Support: NIH R01 NS066974
James G. Hirsch, M.D. Endowed Medical Student Research Fellowship

Title: Decreased locus coeruleus neuronal activity is associated with behavioral arrest in an awake mouse model of focal limbic seizures

Authors: *P. PASZKOWSKI[1], M. VALCARCE-ASPEGREN[1], S. LIU[2], Q. WU[2], L.-A. SIEU[3], S. MCGILL[4], H. BLUMENFELD[3];

[1]Yale Med. Sch., Yale Med. Sch., New Haven, CT; [2]Yale Sch. of Med., New Haven, CT; [3]Yale Univ. Sch. of Med., Yale Univ. Sch. of Med., New Haven, CT; [4]Yale Univ., Yale Univ., New Haven, CT

Abstract: Cholinergic neuronal inhibition in the brainstem and basal forebrain has been implicated in depressed conscious awareness and behavioral arrest during temporal lobe seizures in humans and rats. It is still unknown if other types of subcortical neurons contribute to modulating the aforementioned cortical depression during focal limbic seizures. We attempted to explore one possible parallel pathway through multi-unit recordings of the locus coeruleus (LC) during focal limbic seizures in an awake-behaving mouse model. We aimed to analyze the association between changes in LC activity during seizures and impaired running behavior. Local field potentials of the left orbitofrontal cortex (OFC) and bilateral hippocampi were measured in mice with chronically implanted bipolar electrodes while head-fixed on a running-wheel apparatus. Focal limbic seizures were induced with 2s 60 Hz pulses into the hippocampus, and LC neurons were recorded using a high-impedance tungsten microelectrode. The LC was visualized using anti-tyrosine hydroxylase fluorescent antibodies and electrode placement was verified using DiI staining. When comparing LC multiunit activity between ictal and post ictal periods, a paired t-tests was used and showed a significant ($n = 8$, $p < 0.05$) decrease in average firing. Behaviorally, motor activity quantified as wheel speed (cm/s), showed significant decreases (and frequently complete arrest) during ictal epochs. Mean wheel running speed was found to be 30.1 ± 6.9 in the pre-ictal period, and 3.3 ± 1.4 cm/s in the ictal period ($p < 0.01$). In sum, deep brainstem recordings are feasible in awake, moving mice during focal limbic seizures. Furthermore, significant decreases in LC firing were associated with impaired running behavior during seizures. Future studies can further investigate this subcortical modulation during seizures, potentially leading to novel treatments and insights.

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Poster

364. Epilepsy: Seizure Mechanisms

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Program #/Poster #: 364.15

Topic: B.08. Epilepsy

Support: NIH R01 NS066974

Title: Decreased activity in the nucleus basalis during focal limbic seizures: multi-unit and juxtacellular recordings of cholinergic activity in an awake mouse temporal lobe seizure model

Authors: *S. LIU, M. VALCARCE-ASPEGREN, L.-A. SIEU, W. KHAN, A. DUQUE, S. MCGILL, J. LIU, H. BLUMENFELD;
Yale Univ. Sch. of Med., New Haven, CT

Abstract: Human temporal lobe epilepsy is characterized by impaired consciousness, which often has serious consequences. Our goal is to restore patients' consciousness during seizures, so we need to gain a good understanding of the physiological and neuroanatomical basis of unconsciousness during seizures. Previous studies in our laboratory identified the inhibition of brainstem and basal forebrain cholinergic neurons as potentially responsible for decreased arousal during focal limbic seizures in "lightly anesthetized" rats. Our goal was to determine if this mechanism also plays a role in our new awake, behavioral mouse model. In addition, the use of awake mouse models allows the exploration of changes in pupillary diameter and animal behavior to assess loss of consciousness during temporal lobe epileptic seizures.
In this study, mice were head-fixed to a running wheel. Local field potentials in the right lateral orbitofrontal cortex and bilateral hippocampus were measured using chronically implanted bipolar electrodes. Focal limbic seizures were induced by applying current pulses (2 s, 60 Hz) into the hippocampus. We recorded multi-unit activity (MUA) and juxtacellular single unit activity (SUA) in the nucleus basalis of Meynert, a major subcortical structure, using tungsten microelectrodes and glass capillaries filled with 4% Neurobiotin, respectively. After tissue processing, recorded and juxtacellularly labeled cholinergic neurons were identified by immunofluorescence.
Early results showed a general reduction in neuronal firing frequency in the nucleus basalis of Meynert during focal seizures in MUA recordings. Running-wheel behavior was also suppressed during seizures, and orbitofrontal local field potentials exhibited synchronized slow-wave activity similar to that observed in human patients. SUA recordings were also successfully achieved from neurons in the nucleus basalis of Meynert. These results suggest that stable MUA and SUA recordings of deep subcortical neurons are possible during seizures in awake, behaving mice. Furthermore, inhibition of neurons in the nucleus basalis of Meynert may play a potential role in the regulation of arousal during focal limbic seizures. Further investigations of the specific subcortical neurons and neurotransmitters involved during seizures are necessary to elucidate the mechanisms underlying seizure-related unconsciousness and may offer possibilities for new therapies for people with epilepsy.

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Poster

364. Epilepsy: Seizure Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 364.16

Topic: B.08. Epilepsy

Support: NIH R01 NS066974

Title: Focal limbic seizures with impaired behavioral responses are associated with cortical slow waves and reduced cholinergic arousal in a novel mouse model

Authors: *L.-A. SIEU[1], S. SINGLA[1], J. LIU[1], X. ZHENG[1], A. SHARAFELDIN[1], G. CHANDRASEKARAN[2], M. VALCARCE-ASPEGREN[1], A. NIKNAHAD[3], I. FU[1], N. DOILICHO[1], A. GUMMADAVELLI[1], C. P. MCCAFFERTY[4], R. B. CROUSE[1], Q. PERRENOUD[1], M. R. PICCIOTTO[1], J. A. CARDIN[1], H. BLUMENFELD[1];
[1]Yale Sch. of Med., New Haven, CT; [2]Univ. of Pennsylvania, Philadelphia, PA; [3]John Hopkins school of medicine, Baltimore, MD; [4]Univ. Col. Cork, Cork, Ireland

Abstract: Patients with temporal lobe epilepsy often experience loss of consciousness during seizures. Clinical studies of temporal lobe seizures show sleep-like cortical slow wave activity during seizures with impaired consciousness. Prior investigations in an anesthetized rat focal limbic seizure model showed decreased brainstem and basal forebrain cholinergic neuronal activity during seizures. To better understand the underlying circuit and neuronal mechanisms causing loss of consciousness in temporal lobe seizures, we developed an awake behaving mouse model. Focal limbic seizures were induced by stimulating the hippocampus at 60Hz for 2s via implanted bipolar electrodes in the dorsal hippocampus. Local field potential (LFP) signals were recorded from hippocampus, with simultaneous LFP and multiunit activity (MUA) recordings from orbitofrontal cortex. To assess behavioral responses during seizures, water-restricted mice were head-fixed on a running wheel and trained to associate an auditory stimulus (0-50kHz, 12ms) with the delivery of a drop of water every 10-15s. Cholinergic input to OFC was studied by expressing a genetically encoded fluorescent acetylcholine indicator (GACh) in the OFC and measuring signals via an implanted optic fiber. Focal limbic seizures were induced with a duration of 5-40s and repeatable for several weeks (n=26 animals). Responses to sound and running speed both overall decreased during seizures and recovered postictally ($p<0.01$, n=26 animals) but remained at baseline levels during some seizures. By examining the correlation between behavioral responses and cortical activity during seizures, we found that impaired behavior was associated with cortical slow waves ($p<0.01$, n=26 animals), as observed in humans, with neuronal MUA firing following an Up and Down state firing pattern (n= 11 animals). Furthermore, cholinergic input to the cortex was reduced during seizures, with larger decreases associated with impaired behavior (n=7 animals). In summary, we found that this novel mouse model of temporal lobe seizures shares characteristics with humans and with the rat model, and demonstrates evidence of decreased subcortical cholinergic arousal and altered cortical function during seizures with impaired behavior. With further investigation this approach may lead to novel therapies to improve quality of life for people with temporal lobe seizures and impaired consciousness.

Disclosures: L. Sieu: None. **S. Singla:** None. **J. Liu:** None. **X. Zheng:** None. **A. Sharafeldin:** None. **G. Chandrasekaran:** None. **M. Valcarce-Aspegren:** None. **A. Niknahad:** None. **I. Fu:** None. **N. Doilicho:** None. **A. Gummadavelli:** None. **C.P. McCafferty:** None. **R.B. Crouse:** None. **Q. Perrenoud:** None. **M.R. Picciotto:** None. **J.A. Cardin:** None. **H. Blumenfeld:** None.

Poster

364. Epilepsy: Seizure Mechanisms

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Program #/Poster #: 364.17

Topic: B.08. Epilepsy

Support: R01 NS066974

Title: Locus coeruleus neuronal activity in an awake mouse model of focal limbic seizures investigated by multiunit recordings

Authors: *M. VALCARCE-ASPEGREN, P. PASZKOWSKI, S. LIU, Q. WU, L.-A. SIEU, S. MCGILL, H. BLUMENFELD;
Yale Sch. of Med., Yale Sch. of Med., New Haven, CT

Abstract: Previously, rat models have implicated the inhibition of brainstem and basal forebrain cholinergic neurons as a driving force behind impaired consciousness during temporal lobe seizures. However, much remains unknown about the subcortical pathways modulating depressed cortical function during focal limbic seizures. Recording multiunit activity in the locus coeruleus (LC) in an awake mouse model will help define the role of a separate, parallel neurotransmitter pathway by interrogating ictal noradrenergic modulation associated with temporal lobe seizures. To accomplish this, recordings were performed using head-fixed mice running on a wheel. Chronically implanted bipolar electrodes were used to measure local field potentials in the right orbitofrontal cortex and bilateral hippocampi. Focal limbic seizures were induced via the application of current pulses (2s, 60 Hz) into the hippocampus. Neurons in the locus coeruleus were recorded using a high-impedance, tungsten microelectrode (2-4 MΩ resistance; FHC). The LC was visualized using anti-tyrosine hydroxylase fluorescent antibodies and electrode placement was verified using DiI staining. We observed a statistically significant ($p<0.05$) decrease in multiunit firing of LC neurons during focal limbic seizures. Additionally, there was a simultaneous significant increase in power of the 1-4Hz band in the lateral orbitofrontal cortex (LOFC) ($n=8$). Neurons in the locus coeruleus and electrode traces were successfully identified by histology. In summary, we found that multiunit recordings in the deep brainstem are feasible in awake, moving mice during seizures. Additionally, statistically significant decreases in the firing patterns of neurons in the locus coeruleus were observed with concomitant slow wave activity in the LOFC. This is the first evidence of a parallel noradrenergic pathway involving the LC modulating arousal in a temporal lobe seizure model. To fully understand impaired consciousness in temporal lobe seizures, it will be critical to further investigate the role of subcortical neuronal modulation during seizures. This will provide important insights about how seizures impair consciousness, which may in turn lead to beneficial, novel treatments for people with epilepsy.

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Poster

364. Epilepsy: Seizure Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 364.18

Topic: B.08. Epilepsy

Support: Rowan University
University of Pennsylvania

Title: Electroconvulsive shock selectively alters the transcriptome of hippocampal cells in mice

Authors: *T. N. FERRARO[1], B. C. REINER[2], G. A. DOYLE[2], A. E. WELLER[2], A. I. BATTERMAN[1], W. H. BERRETTINI[2], R. J. BUONO[1], R. CRIST, III[2];
[1]Cooper Med. Sch. of Rowan Univ., Cooper Med. Sch. of Rowan Univ., Camden, NJ; [2]Univ. of Pennsylvania, Philadelphia, PA

Abstract: Electroconvulsive shock (ECS) affects cellular processes relevant to seizure biology and the treatment of depression. We analyzed cell type-specific gene expression in the hippocampus to gain new insight into these aspects of brain function. We utilized 8-10 week old male C57BL/6J (B6) mice (N=4), inducing in them 3 ECS generalized seizures, spaced 15 minutes apart, for 3 consecutive days. Strain, sex and age-matched control mice (N=4) received sham treatment. We euthanized mice 24 hours after the last treatment, collected hippocampal punches from 2 mm coronal sections, extracted nuclei and conducted single-nucleus RNA sequencing using the 10x Genomics microfluidics platform. We performed clustering and cell type identification using Seurat, identifying 13 types of neurons (11 excitatory, 2 inhibitory) and one type each of astrocytes, oligodendrocytes, oligodendrocyte precursor cells, microglia, and endothelial cells. We then performed pseudobulk analysis with the R package Libra, and identified differentially expressed genes (DEGs) between ECS- and sham-treated mice in individual cell populations. After cutoffs, we detected over 500 DEGs in each of two types of excitatory granule cells. We detected fewer DEGs (50 or less) in other cell types. The major gene classes differentially expressed in multiple cell types include those related to calcium channels, potassium channels, inflammation, cell adhesion and cell signaling, especially phosphatases and kinases. Many DEGs represent genes or gene families linked previously to epilepsy such as *Cacna1b*, *Chrna7*, *Gabrd*, *Hcn1*, *Malat1*, *Pcdhb20*, *Pdyn*, *Scnb3* and *Tcf4*. Other DEGs represent genes related to the pharmacotherapy of depression including *Adra1b*, *BDNF*, *Camk2a*, *Camk2b*, *Htr1b*, and *Htr5b*. Transcription factor network analysis revealed DEG upstream regulators also related to epilepsy and depression. Of further note, we found that ECS altered transcription of core clock genes *Cry1* and *Per2*. Importantly, although many of the DEGs we detected relate to epilepsy or depression, the majority are not associated directly with the pathogenesis or treatment of either disease. In conclusion, ECS treatment has a major impact on the transcriptome of specific cell populations in the mouse hippocampus. Subtypes of excitatory neurons are most significantly affected, and responses involve a highly diverse array of genes. Future research aimed at elucidating the relationship between the specific DEGs induced by ECS and the activity of gene expression pathways in identified hippocampal cell types will facilitate the development of novel treatment strategies for epilepsy and depression.

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Poster

364. Epilepsy: Seizure Mechanisms

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Program #/Poster #: 364.19

Topic: B.08. Epilepsy

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Title: Role of testosterone in spike-wave discharges seizures in taiep rats

Authors: *R. CASTILLO[1], C. CORTES[2], A. TRUJILLO[1], J. R. EGUIBAR, Sr.[3];
[1]Benemérita Univ. Autónoma de Puebla, Puebla, Mexico; [2]B. Univ. Autonoma de Puebla, Puebla, Mexico; [3]Behavioral Neurophysiol., Benemerita Univ. Autonoma De Puebla, Puebla, Pue., Mexico

Abstract: The *taiep* rats were obtained at Benemérita Universidad Autónoma de Puebla and they had a motor syndrome characterized by tremor, ataxia, immobility episodes, epilepsy, and paralysis. Additionally, is the first model of the leukodystrophy H-ABC which is a tubulinopathy whit a point mutation in the tubulin β4A (TUBB4A) and similar MRI imagenology to that presented in humans, whit ventriculomegaly and hypomyelination. The *taiep* rats had spike-wave discharges (SWDs) in the electroencephalogram (EEG) similar to those reported in humans with absence seizures. The SWDs are sexually dimorphic because the expression is earlier and with higher frequency in males with respect to female *taiep* rats. In male rats newborn gonadectomy significantly decreased the incidence of SWDs, and the opposite happen with adult orchiectomy significantly increased the frequency of SWDs. The aim of the present work is to evaluate, the role of testosterone propionate (TP) systemic administration as a modulator of SWDs in male and female *taiep* rats. We used 8 adult orchiectomized male and 8 ovariectomized female *taiep* rats. All subjects were implanted for EEG, EMG and EOG recordings to characterize absence seizures. Two 24h EEG recordings, one control after s.c. administration of olive oil and a second recording after s.c. administration of TP with a dose of 2mg/Kg. We measured the frequency, duration, and latency to the first SWDs. Our results showed that after TP administration decreased the frequency and the duration of SWDs in orchiectomized male *taiep* rats with respect to control group ($t=8, 2.8, P<0.05$). However, TP administration in ovariectomized female rats increased the frequency of SWDs with respect to control group ($t=132, 2.1, P<0.05$). In conclusion, testosterone had a dual role depending on the sex of the subjects decreasing SWDs in orchiectomized males and increased the duration of SWDs in ovariectomized female *taiep* rats.

Disclosures: R. Castillo: None. **C. Cortes:** None. **A. Trujillo:** None. **J.R. Eguibar:** None.

Poster

365. Epilepsy: Networks and Oscillations

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 365.01

Topic: B.08. Epilepsy

Title: Neural dynamics of ictogenesis in acute and chronic models of temporal lobe epilepsy in-vivo

Authors: *A. MASHIEL, Y. SCHILLER;
Technion Israel Inst. of Technol., Haifa, Israel

Abstract: Epilepsy is the fourth most common neurological disorder affecting the lives of nearly 50 million people globally. Despite vigorous research, the dynamics leading to seizure onset, and the role of γ-Aminobutyric acid (GABA)-ergic interneurons in seizure initiation are not fully understood and are hitherto a subject of a heated debate. In this study, we aimed to determine the recruitment patterns of excitatory and different GABAergic interneuron subtypes during seizure onset in the hippocampus in-vivo. To this aim, we have used both acute 4-aminopyridine (4-AP) and chronic kainic acid (KA) models of temporal lobe epilepsy (TLE), the most common form of focal epilepsy, in CA1 region of the hippocampus in mice. We utilized two-photon microscopy of GCaMP6f-expressing pyramidal neurons (PNs) and parvalbumin (PV) or somatostatin (SST) interneurons (INs) to image the different cell types simultaneously in a single cell resolution, while recording the corresponding local field potential (LFP) to identify seizures in awake mice. The activation dynamics of the three recorded neural subpopulations (PNs, PV-INs, and SST-INs) differed during seizure onset with respect to both synchronization and neural recruitment. Interestingly, in all three subpopulations different seizures showed comparable neuronal recruitment patterns during seizure onset. Simultaneous recordings of PNs with either PV or SST INs revealed a bimodal ictogenic dynamics, with some seizures showing initial recruitments of inhibitory INs while in other seizures PNs and INs were recruited simultaneously. ChannelRhodopsin2 mediated optogenetic activation of PV-INs showed seizures were initiated primarily early after the cessation of the activation phase, whereas optogenetic activation of SST-interneurons induced seizures early within the activation phase. Taken together, our data exhibits unique dynamics in hippocampal circuits and neural subpopulations during seizure onset, and sheds light on mechanisms of ictogenesis. These findings can in future be used to develop novel treatment approaches to ameliorate seizures in TLE.

Disclosures: **A. Mashiel:** None. **Y. Schiller:** None.

Poster

365. Epilepsy: Networks and Oscillations

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 365.02

Topic: B.08. Epilepsy

Title: Mesoscale excitatory and inhibitory mapping of network activity during focal neocortical seizures

Authors: *J. E. NIEMEYER[1], P. LUO[2,1], F. ZHAN[1], H. MA[1], C. PONS[1], T. H. SCHWARTZ[1]; [1]Neurosurg., Weill Cornell Med., New York, NY; [2]First Hosp. of Jilin Univ., Changchun, China

Abstract: Focal seizures initiate at one location in the brain and then propagate through both contiguous ictal wave expansion and through activity along axonal connections to recruit distal brain regions. Recent studies from our lab and others have reported differences in excitatory (E) and inhibitory (I) neural activity across the brain during seizures, but the specific roles of E:I balance across brain networks that permit seizure spread are not known. Here we test how E:I differs across a defined bilateral seizure network in awake seizing animals. We injected 4-Aminopyridine (4AP, 2.5 mM) into Thy1-GCaMP6f mice and PV-GCaMP6f mice while performing mesoscale imaging. We measured neural activity by GCaMP6f across the dorsal cortex and electrophysiological data (1 kHz) at the 4AP ictal focus. This focus was a subregion of primary somatosensory cortex (SSp-bfd), which has well-defined interconnections with ipsilateral secondary motor cortex (M2) and contralateral SSp-bfd. Allen Atlas brain maps were used to identify this bilateral network, followed by pilot experiments with electrical stimulation and bicuculline to confirm the connectivity of this network. We find that, prior to electrographic seizure onset, PV inhibitory and Thy1 excitatory cell fluorescence activity increases weakly at the seizure focus, though these two groups do not appear significantly different pre-ictally. Following onset, on average, both Thy1 and PV activity show that propagation in contralateral nodes connected by callosal projections (contralateral M2 and S1) is delayed relative to ipsilateral non-callosal node propagation. Further, contralateral S1, despite receiving direct callosal inputs from the seizure focus, is recruited significantly later than the contralateral M2 site (a di-synaptic node). The difference in propagation speeds between the focus and ipsi- vs contralaterally connected brain sites supports the possibility that callosal projections may vary in their function depending on their origin, providing either predominantly excitatory or inhibitory activity. These differences, especially regarding E:I, may underlie seizure propagation patterns and could potentially be leveraged to control seizure networks.

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Poster

365. Epilepsy: Networks and Oscillations

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Program #/Poster #: 365.03

Topic: B.08. Epilepsy

Title: Electrical probing of tetanus toxin induced seizures to measure imminent seizure risk

Authors: *W.-C. CHANG[1], A. LAI[2], M. COOK[2], W. STACEY[1,3];
[1]Neurol., Univ. of Michigan, Ann Arbor, Ann Arbor, MI; [2]Graeme Clark Inst., Univ. of Melbourne, Melbourne, Australia; [3]Biomed. engineering, Univ. of Michigan, Ann Arbor, MI

Abstract: Epileptic seizures are very common and greatly devastate patients' lives. Seizures are abrupt and difficult to predict, so there is great interest in developing biomarkers that can reliably signal their approach. One potential biomarker is to focus on the critical transitions from complex systems theory. Previous work has focused on critical slowing, tardiness in recovery from perturbations that suggests the imminence of transitions. However, a recent theory also postulates a distinct category of seizures in which perturbations produce oscillations rather than critical slowing. In this study, we propose that identifying the type of nearby seizure (dynamotype) helps distinguish which of the two responses are expected, and predicts the specific patterns that would be expected as seizures approach. To test the hypothesis, a rat temporal lobe epilepsy model was employed. Tetanus toxin (TeNT, 25 ng/ul) was slowly infused into the right hippocampal CA3 of six Sprague Dawley rats (3 males and 3 females, 7-8 weeks old). Two EEG electrodes and two depth recording electrodes in both hippocampal CA1s were implanted after the TeNT injection. Four days after TeNT injections, intermittent electrical stimuli (0.1 Hz, 0.5/0.5 ms biphasic, 10 V in amplitudes) were delivered through a bipolar electrode to the right perforant pathway working as a consistent source of perturbations. First seizures presented in the second to the third week after TeNT injections, and the rats remained epileptic for over 8 weeks. Electrographic seizures were categorized based on their dynamotypes. Interictal perturbation responses were characterized and measured as either critical slowing or oscillations; then the responses were quantified using variance and spectral power in the data immediately following the stimuli. We found evidence of both stimulus responses, which were highly correlated with the upcoming seizures. The results provide quantifiable measures demonstrating the distance to seizures in the post hoc analyses, that will be applied in later online seizure prediction.

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Poster

365. Epilepsy: Networks and Oscillations

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Topic: B.08. Epilepsy

Support: NIH/NINDS Grant NS115049

Title: Altered dendritic and axonal maturation of adult-born hippocampal granule neurons in a transgenic mouse model of adult-onset epilepsy

Authors: A. TYULMENKOVA, V. STUBBS, J. LABRADOR PINO, ***C. ISGOR**;
Florida Atlantic University/BIOMED, Boca Raton, FL

Abstract: Dentate granule neurons (DGNs) of the hippocampus gate neuronal information entering the hippocampus proper. Dysregulations in DGN gating properties may create a permissive synaptic environment for spreading hyperexcitability. It is not well understood how adult-born DGNs contribute to seizure prone circuits or how seizure neuro-environment can impact the maturation of DGNs. Our laboratory uses a transgenic mouse strain that over-expresses the brain-derived neurotrophic factor (BDNF) in the forebrain under the calcium/calmodulin-dependent kinase II alpha promoter (termed TgBDNF mice). This model allows for studying progressive synaptic changes that are pro-epileptic and can be differentiated from subsequent seizure-induced changes. TgBDNF mice develop seizures that are elicited by brief tail suspension and cage agitation in mid adulthood, and the seizures become progressively more severe with successive tonic/clonic episodes. We previously reported significant expansion of TgBDNF hippocampal mossy fiber (MF) terminal fields prior to tonic/clonic seizures likely due to excess local BDNF, and this expansion becomes even larger following repeated seizures. The potential contributions of the adult-born neurons on changes in gating properties of the granule neuron synaptic circuits are assessed particularly in dendritic input and MF output regions of immature DGNs. We bred the TgBDNF strain with a transgenic line that expresses green fluorescent protein (GFP) under the GAD67 promoter. Newborn DGNs express GFP transiently (~first 4 wks post mitosis) throughout the cell body and processes, including developing dendrites and axons. Our preliminary data revealed seizure-associated increase in dendritic arbors of the GFP+ immature DGNs in TgBDNF mice in the form of increase in dendritic length and spine density. Moreover, the immature axons innervating the CA3 dendritic fields (i.e., MF boutons) were assembled differently in seizure prone TgBDNF mice compared to WT controls. Specifically, we observed an increase in MF bouton densities at target CA3 stratum lucidum, increased cross-sectional length and post-synaptic contact zones per bouton in TgBDNF mice with repeated tonic/clonic seizure history compared to age- and sex-matched controls. These results provide evidence for BDNF- and seizure-driven changes in adult-born DGN circuits that may play a functional role in increased hyperexcitability and subsequent loss of gating function.

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Poster

365. Epilepsy: Networks and Oscillations

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Topic: B.08. Epilepsy

Support: CURE W81XWH-15-2-0069
NIH R01NS112538
R01HD099397
R35NS116852

Title: Chloride changes during post-traumatic epileptogenesis in living swine and rodent brain slice cultures

Authors: *K. P. LILLIS[1], B. COSTINE-BARTELL[2], L. MARTINEZ-RAMIREZ[2], B. GOLEMB[2], K. STALEY[1];
[1]Neurol., [2]Neurosurg., Harvard Med. School, MGH, Charlestown, MA

Abstract: The most common anatomical feature of post-traumatic epilepsy (PTE) is astrogliosis; however, the link between gliosis and PTE remains unknown. TBI *is* linked to the release of matrix metalloproteinases and subsequent remodeling of the extracellular matrix (ECM). Preliminary data suggest that glia form ECM that is compositionally different from mature neuronal ECM such as that found in perineuronal nets. We hypothesize that this altered ECM has more densely sulfated proteoglycans, which "crowd out" chloride in the ECM, decreasing inhibition and increasing excitability in the injured tissue. Here, we directly imaged chloride in the ECM or in neuronal cytoplasm following acute TBI in 4-6 month or following chronic PTE in 16-18 month Yucatan minipigs. Pigs were chosen because their gyrencephalic brains approximate human anatomy and responses to external force. We used two-photon imaging in living pigs, before and after cortical impact, to quantify the effects of acute trauma on intracellular chloride (using virally delivered chloride sensor) and extracellular chloride (using a novel dye, ABP-dextran). We also measured chronic extracellular chloride 1 year following cortical impact or a sham procedure. We found that control extracellular chloride in brain parenchyma was significantly lower than that in bulk CSF. Four hours post-injury there was a mean decrease in ABP fluorescence lifetime of 25%, corresponding to an extracellular chloride increase of approximately 30mM. Similarly, the SClm YFP/CFP emission ratio decreased by 30%, corresponding to an increase in intracellular chloride. 1 year post-injury extracellular chloride was significantly lower in the injured, epileptic pig brain vs the sham, non-epileptic pig. Mouse hippocampal brain slice cultures were used to test the hypothesis that sulfation of the extracellular matrix is a determinant of extracellular chloride. Slices incubated in ABP-dextran were treated with chondroitinase-ABC, an enzyme which digests chondroitin sulfate, a key component of the ECM, and imaged. Slice cultures also exhibited unexpectedly low extracellular chloride. Acute treatment with chondroitinase altered extracellular chloride, supporting the hypothesis that sulfation of the ECM is correlated with extracellular chloride concentration. Together these preliminary findings are consistent with a model wherein TBI induces changes in the ECM that lead to secondary increases in both extracellular and intraneuronal chloride. If confirmed, this may point to a previously unknown mechanism of cerebral edema as well as pathological changes in GABAergic inhibition that contributes to post-traumatic epileptogenesis.

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Poster

365. Epilepsy: Networks and Oscillations

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Program #/Poster #: 365.06

Topic: B.08. Epilepsy

Support: 5TR01NS038572

Title: Behaviorally salient active coding DGC pool is masked by hyperexcitable neighboring cells in TLE

Authors: *A. GOODMAN[1], S. JOKSIMOVIC[2], D. A. COULTER[3];
[1]Children's Hosp. of Philadelphia, PHILADELPHIA, PA; [2]Neurol., [3]Pediatrics and Neurosci., Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: The pattern separation and functional coding of the dentate gyrus (DG) necessary for spatial competency is predicated on potent inhibitory properties to create a functional sparse coding network (FSCN). The most common form of epilepsy in adults is temporal lobe epilepsy (TLE) which is perpetuated by massively disrupted neuronal circuitry within the DG. Our lab previously demonstrated hyperactivity of dentate granule cells (DGC) in the pilocarpine-treated mouse model of TLE which may serve as therapeutic targets for both seizures and cognitive comorbidities. Since both synaptic and intrinsic properties of individual DGCs dictate which may be incorporated into the FSCN and thus inhibit their neighboring cells, the global excitability tuning of these cells is paramount to healthy DG function. We predict that in the healthy mouse DG, that those cells integrated into the FSCN have greater measures of intrinsic and synaptic excitability than non-integrated cells. However, TLE-driven hyperactivity of DGCs disrupts the FSCN necessary for healthy function of the DG by tonically pushing DGCs closer to action potential (AP) threshold, regardless of behavioral context. These hyperexcitable neighbors fire more readily and obscure the FSCN of behaviorally salient DGC activity, degrading information coding ability. To determine if global DGC hyperexcitability in the TLE model is obscuring the FSCN we compared amplitude and frequency of spontaneous excitatory post-synaptic currents (sEPSCs) in integrated vs unintegrated cells in both healthy and epileptic mice. We used the bitransgenic TetTag model system (fos-tTA x tetO-H2B-GFP) to identify the active pool of DGCs from a novel environment exposure which were patch-clamped in acute slice to determine excitability compared with behaviorally inactive, untagged neighboring cells. Preliminary data shows a significant increase in amplitude and frequency of spontaneous excitatory post-synaptic currents (sEPSCs) in the GFP tagged DGCs compared to adjacent untagged neurons (*t-test*, $p<0.05$, each) from the healthy mouse. This confirms that the active neuronal ensemble of DGCs during a spatial memory task are supported by stronger, more frequent synaptic input. Furthermore, we found a substantially larger number of tagged DGCs in TLE mice and similar measures of excitability between those tagged and untagged cells. These results suggest the FSCN is occluded by hyperactive neighbors and that global DGC inhibition may unmask this sparse coding network and support healthy information coding ability.

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Poster

365. Epilepsy: Networks and Oscillations

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Topic: B.08. Epilepsy

Support: NIH R01 EB014641

Title: Mechanistic neural masses (mNM) to understand seizure susceptibility

Authors: R. TRIPATHI[1,2], R. ABOHTYRA[1], ***B. J. GLUCKMAN**[1,3,4,5];
[1]Ctr. for Neural Engin., Penn State Univ., University Park, PA; [2]Ctr. for Advanced Systems Understanding, Helmholtz Ctr. Dresden-Rossendorf, Dresden, Germany; [3]Engin. Sci. and Mechanics, Penn State, University Park, PA; [4]Neurosurg., Penn State, Hershey, PA; [5]Biomed. Engin., Penn State, University Park, PA

Abstract: A fundamental conundrum in epilepsy research is why epileptic brains aren't seizing all the time. Experimentally, one measures that epileptic tissue has lower thresholds for inducing seizures or spreading depolarization (SD). Brain rhythms emerge from the activity of networks of neurons. There have been many efforts to build mathematical and computational embodiments in the form of discrete cell-group activities - termed neural masses - to understand in particular the origins of evoked potentials, intrinsic patterns of brain activity, and mimic seizure dynamics. As originally utilized, standard neural masses convert input through a sigmoidal function to a firing rate, and firing rate through a synaptic alpha function to other masses. We defined a process to build mechanistic neural masses (mNMs) as mean-field models of microscopic membrane-type (Hodgkin Huxley type models) models of different neuron types that duplicate the stability, firing rate, and associated bifurcations as function of relevant slow variables - such as extracellular potassium - and synaptic current; and whose output is both firing rate and impact on the slow variables - such as transmembrane potassium flux. Small networks composed of just excitatory and inhibitory mNMs demonstrate expected dynamical states including firing, runaway excitation and depolarization block, and these transitions change in biologically observed ways with changes in extracellular potassium and excitatory-inhibitory balance. Additionally, by introducing the change in function in sodium channels associated with genetic mutations associated with specific epilepsies, we similarly obtain networks that are more seizure-susceptible. Going forward, larger networks of mNMs can be constructed and coupled to glial networks, the extracellular space, it's volume fraction, and associated volume-conducted variables such as extracellular potassium and field potential.

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Poster

365. Epilepsy: Networks and Oscillations

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 365.08

Topic: B.08. Epilepsy

Title: Excitatory and inhibitory spatial mismatch mediates the global propagation of interictal spikes

Authors: *H. MA[1], P. LUO[3], F. YANG[3], J. LI[3], J. NIEMEYER[1], M. ZHAO[1], D. LI[4], W. LIN[3], J.-Y. LIOU[2], T. H. SCHWARTZ[1];
[1]Neurolog. Surgery, [2]Anesthesiol., Weill Cornell Med., New York, NY; [3]Neurol., [4]Radiology, First Hosp. of Jilin Univ., Changchun, China

Abstract: Rationale: Epilepsy can spread slowly across the cortex in a classic Jacksonian march, or rapidly through long-range horizontal projections. The recruitment of distant is a crucial component of the modern conception of epilepsy as a network disease. Whether long-range projections recruit excitatory or inhibitory activity in distant nodes, and this E/I balance impacts the spread of seizures is not well understood. **Methods:** Using the Allen Mouse Brain Connectivity Atlas, we injected BMI into the primary sensory cortex (S1), which is known to project to ipsilateral M2 and contralateral S1, and recorded interictal spikes (IIS) with wide-field calcium imaging of excitatory (Thy1-GCaMP6f mice, Jaxlab #024276) and inhibitory neurons (PV-GCaMp6f mice, crossing of Jaxlab #017329 and #028865) across both hemispheres. We quantified the amplitude and participation rate of each of the four visible nodes (iS1, iM2, cS1, cM2) and compared activity to control regions in iV1 and cV2, which are not strongly connected with iS1. We measured the strength of recruitment and coactivation to investigate the impact and dependence of each node on another. **Results:** IIS-associated calcium signal (both Thy-1 and PV) could be recorded from the primary node (iS1) where BMI was injected, as well as from the nodes with monosynaptic connections (iM2 and cS1). Surprisingly, cM2 was also frequently recruited, which was disynaptically connected to iS1. Quantitive analysis showed that both excitatory and inhibitory cells were recruited in all nodes and iS1 was the primary driver of this recruitment. IM2 was most frequently recruited, but all other nodes were activated as well. CS1 displayed more inhibitory cell recruitment, compared with cM2, in which excitatory cells were more frequently recruited. **Conclusions:** We have shown how IISs can hijack the known long-range projections between brain areas to create an epileptic network. Inhomogeneities in E/I cell recruitment explain variable node recruitment. Further studies employing cell-specific/node-specific modulation may uncover potential network-based therapies to control intractable epilepsy.

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Poster

365. Epilepsy: Networks and Oscillations

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Title: Repetitive thalamus stimulation modulates thalamocortical connectivity in humans

Authors: *N. GREGG[1], B. N. LUNDSTROM[1], H. HUANG[2], J. VAN GOMPEL[1], K. J. MILLER[3], G. A. WORRELL[1], D. HERMES[2];
[2]Physiol. and Biomed. Engin., [3]Neurosurg., [1]Mayo Clin., Rochester, MN

Abstract: Thalamocortical circuit dysfunction is involved in neurological disorders, including epilepsy, essential tremor, Parkinson disease, Tourette syndrome, and deafferentation pain. Deep brain stimulation (DBS) is a potential therapy, however, for some conditions treatment is hampered by a lack of real-time markers to guide stimulation parameter optimization. Single pulse electrical stimulation (SPES)—and the resulting evoked potentials in connected brain regions—provides a measure of brain network connectivity and excitability. Thalamocortical evoked potentials (TCEPs) may inform DBS parameter optimization for brain circuit modulation. Here, six subjects with drug resistant epilepsy underwent clinical intracranial stereoEEG (sEEG) monitoring for seizure onset zone localization. Subjects had electrodes targeting the thalamus [5 subjects with anterior thalamic nuclei (ANT) electrodes (bilateral in 1 subject); 1 subject with a pulvinar electrode]. During monitoring SPES (3-6 mA, 200 microsec. pulse width, 0.2 Hz, 10-20 pulses) was delivered to thalamus contacts at baseline and again following a trial of repetitive thalamus stimulation (low frequency (LF) < 10 Hz; high frequency (HF) > 100 Hz; 3-7 V; continuous, or duty cycle (1 minute on, 3-5 minutes off); lasting 80 minutes - 24 hours). TCEP amplitude in band (AIB) (25 to 500 millisec. post SPES; 2-10 Hz band) was used to quantify evoked potential amplitude. One-way ANOVA assessed statistical significance; 0.05 significance level. The study was approved by Mayo Clinic IRB. All five subjects with HF duty cycle stimulation had reduced TCEP AIB in associated thalamocortical circuit regions; one of these subjects had bilateral ANT electrodes and TCEP suppression was limited to the right hemisphere, with no consistent reduction in AIB in the left hemisphere (bifrontal onset seizures, maximal left; hypermotor semiology). Both subjects with continuous LF stimulation had reduced TCEP AIB. One subject had TCEPs assessed at baseline, and at 4-hr and 24-hr post HF duty cycle stimulation, with a dose response effect with greater AIB suppression with extended stimulation. One subject had continuous HF stimulation and did not have suppression of TCEP AIB. Pulvinar stimulation had comparable TCEP AIB suppression to ANT (HF duty cycle), seen in posterior quadrant/visual association regions, and limbic regions, respectively. In summary, we report thalamus stimulation frequency, duty cycle, and duration specific effects on TCEP AIB. TCEPs can provide temporally resolved measures of circuit connectivity and excitability, and may be a valuable tool for neuromodulation parameter titration.

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Poster

365. Epilepsy: Networks and Oscillations

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Topic: B.08. Epilepsy

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Title: A loss of function mutation in SLC35A2 disrupts the glutamatergic/GABAergic balance resulting in asynchronous, hypoactive neural networks

Authors: *D. LAI[1], P. SOSICKA[2], A. K. RESSLER[3], H. H. FREEZE[2], M. J. BOLAND[3], E. L. HEINZEN[1];
[1]Univ. of North Carolina, Chapel Hill, NC; [2]Sanford Burnham Prebys Med. Discovery Inst., La Jolla, CA; [3]Columbia Univ., New York, NY

Abstract: Germline genetic variants in *SLC35A2*, which encode a Golgi-localized, UDP-galactose transporter (UDPgalT) important for cellular glycosylation, have been implicated in one type of congenital disorder of glycosylation associated with intractable seizures and a rare X-linked developmental and epileptic encephalopathy. Our lab was the first to identify post-zygotically-acquired, loss-of-function (LOF) *SLC35A2* variants in brain tissue in intractable focal neocortical epilepsy. Despite this clear link to epilepsy, how *SLC35A2* variants contribute to the epileptic phenotype remain unknown. This study explores how *SLC35A2* LOF variants affect neurodevelopment and neural network connectivity using a human induced pluripotent stem cell (iPSC)-derived neuron model. Healthy male iPSCs (isogenic control) were CRISPR-edited to harbor a patient identified missense variant ($SLC35A2^{S304P/Y}$) or a frameshift indel ($SLC35A2^{-/Y}$). $SLC35A2^{S304P/Y}$ and $SLC35A2^{-/Y}$ encode LOF proteins shown by loss of UDPGalT expression, reduced migration of highly glycosylated proteins (e.g., N-cadherin) on Western blot, and reduced MAL-I lectin binding when compared to the isogenic control. While undergoing dorsal forebrain directed differentiation (dual Smad inhibition), we observed ß3-tubulin+ neurons and lumen formation in $SLC35A2^{S304P/Y}$ and $SLC35A2^{-/Y}$ cultures well before their appearance in the isogenic control suggesting *SLC35A2* variants undergo precocious neurodevelopment. To capture whether *SLC35A2* variants recapitulate the seizure phenotype, neural network activity was evaluated using multielectrode array (MEA). We observed clear, synchronous activity in neural networks formed by the isogenic control that was largely nonexistent in $SLC35A2^{S304P/Y}$ and $SLC35A2^{-/Y}$ networks when quantified by mutual information (P-value < 0.01) and spike train tiling coefficient (P-value <0.01). However, acute bicuculline treatment recovered some synchronous activity in *SLC35A2*-variant harboring networks suggesting a possible glutamatergic/GABAergic imbalance. Consistent with this finding, we detect increased GABAergic markers in $SLC35A2^{S304P/Y}$ and $SLC35A2^{-/Y}$ neurons compared to the isogenic control by qRT-PCR. Together, these data suggest that LOF variants in *SLC35A2* lead to aberrant neurodevelopment and favor the differentiation trajectory towards a GABAergic fate, which consequentially generates asynchronous, hypoactive networks. We are further exploring the underlying cell signaling pathways affected, single cell RNA sequencing to classify cell types, and the possibility of galactose therapy on reversing the phenotypes.

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Poster

365. Epilepsy: Networks and Oscillations

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Topic: B.08. Epilepsy

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Title: Cellular contributions of ictal population signal

Authors: *L. A. LAU, Z. ZHAO, S. N. GOMPERTS, K. J. STALEY, K. P. LILLIS; Massachusetts Gen. Hosp., Boston, MA

Abstract: The amplitude of ictal activity is a defining feature of epileptic seizures, but the determinants of this amplitude have not been studied. Pathological synchronization of neuronal activity is assumed to be the main driver of increased ictal amplitudes. However, cell intrinsic changes that drive pathological neuronal discharges may also contribute to ictal amplitude. Clinically, ictal amplitudes are measured electrographically (using e.g. EEG, ECoG, and depth electrodes), but these methods do not enable the assessment of the contributions of altered activity in individual neurons, as well as the synchronization of those neurons. We therefore measured ictal population activity by recording both local field potentials (LFP) and neuronal calcium levels, the latter measured using optical imaging of neuronal transgenic calcium-sensitive fluorophores. Spontaneous seizure activity was first assessed in an awake, behaving mouse model of focal cortical injury, using paired GCaMP6-based calcium imaging and LFP electrical recording. Spontaneous recurrent seizures were then measured in organotypic hippocampal slice cultures (OHSC), with a combination of GCaMP7 calcium imaging and LFP recordings. OHSC is an in vitro preparation in which all electrical and calcium signals could be unambiguously ascribed to the epileptic network. Population-averaged calcium activity was highly correlated with the time integral of electrographic field recordings, both in vivo and in vitro. We found that cell intrinsic changes were the largest contributor to the population signal during seizure onset. In other words, the network signal was not merely the summation of highly synchronous physiological activity, but that individual neurons were generating pathological discharges. During frank seizure, individual neurons continued to generate pathological activity, which also became highly synchronized. Interestingly, recruitment of newly active neurons accounted for only a small fraction of synchronous activity, particularly in vivo, revealing that network synchrony was largely due to reactivation of the same population of neurons. In conclusion, we introduce here a novel method for the quantification of the relative contributions of inter- versus intra-cellular changes (i.e. pathological synchronization and cell intrinsic pathological discharges) and demonstrate that both are important contributors to the overall population ictal signal.

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Poster

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Title: Synergistic positive feedback loops underlying a critical brain state transition

Authors: *A. TREVELYAN[1], R. GRAHAM[3], L. ALBERIO[2], R. R. PARRISH[4]; [2]Biosci. Inst., [1]Newcastle Univ., Newcastle Upon Tyne, United Kingdom; [3]UCL, London, United Kingdom; [4]BYU, Provo, UT

Abstract: Brain state transitions are a fundamental feature of neocortical physiology, and may also play a major role in its functional pathology. Currently, however, we lack a coherent understanding of how these occur. Different brain states may be stabilized either by recurrent network activity or cellular mechanisms, which constitute negative feedback towards these attractor states. The separation between these stable states is typically modelled as an unstable tipping point (a "saddle node") within the phase space. Critically, the transitions tend to be rapid, and while the transition itself is readily apparent from changes in brain rhythms, the precipitating cause may be invisible to passive recording methods. Identifying such cryptic influences may lead to improvements in predicting brain state transitions, which would have great practical significance in clinical practice.**Here we investigated cryptic network changes associated with the onset of seizure activity, in different acute ictogenic models. We employed an "active probing", optogenetic, stimulation paradigm to investigate seizure tipping points in different ictogenic models. We found that the ictal transition is associated with an all-or-nothing change in the response to an optogenetic stimulation in all models, indicative of a latent change in dendritic excitability. The enhanced dendritic response is accompanied by dendrite-wide Ca^{2+} entry, coupled with action potential bursting, thereby reinforcing the positive feedback into the network. Our data show how the precipitous nature of the transition can be understood in terms of multiple, synergistic positive feedback mechanisms: raised intracellular Cl^- and extracellular K^+, coupled to a reduced threshold for dendritic plateau potentials, and which in turn leads to a switch to pyramidal burst firing. These interlinked feedback loops draw together several major lines of epilepsy research and provide a framework for understanding other brain state transitions.**

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Poster

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Title: Memory-related cognitive mapping in hippocampal neural network: insights from volumetric analysis in temporal lobe epilepsy

Authors: S. HWANG[1], S. KIM[2], ***E. CHO**[4], S. MUN[3], E. KIM[3], Y. CHOI[2], S. KIM[1], H. KIM[1], H. KIM[1], S. KIM[1], J. LEE[1], H. LEE[5];
[1]Neurol., Ewha Womans Univ. Mokdong Hosp., Seoul, Korea, Republic of; [2]Neurol. and Med. Sci., [3]Neurol., Ewha Womans Univ. Sch. of Med., Seoul, Korea, Republic of; [4]Psychology, Univ. of California, Berkeley, Berkeley, CA; [5]Ewha Womans Univ. Mokdong Hospital, Ewha Womans Univ. Sch. of Medicine, Ewha Womans Univ., Seoul, Korea, Republic of

Abstract: Although temporal lobe epilepsy (TLE) involves both neuronal hyperexcitability and memory impairment, a thorough information of hippocampal neural network for cognitive mapping of specific memory components has not been clearly found. The current study aims to investigate (1) the association between hippocampal subfield volume and memory performance, (2) overlapping hippocampal subregions in epileptogenesis and memory impairment, and (3) hippocampal subfield mapping involving specific memory functions. For the study, we recruited total 63 subjects (33 left TLE patients and 30 controls) and assessed various neuropsychological tests for verbal and spatial memory: California verbal learning test (CVLT), Rey-Osterrieth complex figure test (RCFT), and object-place paired associate memory test (OPPA). High resolution 3-dimensional T1 MR images with the FreeSurfer 6.0 was utilized for quantitative measurements of 12 segmented subregional volumes. Analysis of covariance (ANCOVA) was evaluated for TLE and control groups, and Pearson's partial correlation was analyzed for correlations between hippocampal subfield volume and each cognitive function score. The overall volume of left hippocampus was significantly lower in left TLE group, compared to controls (P<0.001). Among 12 segmented subregions, subfield volumes of left granule cell-dentate gyrus (GC-DG), molecular layer (ML), CA4, CA1, and hippocampus tail were significantly reduced in left TLE group, compared to controls. In patient group, left GC-DG, ML, CA1, and CA4 were considered as overlapping hippocampal subregions, associated to both epileptogenesis and severe memory impairment. Specifically, for verbal memory and OPPA, poor performances were commonly correlated to smaller volumes in left CA1, GC-DG, and CA4, with a minor difference of left subiculum for verbal memory and left hippocampal tail for OPPA. In contrast, lower scores for visuospatial memory were associated to lower volumes of both right CA1 and left CA3 regions. To explain the mechanisms of hippocampal neural circuits for memory, the current research investigated the association between each hippocampal subregional volume and specific memory functions. By exploring volumetric analysis of

hippocampal subfields and its association to specific memory functions in TLE patients, the study provides fundamental research findings of memory-related cognitive mapping, involving functional organization of hippocampal neural networks.

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Poster

365. Epilepsy: Networks and Oscillations

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Title: Comparison of High Frequency Oscillation network centrality with proposed resection predicts surgical outcome in patients with intractable epilepsy.

Authors: *J. LIN[1], M. R. ZOCHOWSKI[2], K. A. SHEDDEN[3], W. C. STACEY[4];
[1]Univ. of Michigan, [2]Dept. of Physics and Biophysics Program, [3]Statistics, [4]Neurol., Univ. of Michigan, Ann Arbor, MI

Abstract: For decades, high frequency oscillation (HFO) has been viewed as a promising biomarker of the seizure focus with many studies having shown that regions with relatively high HFO rates tend to correspond with clinically defined seizure onset zones (SOZ). Nevertheless, it has been difficult to translate these findings into clinical care. We believe that one aspect of HFOs that has been underexplored is their network properties. Whereas previous work has focused on counting HFOs across different channels, we have found that HFOs often exist as a network discharge across coupled channels. Thus, HFOs could have important implications on the network structure of epilepsy. Here, we assessed the relationships between HFOs across the array of channels through a network-based approach. We hypothesize that features derived from the analysis of HFO networks will yield complementary information to HFO rate for determining surgical outcomes given SOZ and resected volumes (RV). To this end, we first characterize HFO functional networks through correlational analysis and then evaluate the impact of each channel within the network through a suite of centrality algorithms. As previously implicated with HFO rate, we show that centrality is highly associated with the SOZ in good outcome patients. Furthermore, we find that the resection of a greater proportion of the highly central tissues accounts for better surgical outcome. We then demonstrate how these tools can be used in prospective fashion to predict whether a patient will have a good outcome by comparing centrality measures with the SOZ and planned resected volume. These results not only provide a different approach to evaluating HFOs in epilepsy in the context of a network disorder, but also bolsters the relevance of HFOs as useful biomarker for clinical trials.

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Poster

365. Epilepsy: Networks and Oscillations

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Title: The reticular nucleus of the thalamus modulates the maintenance and termination of spike-wave discharges in the myelin mutant taiep rat

Authors: *J. M. IBARRA-HERNÁNDEZ[1,2], C. CORTES[2], J. R. EGUIBAR[2,3];
[1]Physiol. Department. Fac. of Med., Univ. Autónoma de Nuevo León, Monterrey, Mexico; [2]Lab. Neurophysiol. of Behavior and Motor Control. Inst. of Physiol., [3]Intl. Office, Benemérita Univ. Autónoma de Puebla, Puebla, Mexico

Abstract: The absence seizures are one type of generalized epilepsy which is characterized by a sudden loss of consciousness and with characteristic spike-wave discharges (SWDs) in the electroencephalogram. Absence seizures are due to a dysregulation in the activity of the gamma-aminobutyric acid receptor type A ($GABA_A$) in the thalamo-cortical network, which is modulated by the reticular nucleus of the thalamus (RNT). The aim of this study was to analyze the role of $GABA_A$ receptors in the RNT on the SWDs on adult male *taiep* rats. We used 6 months old *taiep* rats which were implanted with electrodes in the RNT, cerebral cortex, neck muscles, and the right eye orbit for simultaneous recordings. Gaboxadol was infused at 7.5, 15 y 30 µg/µL and bicuculline 50, 100, and 200 ng/µL doses into RNT through a guide cannula at 0800. We did four video-EEG recordings of 8 h, the first one was control, and three additional recordings using an increasing dose scheme every 72 h. We analyzed the frequency and duration of the SWDs, the power spectrum, and cerebral coherence in the thalamo-cortical network. We followed the NIH rules for the care and use of experimental animals and the protocol was approved by the institutional animal care and use committee. The administration of gaboxadol abolished the SWDs during the first 4 h ($P<0.001$) and decreases the frequency of SWDs from 6.25 to 4.69 Hz. After this, there were an increase in cerebral coherence in the thalamo-cortical network. In contrast, the bicuculline increased the number the SWDs ($P<0.05$) and decreased their duration ($P<0.001$), induced SWDs with a main frequency of at 7.2 Hz, and an increase in the cerebral coherence. In conclusion, our results show that RNT is decisive for the maintenance and is an important component for the termination of spike-wave discharges on *taiep* rat, an animal model of the leukodystrophy H-ABC.

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Poster

365. Epilepsy: Networks and Oscillations

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Finnish Epilepsy research association

Title: Development of a functional in vitro model in Dravet syndrome patient hiPSC-derived cortical neurons.

Authors: *R. MZEZEWA[1], T. HYVÄRINEN[1], J. SCHUSTER[2], N. DAHL[2], S. NARKILAHTI[1];
[1]Fac. of Med. and Hlth. Technol., Tampere Univ., Tampere, Finland; [2]Dept. of Immunol. genetics and pathology, Uppsala Univ., Uppsala, Sweden

Abstract: Intractable epilepsies that emerge in childhood, such as Dravet syndrome (DS), have limited response to current antiseizure medications (ASMs). DS patients are often typified by temperature sensitive seizures, along with other complexities such as intellectual disability. New therapeutic drugs are therefore needed, as most patients are unresponsive to current ASMs. However, to obtain this, relevant preclinical models are needed. DS is mainly caused by a mutation in the SCN1A gene which is crucial for generating and propagating action potentials. Heterozygous mutation in SCN1A gene leads to loss of sodium currents and action potentials, resulting in reduced neural excitation and ultimately seizure formations. Mouse models and in vitro based human induced pluripotent stem cell (hiPSCs) models have shown that the reduced sodium current density and impaired excitation due to the mutation, primarily affects the GABAergic inhibitory interneurons. Thus far most of the electrophysiological findings have been performed using whole-cell patch clamp, and only few have studied this phenomenon at the network level. Here we investigated the functional alteration in a hiPSC-derived neurons of Dravet Syndrome patients using microelectrode arrays (MEAs). We generated two different subtypes of neural cultures: glutamatergic and GABAergic enriched cultures from DS patient lines. When cultured on MEAs, we observe that DS neurons display a distinctive neuronal activity pattern compared to control neurons. Furthermore, preliminary data reveals that DS neurons portray more sensitivity in response to specific pharmacological treatments. This study highlights the applicability of disease modelling with hiPSCs and MEAs as a valuable platform to reveal underlying disease mechanisms.

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Poster

365. Epilepsy: Networks and Oscillations

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Title: Neuromodulatory Effects on Hippocampal Subnetwork by High Frequency Electrical Stimulation during Seizure-like Events in Entorhinal-Hippocampal Slice Recording

Authors: *Y. CHOI[1,2], S. KIM[1,2,3], Y. KIM[1], S. AN[4], S. JUN[4,5,6], H. LEE[1,2,3];
[1]Med. Science, Sch. of Med. and Ewha Med. Res. Inst., [2]Neurol., [3]Grad. Program in Syst. Hlth. Sci. and Engin., [4]Electronic and Electrical Engineering, Sch. of Engin., [5]Grad. Program in Smart Factory, [6]Dept. of Brain and Cognitive Sci., Ewha Womans Univ., Seoul, Korea, Republic of

Abstract: Hippocampus plays an important role for seizure generation in temporal lobe epilepsy (TLE), but network interaction inside the hippocampal subnetwork is not fully understood. In this study, we investigated the causal relationship of spatiotemporal dynamics during seizure-like events (SLEs) and the effects of high frequency electrical stimulation (HFS) to abort SLEs in hippocampal subnetworks.
We recorded neuronal activities from entorhinal-hippocampal slices including the dentate gyrus (DG), CA3, CA1, subiculum, medial entorhinal cortex (MEC) and lateral EC (LEC) in 14-day-old male Sprague-Dawley rats' brain using microelectrode array (MEA) recording system. When SLEs induced by 4-aminopyridine (4-AP) administration were clearly identified, we applied 130Hz HFS to layer III of the MEC through an external electrode-connected stimulator. Integrated net causal outflows (iOF) based on Granger Causality (GC) was calculated to identify the location and time of seizure onset. The directional network interaction was analyzed by iOF and GC values between all the electrode pairs, to investigate the strength and directional changes of in 7 different frequencies; delta (1-4Hz), theta (4-8Hz), alpha (8-13Hz), beta (13-25Hz), gamma (25-55Hz), ripple (65-200Hz), and fast ripple (250-500Hz).
The onset time of SLEs, which was identified by the maximum iOF values at the time of iOF increase greater than 3 standard deviations of baseline values, were observed 5.03 ± 3.72 sec earlier compared with the visual analysis. The iOF value was increased first in the area of seizure-onset electrodes, which was propagated into other channels along with the epileptic hippocampal subnetworks. After applied HFS on the MEC during SLEs, the mean duration of SLEs was significantly reduced in the stimulation group compared to the non-stimulation group

(59.02 ± 34.95 sec vs 237.65 ± 269.82 sec, respectively). Also, GC values were significantly different in SLEs before and after HFS (0.14 ± 0.08 vs 0.06 ± 0.03, respectively).
The present study showed that HFS on the MEC significantly reduced seizure durations. Also, GC values were altered by HFS, which could contribute disrupting the propagation of epileptic activities from seizure-onset zones to other hippocampal subregions. Insights into the directional network interaction of hippocampal subnetwork during seizure generation could provide more efficient therapeutic strategies to suppress seizures as well as in-depth understandings of the spatiotemporal dynamic mechanisms involving ictogenesis.

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Poster

365. Epilepsy: Networks and Oscillations

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Title: Seizure-induced neuronal plasticity in apnea and death

Authors: *A. BRODOVSKAYA[1], H. SUN[1], N. ADOTEVI[1], I. WENKER[2], K. MITCHELL[3], R. CLEMENTS[4], J. KAPUR[5];
[1]Dept. of Neurol., [2]Dept. of Anesthesiol., [3]Dept. of Chem., [4]Neurosci. Grad. Program, Univ. of Virginia, Charlottesville, VA; [5]UVA Neurol., Univ. Virginai Hlth. Sci. Ctr., Charlottesvle, VA

Abstract: Repeated generalized tonic-clonic seizures (GTCS) increase SUDEP risk (Sudden Unexpected Death in Epilepsy). We tested whether seizure-induced plasticity of excitatory transmission plays a role in seizure-related death. We induced 10 GTCS (PTZ, 50 mg/kg) every other day and simultaneously recorded EEG, breathing, and heartbeat. C57BL/6 mice, during initial seizures, had brief primary apneas at the seizure onset (2.68 ± 0.14s; n=17). With repeated seizures, some mice developed post-ictal secondary apneas (19.29 ± 5.04s) with recovery. Mice then had secondary apnea without recovery and gradual bradycardia until death (SUDEP-like event). Seizure latency decreased with repetition, from 5.35 to 2.9 min, and mice that died (33%) had longer seizures than survivors (24.73 ± 1.89s vs 19.90 ± 0.50s). The gradual development of secondary apneas, faster seizure onset, and longer seizure duration indicated seizure-induced plasticity leading to death. To determine the cellular basis of this plasticity, we did patch-clamp recordings from motor cortical layer 2/3 in a TetTag mouse that utilizes c-Fos activity to tag activated neurons with eGFP. Seizure-activated neurons were more excitable than non-activated

neurons (8 tagged, 5 untagged pyramidal neurons, n=6 mice) and had larger AMPA receptor-mediated sEPSCs (p=0.0336, 13 tagged, 16 untagged, n=12 mice), demonstrating enhanced AMPA transmission after a single seizure. We used an AMPA receptor- GluA1 subunit global knockout to determine the effect of repeated GTCS on apnea and death. 88.89% of GluA1 KO survived compared to 55.56% of WT littermates (Died: 1 out of 8 mice for KO vs 8 out of 19 for WT). Surviving KO mice never developed stage 6 seizures and had no secondary apneas compared to the WT mice. GluA1 KO had shorter seizures that remained relatively of the same duration (slope 0.16, 15.13 ± 0.44s), whereas seizures of WT mice gradually became longer (slope 0.44, 20.21 ± 0.71s, $p< 0.001$). Hippocampal kindling confirmed that GluA1 KO did not die and had less severe seizures (n=10 each). Even when GluA1 KO mice achieved a stage 5 behavioral seizure score, they could not maintain it compared to WTs. We next used activity reporter TRAP2 mice to visualize activated neurons in the motor cortex and brainstem nuclei after one seizure versus secondary apnea caused by repeated GTCS. We found increased neuronal activation in the specific regions (quantification ongoing).We conclude that GTC-seizure-induced excitatory transmission plasticity contributes to apnea and SUDEP-like death.

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Poster

365. Epilepsy: Networks and Oscillations

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 365.19

Topic: B.07. Network Interactions

Support: Wellcome Trust (102037)
Engineering and Physical 679 Sciences Research Council (A000026)

Title: Closed-loop optogenetic control of seizure activity and opsin optimisation using inhibitory opsins

Authors: *M. TURNBULL, F. MCLEOD, D. WALSH, M. BOCCHIO, A. TREVELYAN, F. E. N. LEBEAU, A. JACKSON;
Newcastle Univ., Biosci., Newcastle, United Kingdom

Abstract: We are investigating feedback control of oscillatory network dynamics by using local field potential (LFP) recordings to drive closed-loop optogenetic stimulation using inhibitory opsins (CLOSi$^-$). The aim is to develop reliable approaches to modulate normal and abnormal activity patterns, such as epilepsy, through the use of optogenetics as a method of treatment. Optogenetics enables real-time, continuous ‘closed-loop’ control of brain activity allowing precise manipulation of neural dynamics compared to alternative techniques such as electrical stimulation, which prevents concurrent electrical recording and, as such, is limited to open-loop or delayed closed-loop stimulation. There are, however, limitations in using inhibitory

optogenetic tools.
Progress has been made in developing Anion-conducting channelrhodopsins (ACRs) which are distinct from ion-pumping opsins, such as Halorhodopsin, due to their ability to conduct multiple ions during each photoreaction cycle. However, there have also been limitations of ACRs which lack specific membrane targeting. As such developments have been made to improve membrane targeting with the specific soma-targeted GtACR2 (stGtACR2).
Here we report a comparison of GtACR2(non-soma targeted) and stGtACR2 from in vivo experiments of freely moving rats that have been injected with either: Lenti-Syn-GtACR2-EYFP or AAV8-CKIIa-stGtACR2-FusionRed, as well as further in vivo experiments under terminal anaesthesia in non-human primates that have also been injected with either: Lenti-Syn-GtACR2-EYFP or AAV8-CKIIa-stGtACR2-FusionRed.
In freely moving rats, we investigated the effect of phase-shifted closed-loop optogenetic stimulation on the endogenous activity as well as on the duration of seizure-like events elicited by the tetanus toxin model of epilepsy. Closed-loop optogenetic stimulation produced a phase-shift-dependent modulation of LFP power of both background activity and seizure-like events relative to conditions with no stimulation. Whilst in the non-human primates, we performed comparable experiments but with seizure-like events being produced by focal application of 4-aminopyridine. Closed-loop optogenetic stimulation again produced phase-shift-dependent modulation of LFP power associated with endogenous activity and, seizure-like events relative to conditions with no stimulation. We conclude that closed-loop optogenetic stimulation with inhibitory opsins can reduce abnormal activity during seizure-like events, with potential application in the treatment of epilepsy.

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Poster

365. Epilepsy: Networks and Oscillations

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Title: Symmetric closed-loop transcranial electric stimulation prevents the generalization of temporal lobe seizures in rats

Authors: T. FOLDI[1,2], M. LORINCZ[1], M. HARANGOZO[1], G. KOZAK[1], A. J. NAGY[1], K. FURUGLYAS[2], Z. SOMOGYVARI[3,2], Z. CHADAIDE[2], ***A. BERENYI**[1,2,4];
[1]Univ. of Szeged, Univ. of Szeged, Szeged, Hungary; [2]Neunos ZRt, Szeged, Hungary; [3]Dept. of Theory, Wigner Res. Ctr. For Physics, Budapest, Hungary; [4]Neurosci. Inst., New York Univ., New York City, NY

Abstract: Transcranial electric stimulation (TES) using weak currents has been extensively used to influence brain activity. The induced electric fields can instantaneously and reproducibly alter neuronal spiking and consequent brain network activity. TES can be best utilized in disorders with sudden, significant electrographic changes such as epileptic seizures. Absence seizures generated within cortico-thalamo-cortical networks can be effectively terminated by closed-loop diffuse TES; however, secondarily generalized temporal lobe seizures proved to be more resistant to diffuse interference interventions. Spatially focused multiple site Intersectional Short-Pulse (ISP) stimulation can achieve increased intracerebral field strength without adverse peripheral effects. This non-invasive technique can interfere with pathological brain activity by predicting its internal dynamics in a validated animal model of temporal lobe epilepsy and restores normal network activity. To evaluate its utility, temporal lobe seizures were induced in rats by electrical kindling and each seizure was automatically detected and silenced by a closed-loop ISP stimulation. By comparing to closed-loop diffuse TES, we found that ISP with bilateral foci is more effective in early seizure termination. Our results suggest that transcranial closed-loop ISP stimulation is a powerful tool with high translational potential for intervening pathological oscillations and other neuropsychiatric disorders.

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Poster

365. Epilepsy: Networks and Oscillations

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 365.21

Topic: B.07. Network Interactions

Support: MH117155
NS109553

Title: A frequency-resolved human functional connectome of spontaneous cortical activity from composite SEEG

Authors: *B. Q. ROSEN[1], S. KAJFEZ[2], S. S. CASH[5], K. A. DAVIS[6], J. A. GONZÁLEZ-MARTÍNEZ[7], A. M. RASLAN[8], S. BEN-HAIM[9], J. J. SHIH[3], E. HALGREN[4];
[1]Neurosci. Grad. Prgm, [3]Dept Neurol., [4]Dept Neurosci., [2]UCSD, La Jolla, CA; [5]Dept Neurol, Mass Genl Hosp, Boston, MA; [6]Dept Neurol., Univ. of Pennsylvania, Philadelphia, PA; [7]Dept Neurosurg., Univ. of Pittsburgh, Pittsburgh, PA; [8]Dept Neurosurg., Oregon Hlth. & Sci. Univ., Portland, OR; [9]Neurosurg., Univ. of Califronia, San Diego, La Jolla, NY

Abstract: A map of cortical functional connectivity with high temporal and spatial resolution remains an elusive goal in human neuroscience. Functional magnetic resonance imaging (fMRI) is only sensitive to low frequency activity, Magneto- and electro-encephalography (M/EEG) sensors have significant spurious correlation due to overlapping sensor lead fields and the localization of these signals' sources relies on assumptions about spatial correlativity that have little experimental validation. Human stereo-electro-encephalographic depth-electrode recordings (SEEG) in intractable epilepsy patients provide focal, true-scale measurements of transcortical potentials but due to limited implant coverage, standard SEEG analyses can miss the larger-scale cortical patterns integral to commonly observed cognitive and neural phenomena. To address this limitation, SEEG data from several hundred patients from seven clinical sites was statistically integrated to produce a composite group-level complex cross-spectral matrix, a population-level statistical description of the underlying spatiotemporal activity, which when normalized yields the coherence matrix. In aggregate, all 180 areas of the HCP-MMP parcellation atlas are sampled in at least one hemisphere. Spontaneous interareal coherence was quantified as a function of frequency and interareal distance during behavioral states of N2 and N3 non-REM sleep as well as during putative active and quiescent waking. We find that coherence generally decays exponentially with fiber-tract distance, and decays over shorter distances at higher frequencies. The magnitude of phase offsets increases sub-linearly but monotonically with distance. In addition to being a normative atlas of spontaneous multivariate correlativity, the composite SEEG patterns presented may be useful for tuning M/EEG localization assumptions, by providing empirically validated and frequency specific falloff coefficients. While the scope of these composite cross-spectral estimates may be limited by the spontaneous brain states and patient population explored, the empirical characterizations of neural correlativity presented here, being frequency-resolved, volume-conduction-insensitive, and having broad spatial coverage, offer a unique view of spontaneous human neural activity.
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Poster

365. Epilepsy: Networks and Oscillations

Location: SDCC Halls B-H

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Program #/Poster #: 365.22

Topic: B.07. Network Interactions

Support: NIH Grant RF1MH114276-01

Title: State-dependent functional connectivity in epilepsy patients

Authors: *H. ZHU, E. M. HILLMAN, C. SCHEVON;
Columbia Univ. in the City of New York, New York, NY

Abstract: Large-scale functional brain connectivity in humans has been established using functional magnetic resonance imaging, however, the understanding of connectivity in the context of neural activity remains limited. In this study, we aim to investigate the origins of functional connectivity using the stereoelectroencephalography (sEEG) recordings, and its relationship to awake behavioral state transitions.
We recruited epilepsy patients (n=4) undergoing bilateral sEEG implantation with long-term video-EEG monitoring. A 60-minute period of wakefulness with a range of different behaviors was selected for each patient. The averaged power spectrum curves of each channel from all subjects were clustered into 4 groups. These groups were mapped to brain locations, demonstrating regional groupings in a bilateral, symmetric distribution. For each channel, we also computed the whitened dynamic spectral density in 2 second epochs. We then identified two “states" represented by shifts in the whitened power spectrum curve, representing resting vs active states.
To study the relationship between functional connectivity and behavioral state transition, we used language engagement as a target behavioral state. Behavioral states were manually labeled based on video analysis on a time scale of one second, producing a binary state vector (0: silence, 1: engaged in conversation). For each patient, we spatially clustered the binary state vectors of all channels into groups based on temporal similarity to investigate which brain regions show synchronized state switching, with results validated using permutation tests. The state vectors for each channel were compared to the observed behavioral state vector. Optimal clustering revealed 7 large-scale clusters each showing different modulation patterns of simultaneous state switching, suggesting distinct networks with different functional state switches. The majority of channels (77.3%) showed significantly higher correlation within their cluster than outside the cluster ($p<0.05$, permutation test, FWER corrected). 6 of the clusters included multiple brain sites. One cluster demonstrating the highest correlation with the behavioral state transition included bilateral, symmetric temporal lobe sites, a finding that was consistent across patients ($p=9.5e-10$ and $1e-8$, $n=2$, Kruskal-Wallis test). However, this relationship could not be consistently detected using conventional band-limited or wideband EEG synchronization measures.
Our results reveal the organization of connectivity networks identified by neural activity and demonstrate its underlying relationship to subject behavioral states.

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Poster

365. Epilepsy: Networks and Oscillations

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Topic: B.07. Network Interactions

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Title: Sensory Flicker Modulates Widespread Brain Networks via Circuit Resonance and Attenuates Interictal Epileptiform Discharges in Humans

Authors: *L. T. BLANPAIN[1], E. CHEN[3], J. PARK[4], M. Y. WALELIGN[5], R. E. GROSS[1], B. T. CABANISS[2], J. T. WILLIE[6], A. C. SINGER[5];
[2]Neurol., [1]Emory Univ. Sch. of Med., Atlanta, GA; [3]Emory Univ., Atlanta, GA; [4]Emory Univ., Altanta, GA; [5]Georgia Inst. of Technol., Atlanta, GA; [6]Washington Univ. In St Louis, Saint Louis, MO

Abstract: Modulating brain oscillations has therapeutic potential, but standard noninvasive interventions have a limited ability to target deep brain structures. One potential approach to drive brain oscillations is to harness the brain's natural tendency to follow the oscillatory components of sensory stimuli, which is thought to facilitate effective sensory processing. Repeated audiovisual stimulation, or sensory flicker, in mice modulates the local field potential (LFP) of higher cognitive regions involved in disease. However, using this method in humans requires a clear understanding of this naturally occurring process, including the extent of modulation across the brain and the mechanisms involved, as well as its effects on pathological neural activity. To address these gaps, we worked with 14 treatment-resistant epileptic patients undergoing presurgical intracranial monitoring and exposed them auditory and/or visual stimulation, including single pulses or flicker at frequencies spanning the 5.5-80Hz range.
We found that sensory flicker modulates LFP across widespread functional networks, including the limbic, default-mode, attention, and fronto-parietal networks. Next, we examined potential mechanisms by which sensory flicker affects neural activity. We determined whether sensory flicker responses were explained by linear superposition of single sensory pulse evoked potentials or entrainment of endogenous oscillations. Our data did not match any of the predicted outcomes from the superposition mechanism, while the entrainment hypothesis explained observed modulation in about up to half of recording locations. In fact, we observed a stronger amplitude of the flicker response to specific frequencies of stimulation, consistent with circuits resonating at preferred frequencies of stimulation. Finally, we tested the effects of flicker on interictal epileptiform discharges (IEDs), which represent pathognomonic activity in epilepsy that also occurs in other diseases such as Alzheimer's disease and autism. We found that short, 10-second exposure to sensory flicker mildly decreases IED frequency in our epilepsy patient population, suggesting that this modulation may have a meaningful impact on various diseases that manifest IEDs.
Our study determined with high spatiotemporal resolution that flicker modulates widespread networks in humans, including those involved in disease, likely via resonance of circuits

involved. Moreover, we showed that flicker decreases the rate of IEDs in our epilepsy patient population, advancing this approach as an advantageous non-invasive means of modulating the human brain for therapeutic purposes.

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Poster

365. Epilepsy: Networks and Oscillations

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 365.24

Topic: B.07. Network Interactions

Title: Median nerve stimulation can induce a coherent high frequency response

Authors: *S. DAS[1], D. J. ZHOU[2], O. TARASCHENKO[2], V. GUMENYUK[2], S. V. GLISKE[1]; [1]Neurosurg., [2]Neurolog. Sci., Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: High frequency oscillations (HFOs) in intracranial EEG are a promising biomarker of epileptic tissue. Spontaneous HFOs have also been observed in interictal MEG data, but techniques for analysis of HFOs in MEG are still in the early stages. We hypothesize that stimulus mapping is an ideal signal for developing MEG HFO techniques as the HFOs are expected to coincide with the lower-frequency evoked response. The objective of this study was to test that hypothesis by assessing whether the stimulus triggered average increases in the high frequency power at the same location and latency as the lower frequency response. We selected median nerve stimulation and analyzed data from N=5 patients receiving this as part of standard clinical MEG scans. The research was conducted under an approved IRB protocol. Each subject received approximately 100 electrical stimulations to the median nerve of each arm, with 10 ms pulses every 500 ms. Following standard clinical procedures, the stimulus threshold was adjusted to cause a visible twitch of the thumb. MEG data were acquired at 1 kHz and analyzed in MNE-Python. Independent component analysis was used to remove ocular and heart artifacts. Gradiometer data were filtered into the 8-40 Hz (low frequency) or 80-300 Hz (high frequency) bands, epoched from -50 ms to 250 ms around each stimulation, and then averaged. All subjects had a discernable evoked response over their primary somatosensory cortex. The instantaneous power was assessed using the magnitude of the Hilbert transform, and the average power was computed at baseline (-50 to -10 seconds before the stimulus) and during the evoked response (30-50 ms after the stimulus). In all five subjects, the power in the high frequency band during the evoked response was significantly higher than at baseline ($p<10\text{-}25$, Wilcoxon Sign Rank). Additionally, the Pearson correlation between the power in the low and high frequency bands during the evoked response was >0.94 in all five subjects. Thus, we observe a distinct and

coherent high frequency response with strong amplitude-to-amplitude coupling between the low and high frequency data.

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Poster

365. Epilepsy: Networks and Oscillations

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 365.25

Topic: B.07. Network Interactions

Support: Tiny Blue Dot Foundation

Title: Changes in functional network responses to intracranial electrical stimulation across waking, sedative and unconscious states

Authors: F. TIAN[1], P. KAHALI[1], R. ZELMANN[1], G. BALANZA VILLEGAS[1], A. C. PAULK[1], N. PELED[2], D. SOPER[1], L. A. SANTA CRUZ MERCADO[1], R. A. PETERFREUND[1], L. S. AGLIO[3], G. COSGROVE[3], Z. M. WILLIAMS[1], R. M. RICHARDSON[1], S. S. CASH[1], P. L. PURDON[1];
[1]Massachusetts Gen. Hospital/Harvard Med. Sch., Boston, MA; [2]MGH/HST Martinos Ctr. For Biomed. Imaging, Charlestown, MA; [3]Brigham and Women's Hospital/Harvard Med. Sch., Boston, MA

Abstract: It is known that resting-state brain network dynamics vary across different states of arousal, but the ability of the brain to respond to perturbations during these states, elicited by external stimuli or by invasive stimuli, is not well understood. Here we use cortico-cortical evoked potential (CCEP) to characterize changes in brain network activity during waking, sedative and unconscious states induced by propofol. Our subjects are patients with medication-refractory epilepsy implanted with intracranial depth electrodes for detection of seizure onset zone. We recorded intracranial EEG during either propofol-induced monitored anesthesia care (MAC) sedation or general anesthesia prior to the electrode removal surgery. For the MAC cases, we recorded signals for 5 minutes during the waking state, and then 10 minutes after the first dose of propofol. For general anesthesia, we first recorded signals for 5 minutes during the waking state. A bolus of propofol was then given to the patients to induce general anesthesia. We continued recording until 5 minutes after the loss of consciousness. Each patient received single-pulse direct electrical stimulation (SPES) applied in random order across 4 or 5 different regions of the brain during the whole process.We collected the data from 3 patients under MAC sedation and 16 patients under general anesthesia. We analyzed the CCEP in response to SPES for each bipolar channel. Responses were considered as significant if the average waveform poststimulation (50~500ms) exceeded a statistical threshold of 6 SDs of prestimulation (-500~-3ms). Data from 12 subjects under general anesthesia showed that the number of channels with

significant response decreased with frontal channel stimulation. However, with posterior channel stimulation, the number of channels with significant response showed little change. An electrical labeling algorithm was used to identify the anatomical label for each bipolar channel. Electrodes from the 12 subjects were distributed across 35 anatomical locations. A score was calculated for each anatomical location to show the percentage of channels with significant response. We found a decrease of responsive channels all over the brain when middle frontal channels were stimulated. However, stimulating the cingulate cortex induced a decrease of responsive channels in anterior regions of the brain, and an increase of responsive channels in posterior regions, suggesting that the brain's response to electrical stimulation is location specific. This study will contribute to our understanding of how brain processes sensory information under different states of anesthesia-induced altered arousal.

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Poster

365. Epilepsy: Networks and Oscillations

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 365.26

Topic: B.07. Network Interactions

Title: Network of higher order sensorimotor function induced by intracerebral stimulation of periventricular nodular heterotopia: a single patient sEEG case study

Authors: *R. C. EVANGELISTA, A. M. WEAKLEY, A. N. MOGHBEL, S. NAMBOODIRI, D. M. BRANDMAN, K. J. PARK, J. KENNEDY, A. ALIZADEH, S. C. TOPRANI;
UC Davis Med. Ctr., Sacramento, CA

Abstract: Rationale: Periventricular nodular heterotopia (PVNH) is characterized by the presence of abnormal gray matter along the lateral ventricles. Functionality of PVNH has rarely been directly tested. We performed functional examination during electrical stimulation of periventricular nodules and along cortical trajectories. This clinical case report demonstrates sensorimotor brain function within a PVNH region. **Method**: A 24-year-old, right-handed, Caucasian female with refractory focal epilepsy, PVNH, and atypical overlying gyral anatomy underwent stereoelectroencephalography (sEEG) for treatment planning, during which electrical stimulation mapping (ESM) was performed. 12 electrodes were implanted throughout the right hemisphere covering sensorimotor and premotor cortices as well as the anterior cingulate areas. The electrode tips penetrated the PVNH linearly along the right lateral ventricle with cortical

entry points vastly distributed throughout the right hemisphere at all lobes. During electrical stimulation across electrodes with parameters for mapping, non-verbal attention and working memory, visual field, and sensorimotor testing was performed and behavioral responses were recorded. **Result:** Stimulation of PVNH nodules, white matter, and patient's atypical cortical tissue in regions correlating with sensorimotor cortices in a normal brain induced sensorimotor symptoms such as spontaneous clenching and withdrawal of limbs. Symptoms were primarily lateralized to the left side of her body, although symptoms on her right face also occurred. Stimulation of nodules, white matter, and cortical tissue in regions that would correlate with emotion regulation in a normal brain elicited emotional distress. Pain was denied. Stimulation of overlying tissue revealed unexpected functional anatomy of right hemisphere white matter and gyri. **Conclusion:** We describe functionality in the nodules distinct from what has been previously reported that demonstrates variability and individuality of neurologic function that can be present within nodules. Despite the patient's atypical gyral anatomy, ESM revealed higher-order sensory, motor, and emotional functionality with stimulation of nodules and overlying cortex at corresponding regions in a normal brain. Such findings bear implications for surgical treatments such as resection or ablation of PVNH nodules in epilepsy patients. Determination of both extraneous as well as necessary function in the nodules as well as of the presence of that function in other brain regions is important prior to surgical removal of nodules to minimize functional losses with treatment and optimize outcomes.

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Poster

365. Epilepsy: Networks and Oscillations

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Program #/Poster #: 365.27

Topic: B.07. Network Interactions

Support: DARPA N66001-14-2-4032
NIH U01-NS113198

Title: Frequency-specific response to intracranial theta-burst stimulation in the human hippocampal-cortical network

Authors: *M. S. HERMILLER[1], U. R. MOHAN[2], J. JACOBS[1];
[1]Columbia Univ., New York, NY; [2]NIH, Bethesda, MD

Abstract: The hippocampal interacts with a distributed set of brain regions, including the medial temporal, ventromedial prefrontal, lateral parietal, dorsal thalamus, precuneus and posterior medial cortex, to give rise to the hippocampal-cortical network (HCN). Theta-band activity is a putative neural mechanism that coordinates the interactions between the hippocampus and

regions of the HCN, facilitates interregional communication, provides content for binding into memory, and supports recreation of content during retrieval. Proposed anatomical and functional subsystems of the HCN, including the anterior-temporal (AT) and posterior-medial (PM) subnetworks, have been proposed to support specific memory processes. Additionally, there is evidence of distinctions in theta-band activity in the human hippocampus, with low theta frequencies (~2-4 Hz) more prominent in the anterior hippocampus, and higher theta (~5-8 Hz) in the posterior hippocampus. Here, we tested the hypothesis that the AT and PM subnetworks would respond preferentially to intracranial brain stimulation delivered at different theta frequencies. Electrocorticographic recordings were collected from neurosurgical epilepsy patients while simultaneously delivering direct electrical theta-burst stimulation varying in theta frequency (i.e., 3,4,5,6,7,8 Hz). We focused our analyses on the electrodes located in the AT (150 electrodes) and PM (40 electrodes) subnetworks. We measured the change in connectivity between each recording electrode to the stimulation electrode, as well as the change in amplitude and frequency of narrowband oscillations before and after stimulation events. We found that electrodes in the AT exhibited power in lower theta-frequencies (~2-4 Hz) following stimulation relative to pre-stimulation, whereas electrodes in the PM showed an increase in higher theta (~5-8 Hz) power following stimulation, and these changes were related to baseline connectivity between the recording electrodes and the stimulation electrode. These results indicate that the AT and PM subdivisions of the human HCN may respond differently to theta-burst stimulation, possibly related to the endogenous theta oscillations identified in the anterior versus posterior hippocampus. Our findings provide insight into how brain stimulation may be used to target endogenous network oscillations and motivate future interventions for therapies utilizing brain stimulation to treat disorders and impairments with an associated disruption in network oscillatory activity.

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Poster

365. Epilepsy: Networks and Oscillations

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Program #/Poster #: 365.28

Topic: B.07. Network Interactions

Title: Spatial and temporal correlations in human cortex are inherently linked, predicted by functional hierarchy, vigilance state and antiepileptic drug load

Authors: *C. MEISEL, P. MÜLLER;
Charité Berlin, Berlin, Germany

Abstract: The ability of neural circuits to integrate information over time and across different cortical areas is believed an essential ingredient for information processing in the brain. Temporal and spatial correlations in cortex dynamics have independently been shown to capture these integration properties in task-dependent ways. A fundamental question remains if temporal

and spatial integration properties are somehow linked and what internal and external factors shape these correlations to govern cortical information integration. Previous research on spatio-temporal correlations has been limited in duration and coverage, thus providing only an incomplete picture of their interdependence and variability.
Here, we use long-term invasive EEG data to comprehensively map temporal and spatial correlations according to cortical topography, vigilance state and drug dependence over extended periods of time. Specifically, we assessed spatial and temporal correlations (STCs) in multiday, invasive EEG recordings from 23 patients with epilepsy undergoing presurgical monitoring. STCs were determined at the individual channel level of power band fluctuation in the high gamma band. A validated algorithm classified vigilance and slow-wave sleep (SWS) states. Anatomical channel locations were determined from MNI coordinates. Evaluations included high and low ASM drug days (24 h each).
We report that temporal and spatial correlations in cortical networks are intimately linked, decline under antiepileptic drug action, and break down during slow-wave sleep. Further, we report temporal correlations in human electrophysiology signals to increase with the functional hierarchy in cortex. Systematic investigation alongside a companion neural network model links these findings to dynamics being poised near a critical point.
Our results provide novel mechanistic and functional links between specific measurable changes in the network dynamics relevant for characterizing the brain's changing information processing capabilities.

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Poster

365. Epilepsy: Networks and Oscillations

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 365.29

Topic: B.07. Network Interactions

Title: Tracking interhemispheric electrical cortical stimulation responses

Authors: C. KAPELLER[1], K. KAMADA[3], M. JORDAN[1], F. CAO[2], M. CHING[2], ***C. GUGER**[4], K. MAYR[5];
[1]g.tec medical engineering GmbH, Schiedlberg, Austria; [2]g.tec medical engineering GmbH, Albany, NY; [3]Neurosurg., Megumino Hosp., Eniwa, Japan; [4]g.tec neurotechnology GmbH, Schiedlberg, Austria; [5]g.tec medical engineering GmbH, Albany, NY, Albany, NY

Abstract: *Introduction:* Focal and generalized epilepsy may involve networks spreading focal seizure activity. Localizing the seizure onset zone (SOZ) on the cortex is an important part of the pre-surgical evaluation. Electrical cortical stimulation of the SOZ may elicit cortico-cortical evoked potentials (CCEP) on distant brain locations, revealing pathological networks that can be targeted for disconnection. A combined method of CCEP mapping and tractography can identify connecting fibers in such networks. However, this may be difficult for interhemispheric

connection due to the limitations of single tensor fiber tracking. *Objective* This work investigates whether two-tensor UKF fiber tracking can reveal connected interhemispheric cortex locations, which have been identified by CCEP. *Methods:* A 24y-old man underwent corpus callosotomy as part of the treatment of intractable epilepsy. The SOZ was located on the right pars triangularis. Single pulse electrical stimulation of the SOZ was performed with 60 monophasic, alternating pulses of 300µs and 15mA. Elicited CCEPs were recorded at 2400Hz and analyzed between -100 to 800ms relative to stimulation onset. Two-tensor UKF tractography was applied on DTI imaging, obtained by a 3T MRI, using one seed/voxel, FA>=0.15, mean signal GA>=0.1, and a maximum fiber length of 250mm. A sphere with a 6mm radius around the SOZ and related CCEP locations defined the region of interest (ROI). *Results:* The corpus callosum (CC) and four locations on the left pars triangularis with high CCEPs were selected for tractography. Nine fiber tracts connected the ROIs through the CC, three of them propagated through the mid-anterior and the rest through the anterior CC. The CCEPs were monitored multiple times along the corpus callosum, from the central to the anterior CC, and diminished after resection of the anterior CC. *Conclusion:* Intra-operative CCEP monitoring and two-tensor UKF show connecting fibers of pathological networks. This may help minimize the resected volume necessary to disconnect epilepsy networks.

Disclosures: **C. Kapeller:** A. Employment/Salary (full or part-time):; Full. **K. Kamada:** None. **M. Jordan:** A. Employment/Salary (full or part-time):; full. **F. Cao:** A. Employment/Salary (full or part-time):; full. **M. Ching:** A. Employment/Salary (full or part-time):; full. **C. Guger:** A. Employment/Salary (full or part-time):; CEO. Other; g.tec medical engineering is the manufacturer of devices used in this study. **K. Mayr:** A. Employment/Salary (full or part-time):; Full.

Poster

365. Epilepsy: Networks and Oscillations

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 365.30

Topic: B.07. Network Interactions

Support: MH097216
P2SKP3_161675

Title: Prrt2 is a driver of circuit hyperconnectivity and neuropsychiatric phenotypes in 16p11.2 microduplication mice.

Authors: *M. FORREST[1], M. DOS SANTOS[1], N. H. PIGUEL[4], Y.-Z. WANG[1], S. YOON[2], V. BAGCHI[5], L. E. DIONISIO[6], N. HAWKINS[7], M. S. LEDOUX[8], J. A. KEARNEY[3], J. N. SAVAS[1], P. PENZES[2];
[2]Physiol., [3]Pharmacol., [1]Northwestern Univ., Chicago, IL; [4]Physiol., Feinberg Sch. of Med., Chicago, IL; [5]Northwestern, Chicago, IL; [6]UCLA, Los Angeles, CA; [7]Pharmacol., Northwestern Univ. - Chicago, Chicago, IL; [8]Neurosci., Univ. of Memphis, Memphis, TN

Abstract: Neuropsychiatric disorders (NPDs) such as autism and schizophrenia have overlapping symptoms and genetics, but the biological basis of shared risk remains obscure. Copy number variants (CNVs) confer risk to a wide variety of NPDs providing an entry point to understand the mechanisms of this shared susceptibility. We used a mouse model of the *16p11.2* microduplication (*16p11.2*$^{dup/+}$) to uncover molecular and circuit phenotypes which may predispose to multiple disorders, and examined genes within the locus capable of disease reversal. Quantitative proteomics of cortical membranes revealed alterations to synaptic protein networks and products of diverse NPD risk genes in *16p11.2*$^{dup/+}$ mice. In particular, an epilepsy-associated protein subnetwork was dysregulated in *16p11.2*$^{dup/+}$ mice and human NPDs, suggesting that epilepsy endophenotypes may be relevant for causing shared risk. We investigated underlying circuit properties in *16p11.2*$^{dup/+}$ mice and found they exhibited hypersynchronous activity, enhanced network glutamate release and were susceptible to seizures. We hypothesized that a regulator of the synaptic and epilepsy-associated protein networks could have an important impact on pathophysiology. Human brain co-expression and interactome analysis revealed PRRT2 as a major hub in the dysregulated epilepsy subnetwork. Remarkably, restoring *Prrt2* copy number to *wild-type* levels rescued aberrant circuit and behavioral deficits in *16p11.2*$^{dup/+}$ mice. We show that proteomics and network biology can identify important disease hubs in complex CNVs, and reveal molecular and circuit phenotypes which may predispose to shared NPD risk.

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Poster

366. Microglia and Neurodegeneration

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 366.01

Topic: B.09. Glial Mechanisms

Support: Brain Canada (0030547)
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SynAD/ADRD Studentship

Title: Microglia activation in Huntington's disease and modulatory effects by the ganglioside GM1

Authors: *N. STEINBERG[1,2], D. GALLEGUILLOS[2], A. ZAIDI[2], O. SUCHOWERSKY[1,3], S. SIPIONE[1,2];
[1]Neurosci. and Mental Hlth. Inst., [2]Pharmacol., [3]Med. Genet., Univ. of Alberta, Edmonton, AB, Canada

Abstract: Microglia are the immune cells of the brain. They fight pathogens and play important roles in brain health, by removing damaged cells, remodelling synapses, and secreting molecules that modulate neuronal functions and survival. In many neurodegenerative disorders, loss of protective microglia functions, together with dysregulated microglia inflammatory activity, contribute to neurodegeneration. Microglia activation has also been shown in Huntington disease (HD), an inherited neurodegenerative disease caused by a mutation in the *Huntingtin* (*HTT*) gene. However, whether this activation is primarily due to endogenous expression of mutant HTT (mHTT) or is secondary to neurodegeneration in microglia is still unclear. Therefore, we aimed to characterize the activation of isolated primary HD microglia in culture, in the absence of confounding effects deriving from the brain environment. Furthermore, we sought to determine if HD microglia activation is affected by administration of GM1, a sialic acid-containing glycosphingolipid with therapeutic effects in HD animal models, which was recently shown to have anti-inflammatory properties on wild-type (WT) microglia. We measured the expression and secretion of pro-inflammatory cytokines and nitrite levels in murine WT and HD microglia in response to activation with inflammatory stimuli (LPS, LTA and necrotic cells). No differences were found between microglia of the two genotypes. However, when we studied the expression of pro-inflammatory cytokines in monocyte-derived macrophages from HD patients, used as a surrogate for human microglia, we found that M1-polarized HD macrophages expressed higher mRNA levels of pro-inflammatory cytokines compared to healthy controls. Repeated HD mouse microglia stimulation with LPS, revealed a potential impairment in the development of tolerance in HD microglia, which could contribute to microglia activation in HD. GM1 administration following microglia activation reduced RNA and protein levels of pro-inflammatory cytokines in mouse microglia and human macrophages of both genotypes. GM1 also restored tolerance in HD microglia. Overall, our study suggests that expression of mHTT in HD microglia by itself is not sufficient to induce microglia activation or to increase microglia response to inflammatory stimuli. Although we observed an impaired induction of tolerance in HD microglia, which could predispose HD microglia towards chronic activation, an external stimulus (LPS) was still needed to activate microglia. GM1 administration significantly decreased microglial activation, an effect that might contribute to the therapeutic properties of GM1 in HD.

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Poster

366. Microglia and Neurodegeneration

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 366.02

Topic: B.09. Glial Mechanisms

Support: NS12 4226

Title: Aggregated alpha synuclein induces proinflammatory signaling events concomitant with fyn kinase activation in murine macrophage cells

Authors: *C. KARTHICK, C. JANARTHANAM, M. PARKER, H. JIN, G. ZENITSKY, A. VELLAREDDY, A. G. KANTHASAMY, A. KANTHASAMY;
Univ. of Georgia, Athens, GA

Abstract: Parkinson's disease (PD) is an age related disorder characterized by pronounced neuroimmune dysfunction. Aggregated alpha-synuclein (α-syn), a major component of Lewy body has been shown to trigger microglia mediated neuroinflammation and infiltration of peripheral immune cells into the CNS yet the molecular mechanisms underlying deregulated monocyte/macrophage activation remains poorly understood. Previously, we demonstrated that early stage microglia mediated neuroinflammation precedes nigral dopaminergic neurodegeneration in a synucleinopathy mouse model of PD. Herein, we have used both in vitro cell culture systems as well as in vivo LPS neuroinflammation mouse model to investigate the role of innate immune signaling events in monocyte/macrophage activation response as well as their recruitment into the brain. We find that following stimulation of murine macrophage cells, RAW264.7 cells, with aggregated α-syn, FYN kinase was significantly upregulated concomitant with striking induction of chemokine receptors namely CCR1, CCR3 and CCR5. Additionally, this response was accompanied by elevated mRNA expression of proinflammatory signaling mediators namely IL-1β, IL-6, TNF-α production suggesting that aggregated α-syn skews monocyte polarization toward an M1 like proinflammatory phenotype. Furthermore using an LPS model we further demonstrated that striatal CSF1R upregulation was accompanied by pronounced CD14+ monocyte infiltration as well as elevated expression of NLRP3 inflammasome related activation markers. Taken together our findings indicate the involvement of both innate and peripheral immune response in preclinical models of PD and suggest that targeting innate immune signaling events may serve as a novel disease modifying therapy for this disease.

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Poster

366. Microglia and Neurodegeneration

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 366.03

Topic: B.09. Glial Mechanisms

Title: The mTOR expression in microglia and astrocyte of temporal lobe epilepsy model

Authors: *M. KIM[1], H. PARK[1], Y. YI[3], J. KANG[2,4], D. KIM[1];
[2]Chungnam Natl. Univ., [1]Chungnam Natl. Univ., Daejeon, Korea, Republic of; [3]Dept. of

Pediatrics, Col. of Med., Hallym Univ. and Gangdong Sacred Heart Hosp., Seoul, Korea, Republic of; [4]Chungnam Natl. Hosp., Daejeon, Korea, Republic of

Abstract: Epilepsy is a chronic disease that causes repetitive seizures by generating excessive electrical discharge due to functional and structural abnormalities of neuronal cells. Recent studies have implicated those reactive astrocytes and microglia play a key role in epilepsy. When extensive pathological activation in astrocytes and microglia at the injury core, it contributes to epileptic seizures and causes neuronal hyperexcitability. The mTOR signaling pathway, which is involved in brain development, neuronal morphology, and plasticity, is important for understanding the mechanism of epilepsy, following that a consistent activation of the mTOR pathway in astrocytes and microglia have been reported. In this study, we used Kainic acid-induced status epilepticus model and recorded behavioral SE induction rates. After 7 days, we confirmed that mTOR expression was increased not only reactive astrocyte but also microglia in the CA3 regions of hippocampus, accompanied by mossy fiber sprouting. For therapeutic applications targeting mTOR, we applied mTOR targeting siRNA-encapsulated PLGA nanoparticles and confirmed the suppression of seizure activities and inflammatory responses. Taken together, our data suggests that down-regulation of mTOR with nanoparticle applications significantly ameliorated reactive astrocytes and microglia activation, and neuroinflammation in the hippocampus, resulting the alleviation the epileptic seizures.

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Poster

366. Microglia and Neurodegeneration

Location: SDCC Halls B-H

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Program #/Poster #: 366.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AG062021
AG053150

Title: Preclinical evaluation of the exposure and safety of a small molecule inhibitor of tau self-association

Authors: J. G. MOE[1], *E. DAVIDOWITZ[1], B. S. LEVINE[2];
[1]Oligomerix, Inc., White Plains, NY; [2]Levine Tox Consulting, Chicago, IL

Abstract: The overall goal of this program is to develop a disease modifying therapeutic for Alzheimer's disease and related dementias characterized by the formation of tau aggregates. We have shown that tau oligomers cause disruption of neuronal signaling and inhibit the formation of memory in mice (Moe J. et al., 2010; Fá M et al., 2016).We also found that certain forms of tau oligomers are toxic when applied to cultured neurons, whereas tau monomer was not toxic at the same concentrations (Tian H et al., 2013). Tau self-association was chosen as the target for drug discovery as it is necessary for the initiation and growth of tau aggregates. A small

molecule approach was used to enable access to the CNS and cells where tau is aggregating in disease, with good oral bioavailability and ease of manufacture and distribution. We have demonstrated *in vivo* efficacy in studies with lead compound in the human tau/htau (Davidowitz EJ et. al., 2020) and P301L tau JNPL3 mouse models of tauopathy. The purpose of these IND enabling studies was to characterize the safety and pharmacokinetic (PK) profile of OLX-07010 in established in-vitro and in-vivo pre-clinical models to inform the design of human studies. Single dose oral PK studies were performed in rodents and non-rodents. The in-vitro profiling of metabolites generated in hepatocytes from rat, dog and human was performed. In-vitro cytochrome P-450 induction and inhibition and transporter studies were performed using human liver hepatocytes and standard cell systems, respectively. 28-day GLP toxicity studies with 28-day recovery were performed in rats and dogs. In the genotoxicity program, OLX-07010 was negative in the Ames test and Micronucleus Assays. In PK studies, OLX-07010 demonstrated good oral bioavailability and a moderate half-life in multiple species. The drug is extensively metabolized in vitro by rat, dog, and human hepatocytes, and does not appear to be a substrate or inhibitor for human transporters. In addition, it did not inhibit cytochrome P450s, but did result in some induction. In 28-day rat and dog GLP toxicity studies, the toxicology of OLX-07010 was similar in both species, as the liver was the only target organ. No adverse effects were observed, and the NOAEL was the highest dose tested in each species. Based on the above-described non-clinical pharmacokinetic and associated in vitro studies, the absence of cytochrome P450 inhibition with minimal induction, no effects on transporters, and the relatively modest toxicity seen in the 28-day GLP toxicity studies, OLX-07010 appears to be an excellent candidate for clinical development for neurodegenerative diseases.

Disclosures: **J.G. Moe:** A. Employment/Salary (full or part-time):; Oligomerix, Inc.. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIA, NIH. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Oligomerix, Inc. **E. Davidowitz:** A. Employment/Salary (full or part-time):; Oligomerix, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Oligomerix, Inc. **B.S. Levine:** A. Employment/Salary (full or part-time):; Levine Tox Consulting. F. Consulting Fees (e.g., advisory boards); Oligomerix, Inc..

Poster

366. Microglia and Neurodegeneration

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 366.05

Topic: B.09. Glial Mechanisms

Title: Identification of THIK-1 as a therapeutic target for Alzheimer’s disease and characterisation of a selective and novel blocker

Authors: *M. DEY, B. OSSOLA, A. ROWLAND, H. HUNTER, R. BURLEY, C. BENDER, D. BARKER, D. CADWALLADR, L. DICKSON, J. LAWRENCE, J. HARVEY, M. LIZIO, X. XU, E. KAVANAGH, T. CHEUNG, S. SHEARDOWN, K. MATTHEWS, K. DOYLE, K. PAGE, J. POWELL, N. L. BRICE, R. W. BÜRLI, M. B. CARLTON, L. A. DAWSON; Cerevance Ltd., Cambridge, United Kingdom

Abstract: Neuroinflammation is a common underlying pathological feature of most neurological disorders and chronic neuroinflammation is evident in most, if not all, progressive neurodegenerative diseases. The biochemical processes involved are complex, but several pathways have been identified as key to the disease processes and as potential intervention points for therapeutic approaches; one such process is the NLRP3 inflammasome cascade. In the brain, NLRP3 functions as a damage-associated molecular patterns (DAMPs) receptor resulting in microglial release of the proinflammatory cytokine IL-1β. Evidence suggests that NLRP3 activation involves changes in intracellular potassium actuated by potassium channels. Our proprietary Nuclear Enriched Transcript Sort Sequencing (NETSseq) platform, which allows for deep sequencing of purified cell types from human post-mortem brain tissue, demonstrated the highly specific expression of the tandem pore domain halothane-inhibited K+ channel 1 (THIK-1) in microglia compared to other cell types in the human brain. Also, NETSseq showed a significant increase in expression of THIK-1 in microglia isolated from cortical regions of brains with Alzheimer's Disease (AD) relative to control donors.

Herein we report the discovery and pharmacological characterisation of C101248, the first potent and selective small-molecule inhibitor of THIK-1. C101248 showed a concentration-dependent inhibition of both mouse and human THIK-1 (IC50: ~50 nM) and was inactive against K2P family member TREK-1, the closest homologue to THIK-1, TWIK-2 and Kv2.1. THIK-1's constitutive activity was confirmed by patch clamp electrophysiology and blocked by C101248 at a similar potency. C101248 blocked THIK-1 in isolated microglia and prevented NLRP3-dependent release of IL-1β to the same degree as a genetic depletion of the channel. Importantly, C101248 showed no reduction of IL-1β when treating THIK-1-depleted microglia, underscoring its selectivity and mode of action.

In conclusion, we have demonstrated an AD related increase in the microglial specific expression of THIK1 suggesting that this channel may be a potential therapeutic target. For the first time, we herein report C101248 as a novel, potent and selective THIK-1 blocker that prevented the NLRP3-dependent release of IL-1β from microglia, promoting its further use to investigate the biology of THIK-1 in AD.

Disclosures: **M. Dey:** A. Employment/Salary (full or part-time):; M.D. is employed by Cerevance Ltd. **B. Ossola:** A. Employment/Salary (full or part-time):; B.O. is employed by Cerevance Ltd. **A. Rowland:** A. Employment/Salary (full or part-time):; A.R. is employed by Cerevance Ltd. **H. Hunter:** A. Employment/Salary (full or part-time):; H.H. is employed by Cerevance Ltd. **R. Burley:** A. Employment/Salary (full or part-time):; R.B. was employed by Cerevance Ltd. for the research conducted. **C. Bender:** A. Employment/Salary (full or part-time):; C.B. is employed by Cerevance Ltd. **D. Barker:** A. Employment/Salary (full or part-time):; D.B. is employed by Cerevance Ltd. **D. Cadwalladr:** A. Employment/Salary (full or part-time):; D.C. is employed by Cerevance Ltd. **L. Dickson:** A. Employment/Salary (full or part-time):; L.D. is employed by Cerevance Ltd. **J. Lawrence:** A. Employment/Salary (full or part-time):; J.L. is employed by Cerevance Ltd. **J. Harvey:** A. Employment/Salary (full or part-time):; J.H. is employed by Cerevance Ltd. **M. Lizio:** A. Employment/Salary (full or part-time):;

M.L. is employed by Cerevance Ltd. **X. Xu:** A. Employment/Salary (full or part-time):; X.X. is employed by Cerevance Ltd. **E. Kavanagh:** A. Employment/Salary (full or part-time):; E.K. is employed by Cerevance Ltd. **T. Cheung:** A. Employment/Salary (full or part-time):; T.C. is employed by Cerevance Ltd. **S. Sheardown:** A. Employment/Salary (full or part-time):; S.S. is employed by Cerevance Ltd. **K. Matthews:** A. Employment/Salary (full or part-time):; K.M. is employed by Cerevance Ltd. **K. Doyle:** A. Employment/Salary (full or part-time):; K.D. is employed by Cerevance Ltd. **K. Page:** A. Employment/Salary (full or part-time):; K.P. is employed by Cerevance Ltd. **J. Powell:** A. Employment/Salary (full or part-time):; J.P. is employed by Cerevance Ltd. **N.L. Brice:** A. Employment/Salary (full or part-time):; N.L.B. is employed by Cerevance Ltd. **R.W. Bürli:** A. Employment/Salary (full or part-time):; R.W.B. is employed by Cerevance Ltd. **M.B. Carlton:** A. Employment/Salary (full or part-time):; M.B.C. is employed by Cerevance Ltd. **L.A. Dawson:** A. Employment/Salary (full or part-time):; L.A.D. is employed by Cerevance Ltd..

Poster

366. Microglia and Neurodegeneration

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 366.06

Topic: B.09. Glial Mechanisms

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Title: A matrix-microglia feedback loop underlies alterations in the brain extracellular space in parkinsonian mice

Authors: *F. N. SORIA[1,2], C. PAVIOLO[3], E. DOUDNIKOFF[2], M.-L. AROTCARENA[2], I. TOMÉ-VELASCO[1], C. MATUTE[1], B. DEHAY[2], L. COGNET[3], E. BEZARD[2];
[1]Achucarro Basque Ctr. for Neuroscience, UPV/EHU and CIBERNED, Leioa, Spain; [2]Inst. des Maladies Neurodégénératives (IMN), CNRS UMR 5293, Bordeaux, France; [3]Laboratorie Photonique, Numerique et Nanosciences (LP2N), CNRS UMR 5298, Talence, France

Abstract: Beyond neurons and glia, the Central Nervous System (CNS) holds a dynamic extracellular matrix, which in the brain is composed mainly by hyaluronan, a versatile polymer with structural and signalling properties. Microglia, the never-resting immune cells of the CNS, constantly survey the brain parenchyma and respond to “danger” signals by altering their homeostatic status. Here we show that in the substantia nigra of adult mice, alpha-synuclein (a-syn)-induced neurodegeneration triggers matrix fragmentation, which in turn alters microglia

homeostasis. We demonstrate that this altered microglia remodels the matrix by engulfing hyaluronan through CD44. Electron microscopy in cryofixed tissue and carbon nanotube-tracking in live brain show that these matrix alterations, induced either by the pathology or by inhibiting hyaluronan synthesis, widen the extracellular space (ECS) and increase nanoscale diffusion in the parkinsonian substantia nigra. Finally, we demonstrate in vivo that manipulation of the matrix can influence inflammation directly and cell death indirectly, triggering microglia phagocytic state and reducing a-syn load. These findings indicate that matrix-microglia interplay is altered in the parkinsonian brain, affecting ECS nanoscale parameters and disease progression. Our results also highlight hyaluronan as local tissue organizer and diffusion barrier and suggest matrix manipulation as a disease-modifying strategy.

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Poster

366. Microglia and Neurodegeneration

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 366.07

Topic: B.09. Glial Mechanisms

Title: Translocator protein (18 kDa) deficiency attenuates excessive synaptic elimination by microglia in synucleinopathy

Authors: *M. CUI[1,2,3], J. ZU[1,2,3], Y. SHI[1,2,4], J. HERMS[1,2,4];
[1]Ctr. for Neuropathology, Ludwig Maximilian Univ. Munich, Munich, Germany; [2]German Ctr. for Neurodegenerative Dis. (DZNE), Munich, Germany; [3]Munich Med. Res. Sch., Ludwig-Maximilians-University Munich, Munich, Germany; [4]Munich Cluster for Systems Neurol. (SyNergy), Munich, Germany

Abstract: In synucleinopathy such as Parkinson's disease dementia or dementia with Lewy bodies, one of the early pathological hallmarks – abnormal loss of synapses, and the elevation of 18 kDa translocator protein (TSPO) of activated microglia have been repeatedly observed. TSPO, previously known as the peripheral-type benzodiazepine receptor (PBR), is a five-transmembrane domain protein located at the outer membrane of mitochondria. Recently, we demonstrated that prolonged administration of synthetic TSPO ligands, such as diazepam, aberrantly increases microglial TSPO and causes excessive microglial engulfment of synaptic materials. However, how TSPO affects synaptic elimination via microglia in synucleinopathy remains elusive. To this end, we first investigate the expression pattern of microglial TSPO in cortical areas of *PDGF-h-α-syn* mice – a well-established mouse model of synucleinopathy characterised by overexpressed neuronal human wild-type α-synuclein accompanied by abnormal synaptic elimination. We found a rising microglial TSPO expression and a heightened microglial engulfment of synaptic materials in *PDGF-h-α-syn* mice compared to age-matched

WT controls. To further investigate the role of TSPO in the microglial-mediated synaptic elimination in synucleinopathy, we crossed the *PDGF-h-α-syn* mice to the *TSPO knockout* mice. Upon TSPO knockout, we observed a significant reduction of microglial engulfment of synaptic materials and an attenuated synaptic elimination. Collectively, we highlighted the pivotal role of TSPO in the synaptic pathology of synucleinopathy. Our findings indicate the TSPO signalling pathway as both a new aspect of underlying mechanisms of synaptic dysregulation and a potential target for rescue strategies of synapses in synucleinopathy.

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Poster

366. Microglia and Neurodegeneration

Location: SDCC Halls B-H

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Program #/Poster #: 366.08

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Prevention of microglia-mediated synaptic loss in Alzheimer's by targeting NRG1

Authors: J. LIU, J. GERAGHTY, J. LEI, J. LOEB, ***F. SONG**;
UIC, Univ. of Illinois at Chicago, Chicago, IL

Abstract: Microglia-mediated synaptic loss is thought to contribute to the development of cognitive impairments in Alzheimer's disease (AD). However, the basis for this neuroinflammatory-mediated attack on synapses remains to be elucidated. The gliotrophic factor neuregulin-1 (NRG1) regulates developmental neuronal survival and synaptogenesis, astrocytic differentiation, and microglial activation. Given these NRG1 actions, we hypothesize that NRG1 signaling from degenerating neurons to surrounding microglia promotes the local spread of AD pathology through microglial activation that could lead to synaptic loss and neurodegeneration. We found that intraventricular NRG1 augments microglial activation and A-beta plaque formation in early-stage 5XFAD mice. Blocking endogenous NRG1 activity with CNS-specific delivery of a novel biologic (GlyB4) prevents microglial activation and A-beta plaque formation in early-stage and reduces microglial activation and A-beta plaque formation in later-stage disease. As clinical neuropathologic studies suggest that the selective vulnerability of hippocampal cornu ammonis (CA1) pyramidal projection neurons plays a key role in the onset of cognitive impairment during the early phases of AD, our GlyB4 treatment alters microglial morphology and reduces synaptic loss in the hippocampal CA1 in 5XFAD mice. Mechanistically, NRG1 induces pro-inflammatory cytokine expression and promotes phagocytic activity in cultured microglia. Our results suggest that blocking NRG1 signaling prevents and reduces AD pathology and supports the use of GlyB4 to protect synapses from damage by microglial mediators to slow pathological progression in AD.

Disclosures: J. Liu: None. **J. Geraghty:** None. **J. Lei:** None. **J. Loeb:** None. **F. Song:** None.

Poster

366. Microglia and Neurodegeneration

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 366.09

Topic: B.09. Glial Mechanisms

Support: ECS24107121
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C4033-19EF
NSF-32022087
AARF-17-531566
Ref. 133

Title: Alcohol misuse and its implications in neuroinflammation, metabolic reprogramming of microglia, and accelerated brain ageing

Authors: *K. SUN[1], H. CHOW[2];
[1]The Chinese Univ. of Hong Kong, [2]Sch. of Life Sci., The Chinese Univ. of Hong Kong, Hong Kong, Hong Kong

Abstract: Over the past two decades, global adult consumption of alcohol per capita has been increasing steadily and it is continuing to rise in the foreseeable future. Chronic and overconsumption of alcohol has been identified as a risk factor for accelerated brain ageing and cognitive decline. Alcohol (ethanol), a potent neurotoxin, interferes with central carbon metabolism due to its amphipathic properties. Microglia, as resident macrophages of the central nervous system, regulate their functional status and survival by flexibly changing their metabolism. With an appreciation for this concept of immunometabolism, we hypothesised that chronic exposure to ethanol may remodel its physiology, thereby introducing a low-grade chronic inflammatory microenvironment that promotes accelerated brain ageing and neurodegeneration in association with alcohol misuse.
Transcriptomic analyses performed in alcohol-exposed mice revealed disruption to pathways in relation to memory and cognition, and this finding is confirmed with poorer performance in memory-related behavioural paradigms performed in 3-month-old C57BL/6 mice that were exposed to alcohol using a voluntary drinking program. Immunohistochemical analyses showed activation and proliferation of microglia *in vivo* and *in vitro* after ethanol exposure. Single-cell transcriptomic data further revealed disruptions in metabolic pathways involved in glycolysis, and hallmark pathways such as the activation of mTORC1 and NFκB signalling pathways. The three interacting pathways were explored and indicated ethanol's disruption to the central carbon network. Ethanol metabolism drives *de novo* lipogenesis and hijacks the homeostatic metabolic pathway, and this subsequently causes an upregulation of dihydroxyacetone phosphate (DHAP), which has been shown to serve as a glucose-independent activator of mTORC1 and a regulator of lipid biosynthesis. This activation of mTORC1 results in downstream activation of NFκB signalling, which regulates neuroinflammation mediated by microglia due to ethanol exposure. Subsequent inhibition of ethanol metabolism in microglia shows a mediation of the mTORC1

and NFκB pathway and the downstream activation of microglia. Together, these findings identify that alcohol exposure activates microglia and disturbs the homeostatic metabolism, and intervention to inhibit ethanol exposure and mTORC1 signalling can mediate microglial activation.

Disclosures: K. Sun: None. **H. Chow:** None.

Poster

366. Microglia and Neurodegeneration

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 366.10

Topic: B.09. Glial Mechanisms

Support: R01NS102382
R01AG073779

Title: Type I Interferon Signaling Drives Microglial Dysfunction and Senescence in Human iPSC Models of Down Syndrome and Alzheimer's Disease

Authors: *M. JIN, P. JIANG;
Rutgers Univ., Piscataway, NJ

Abstract: Type I Interferon Signaling Drives Microglial Dysfunction and Senescence in Human iPSC Models of Down Syndrome and Alzheimer's DiseaseAuthors:Mengmeng Jin *, Peng Jiang**Disclosures:** Mengmeng Jin: None, Peng Jiang: None.Microglia are critical for brain development and play a central role in Alzheimer's disease (AD) etiology. Down syndrome (DS), also known as trisomy 21, is the most common genetic origin of intellectual disability and the most common risk factor for AD. Surprisingly, little information is available on the impact of trisomy of human chromosome 21 (Hsa21) on microglia in DS brain development and AD in DS (DSAD). Using our new induced pluripotent stem cell (iPSC)-based human microglia-containing cerebral organoid and chimeric mouse brain models, here we report that DS microglia exhibit enhanced synaptic pruning function during brain development. Consequently, electrophysiological recordings demonstrate that DS microglial mouse chimeras show impaired synaptic functions, as compared to control microglial chimeras. Upon being exposed to human brain tissue-derived soluble pathological tau, DS microglia display dystrophic phenotypes in chimeric mouse brains, recapitulating microglial responses seen in human AD and DSAD brain tissues. Further flow cytometry, single-cell RNA-sequencing, and immunohistological analyses of chimeric mouse brains demonstrate that DS microglia undergo cellular senescence and exhibit elevated type I interferon signaling after being challenged by pathological tau. Mechanistically, we find that shRNA-mediated knockdown of Hsa21encoded type I interferon receptor genes, *IFNARs*, rescues the defective DS microglial phenotypes both during brain development and in response to pathological tau. Our findings provide first *in vivo* evidence supporting a paradigm shifting theory that human microglia respond to pathological tau by

exhibiting accelerated senescence and dystrophic phenotypes. Our results further suggest that targeting IFNARs may improve microglial functions during DS brain development and prevent human microglial senescence in DS individuals with AD.

Disclosures: M. Jin: None. **P. Jiang:** None.

Poster

367. Glioblastoma

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 367.01

Topic: B.11. Neuro-Oncology

Support: CONACYT 320621

Title: Expression of the kynurenine pathway enzymes in glioblastoma subtypes and their relationship with hallmarks of cancer: a bioinformatic study

Authors: *J. NAVARRO COSSIO[1], G. I. VÁZQUEZ CERVANTES[1], V. PEREZ DE LA CRUZ[1], G. PÉREZ DE LA CRUZ[2], B. PINEDA OLVERA[3];
[1]Lab. de Neurobioquímica y Conducta, Inst. Nacional De Neurologia Y Neurocirugia, Mexico City, Mexico; [2]Facultad de Ciencias, Univ. Nacional Autónoma de México, Mexico City, Mexico; [3]NEUROINMUNOOGIA, INSTITUTO NACIONAL DE NEUROLOGIA Y NEUROCIRUGIA, MEXICO CITY, Mexico

Abstract: Glioblastoma multiforme (GBM) is the most frequent and lethal of the primary brain tumor. Its poor prognosis has been related to the presence of immunosuppressive mechanisms. In this line, the tryptophan catabolism through the kynurenine pathway (KP) is an important piece to understand this problem, since it has been described that enzyme such as indoleamine-2, 3-dioxygenase (IDO) and kynurenine monooxygenase (KMO) have an important role in the immunosuppressor environment. Although the immunomodulatory properties of some KP enzymes have been well described, it has not been pointed out whether the behavior of KP differs among GBM subtypes. Two very well-established classifications of GBM are the revised fourth edition of the World Health Organization (WHO) Classification of CNS tumors published in 2016, which classifies gliomas based on the presence of point mutations in isocitrate dehydrogenase 1 and 2 (IDH1/ IDH2), and the other one a robust gene expression-based molecular classification of GBM that divide into Proneural, Neural, Classical, and Mesenchymal subtypes. Thus, the aim of this work is to find out differences on the KP among the subtypes of these two classifications of GBM by means of a bioinformatic analysis on the Xena platform of a combined cohort of the Cancer Genomic Atlas (TCGA) and the Genotype-Tissue Expression (GTEx) samples and correlate these differences with the expression of marker genes related to the “Hallmarks of Cancer”. The results show significant differences in the expression of KMO for the mesenchymal subtype, which is consistent with the fact that this subtype is the most aggressive and with the worst prognosis compared to the other 3, in addition to presenting a

greater immunosuppressive environment. On the other hand, in the WHO classification we found that the KP enzymes are overexpressed in the IDH-wildtype subtype while they are not in the IDH-mutant subtype, which is also consistent with the fact that these have a better prognosis. These results are important, since they allow us to understand how the activity of the pathway is related to the different Hallmarks of cancer and, also several of these enzymes are potential therapeutic targets, so knowing how they are found in the different subtypes can help us to use this metabolites as prognostic markers and could be used as a potential therapeutic approach in a personalized medicine frame.

Disclosures: **J. Navarro Cossio:** None. **G.I. Vázquez Cervantes:** None. **V. Perez De La Cruz:** None. **G. Pérez De La Cruz:** None. **B. Pineda olvera:** None.

Poster

367. Glioblastoma

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 367.02

Topic: B.11. Neuro-Oncology

Support: NIH Grant NINDS R21NS120003
Lucille P. Markey Special Emphasis Pathway in Human Pathobiology

Title: Glucocorticoid signaling synchronizes tumor and host circadian rhythms and promotes tumor growth in a murine model of glioblastoma

Authors: *M. GONZALEZ[1], A. R. DAMATO[2], T. SIMON[3], E. D. HERZOG[4];
[1]Washington Univ. in St. Louis, St. Louis, MO; [2]Biol., Washington Univ. in St. Louis, Saint Louis, MO; [3]Biol., Washington Univ. in St. Louis, St. Louis, MO; [4]Washington Univ., St. Louis, MO

Abstract: Glioblastoma (GBM) is the primary and most aggressive brain tumor in adults. The current standard of care consists of maximal surgical resection, followed by radiation and chemotherapy with Temozolomide (TMZ). Additionally, patients often receive daily doses of the synthetic glucocorticoid, Dexamethasone (DEX), to reduce brain edema. Despite extensive research and clinical trials, average survival post-treatment remains at 15 months. Thus, all opportunities to optimize current treatments and improve patient outcomes should be considered. Previous work from our lab has shown that murine and human models of GBM have cell-intrinsic circadian rhythms in the expression of the core clock genes *Bmal1* and *Per2* and their sensitivity to TMZ. Here, we tested the hypothesis that tumors have daily rhythms in their sensitivity to glucocorticoids which can synchronize their daily rhythms to the host and influence growth. We transduced a murine model of GBM with a *Per2* luciferase reporter (GL261-P2L) and found these cells expressed glucocorticoid receptor (GR) mRNA and protein and daily rhythms in clock gene expression. Viral-mediated knock-down of GR reduced expression of GR but had no effect on daily rhythms in *Per2* expression in vitro. We next implanted these cells into

the basal ganglia of male and female mice and found tumor *Per2* expression reliably peaked in the middle of the night, but peaked midday for GL261 cells without GR. Preliminary results from *ex vivo* slices of brain containing GR-deficient tumors showed daily rhythms for less than 3 days, while GR-intact tumors show rhythms for at least 8 days. Further, GR-deficient tumors grew slower, and hosts survived longer compared to mice with GR-intact tumors. We are also examining sex differences in synchronization, tumor growth, and host survival in mice implanted with GR intact or knock-down tumors. These results suggest that glucocorticoid signaling promotes tumor growth and synchronizes tumor and host daily rhythms. This work may eventually inform personalized chronotherapy (e.g., timed TMZ and DEX) to improve individual patient outcomes.

Disclosures: **M. Gonzalez:** None. **A.R. Damato:** None. **T. Simon:** None. **E.D. Herzog:** None.

Poster

367. Glioblastoma

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 367.03

Topic: B.11. Neuro-Oncology

Support: 6195/2020CIB. Universidad Autonoma del Estado de Mexico

Title: Expression of Bcl-2 and active caspase-3 in human glioblastoma cells supplemented with pyridoxine

Authors: *I. CONTRERAS[1], C. A. MARTINEZ-MENDIOLA[2], L. A. ZAPI-COLIN[2], J. A. ESTRADA[2];
[1]Facultad de Medicina, Univ. Autonoma del Estado De México, TOLUCA, Mexico; [2]Facultad de Medicina, Univ. Autonoma del Estado de Mexico, TOLUCA, Mexico

Abstract: Cancer therapy usually involves multi-targeted approaches combining chemotherapy/radiotherapy, surgical tumor resection, immunotherapy, and nutritional interventions, offering greater chance of success to eradicate tumor cells. Among nutritional interventions, polyunsaturated fatty acids, vitamins, minerals, phytochemicals, hormones, and amino acids have been used to modulate cancer cell fitness, survival and invasiveness. Glioblastomas are the most common and aggressive type of brain tumors, with 5-year survival rates of only 5%. Group B vitamins play an important role in central nervous system development and functions; nonetheless, their effects on glioblastoma development and progression are unknown. The aim of this study was to determine changes in the expression of Bcl-2 and active caspase 3 proteins in human glioblastoma cell line U87MG. Glioblastoma cells were cultured and supplemented with 1, 2, and 4 mM concentrations of pyridoxine for 24, 48 and 72 hours. After supplementation, total protein extracts were obtained, quantified, dosed and analyzed by western blot using anti-Bcl-2 and anti-active caspase 3 antibodies, with Beta-actin as loading control. Densitometry analysis was performed using imageJ software. Our results show

that Bcl-2 expression did not change after pyridoxine supplementation; however, active caspase 3 expression showed a 1.5-fold increase compared to unsupplemented controls after 72 hours with 4 mM pyridoxine. To further assess this change, flow cytometry analysis for intracellular active caspase 3 was performed, finding a significant increase in active caspase 3 staining in the same group of pyridoxine-supplemented cells, compared to untreated controls. Our results suggest that pyridoxine supplementation can induce apoptosis in a dose- and time-dependent manner in U87MG cells.

Disclosures: I. Contreras: None. **C.A. Martinez-Mendiola:** None. **L.A. Zapi-Colin:** None. **J.A. Estrada:** None.

Poster

367. Glioblastoma

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 367.04

Topic: B.11. Neuro-Oncology

Title: Cytotoxicity screening of Ibervillae sonoroe root extracts in glioblastoma cells.

Authors: *C. E. RODRÍGUEZ-PÉREZ[1], J. Y. JIMÉNEZ-PEREYRA[1,2], M. A. TORRES-RAMOS[1], E. E. ORTIZ-ISLAS[1];
[1]Natl. Inst. of Neurol. and Neurosurg., MEXICO CITY, Mexico; [2]Metropolitan Autonomous Univ., Mexico city, Mexico

Abstract: Glioblastoma multiforme is a very malignant tumor that develops and originates in glial cells of the brain. The treatment of choice is surgery followed by adjuvant radiotherapy and chemotherapy; despite this, the survival is minimal. The use of plants for the prevention and treatment of cancer is gaining more attention due to their diverse phytochemical constituents and fewer adverse effects. This study investigated the root extracts from *Ibervillae sonoroe* for their cytotoxic potential against two glioma cell lines (U87 and LN18). MTT [3-(4,5-dimethylthiazol-2-yl)-2,5 dipenyltetrazolium bromide] and neutral red assays were performed to assess cytotoxic activity as well morphology cell effects were observed at 24 and 48h. The levels of cytotoxicity for extracts were time- and dose-dependent at lower concentrations. These preliminary results suggest that *Ibervillae sonoroe* root extracts have the potential for development as therapeutic agents for cytotoxicity on glioma cells.

Disclosures: C.E. Rodríguez-Pérez: None. **J.Y. Jiménez-Pereyra:** None. **M.A. Torres-Ramos:** None. **E.E. Ortiz-Islas:** None.

Poster

367. Glioblastoma

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 367.05

Topic: B.11. Neuro-Oncology

Title: Effects of pharmacological blockade of WNT and TBK1 signaling pathways on the expression of TLR 3/4 receptors and its relation to cell cycle arrest in human glioblastoma cells

Authors: *G. CONTRERAS CHÁVEZ[1], I. CONTRERAS[2], J. A. ESTRADA[1];
[1]Univ. Autonoma del Estado de Mexico, Toluca, Mexico; [2]Univ. Autonoma del Estado De México, Toluca, Mexico

Abstract: Glioblastoma multiforme (GBM) is a central nervous system tumor derived from glial cells. GBM is the most aggressive brain tumor, with an average survival time of only 15 months post-diagnosis. Like other types of cancer, GBM creates immunosuppressive tumor microenvironments, leading to low survival rates. Therefore, it is important to understand the molecular mechanisms used by GBM to modulate the immune response, in order to improve disease therapy and prolong patient survival. The main objective of this study was to determine the effects of pharmacological blockage of the WNT and TBK1 signaling pathways on the expression of TLR 3/4 receptors in human glioblastoma cell line U87MG To do so, U87MG cells were cultured in the presence of WNT (IWP12) and TBK1 (BX795) inhibitors, at 0.1, 1 and 10 μM concentrations for 24, 28 and 72 hrs. After treatment, expression of TLR 3 and TLR 4 receptors was analyzed by flow cytometry, total protein extracts from cell cultures were used to determine the expression of AKT and STAT3 proteins by western blot. Finally, cell proliferation and apoptosis in treated cultures were evaluated by flow cytometry with propidium iodide staining and with crystal violet assay by spectrophotometry. Preliminary results from the cell proliferation assay show, that there was no difference in the percentage of cells in the G0/G1 phase between treated and untreated cells, however, there was a reduction from 12.4% of untreated cells to 5.5% of cells treated with 10 μM of BX795 in the S phase, suggesting that the TBK1 inhibitor could reduce the DNA synthesis. Nonetheless, this finding must be further studied with the viability and apoptosis assays.

Disclosures: G. Contreras Chávez: None. **I. Contreras:** None. **J.A. Estrada:** None.

Poster

367. Glioblastoma

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 367.06

Topic: B.11. Neuro-Oncology

Title: Predicting Survival Outcomes in Glioblastoma Multiforme Patients with Machine Learning

Authors: *M. O. OLUFAWO, P. LUCKETT, K. Y. PARK, B. LAMICHHANE, G. TREVINO, D. DIERKER, P. H. YANG, J. J. LEE, A. H. KIM, J. S. SHIMONY, E. C. LEUTHARDT;
Washington Univ. Sch. of Med., Saint Louis, MO

Abstract: Introduction: Glioblastoma multiforme (GBM) is the most common brain malignancy in adults, and generally leads to poor overall survival. Techniques capable of predicting survival outcomes could lead to improved patient care and treatment outcomes. **Objectives:** To use machine learning models to predict survival using clinical and imaging data in GBM patients. **Methods:** Cross sectional clinical and neuroimaging (volumetric and resting state functional MRI) data were acquired in 141 GBM patients (Table 1). Random forest models were used to classify length of survival (<1, 1-2, 2-3, >3 years) using clinical and neuroimaging features. Estimates of feature importance were calculated using out-of-bag predictor permutations. All models were optimized with Bayesian optimization, and validated with 10 fold cross validation. Model results were further evaluated in the context of extent of resection and genetic mutations. **Results:** The random forest model was able to classify survival with 98% accuracy (Figure 1). The strongest predictive features identified in the model were predominantly resting state network correlations involving subcortical (thalamus and basal ganglia) regions (Figure 2 and 3). When evaluating genetic features, IDH1 and MGMT showed significant differences based on classification results, with longer survival associated with a higher rate of mutation positive participants (Table 2, Figure 4). Participants who received gross total resections also had significantly higher rates of long term survival (Table 2, Figure 4). **Conclusion:** Techniques capable of predicting survival outcomes in GBM patients could lead to improved pre-surgical planning and post-surgical care. Our research suggest machine learning is capable of highly accurate survival predictions based predominantly on resting state network correlations.

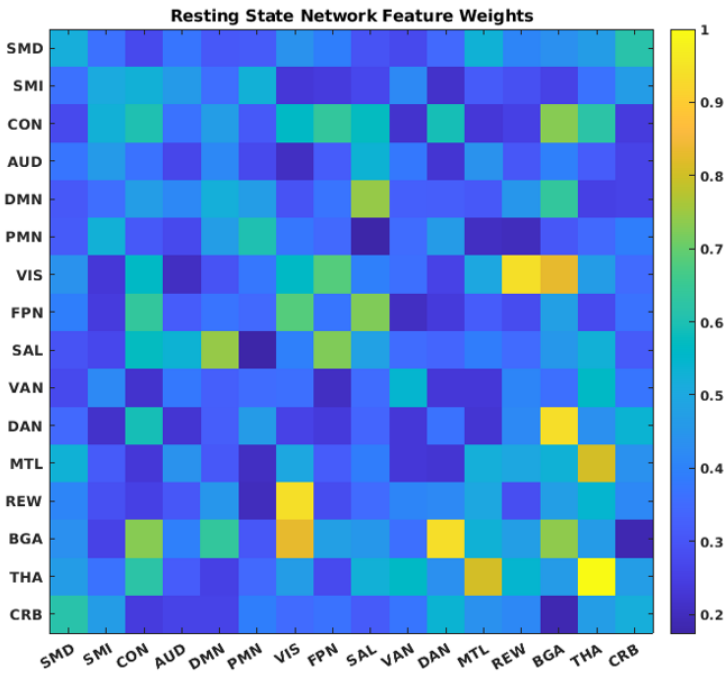

Figure 1. Strongest predictive resting state network features.

Disclosures: **M.O. Olufawo:** None. **P. Luckett:** None. **K.Y. Park:** None. **B. Lamichhane:** None. **G. Trevino:** None. **D. Dierker:** None. **P.H. Yang:** None. **J.J. Lee:** None. **A.H. Kim:** None. **J.S. Shimony:** None. **E.C. Leuthardt:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurolutions, Sora Neuroscience, Inner Cosmos.

Poster

367. Glioblastoma

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 367.07

Topic: B.11. Neuro-Oncology

Support: KFR Research Grant

Title: Resveratrol affects ganglioside remodeling and inflammatory markers in glioblastoma multiforme

Authors: ***D. M. BYERS**, B. G. JETER;
West Texas A & M Univ., West Texas A & M Univ., Canyon, TX

Abstract: Glioblastoma multiforme (GBM), a grade IV astrocytoma, is the most aggressive form of brain cancer with a 15 - 18 month survival rate. Challenges to its successful treatment include the invasive growth of tumor cells into surrounding healthy tissue. Studies have attributed GBM progression partly to inflammation created through communication with tumor-invading microglia cells. Studies have also shown that gangliosides, a family of glycolipids known for their involvement in specific neuropathologies, play a role in tumorigenesis, metastasis, angiogenesis, and the immune response. GBM stem cells are known to over-express ganglioside GD3 and its synthetic enzyme, GD3 synthase. Targeting of GD3 synthase has therefore become a focus of GBM intervention. Resveratrol (RSV) is a natural polyphenol known for its potent anti-oxidant and anti-inflammatory properties. RSV has also demonstrated ganglioside-altering effects, as well as powerful antitumor effects on GBM including inhibition of tumor growth, invasiveness and the sphere-forming ability of GBM stem cells, and activation of p53. This study sought to examine the influence of RSV on ganglioside remodeling and tumor cell immune response in GBM and evaluate its potential as a co-therapeutic in GBM intervention. GBM and Adult Pigmented Retinal Epithelial cells (ARPE-19) were treated with RSV for 24h and assayed for viability and ganglioside profiles by HPTLC. Expression of ganglioside synthetic enzymes was evaluated by qPCR using gene specific primers and RT2 Profiler Arrays were used to evaluate inflammatory response and cancer markers. Expression of selected genes was validated by qPCR. RSV had a significant effect in reducing cell viability in GBM without negatively affecting ARPE control cells. RSV also significantly down-regulated expression of all four ganglioside synthetic enzymes. HPTLC analysis showed an effect of RSV treatment on ganglioside profiles however this was less robust. qPCR Arrays yielded more than forty immuno-regulating factors that were either up- or down-regulated by RSV and more than twenty genes involved in cell proliferation and cell death pathways were also affected by RSV treatment. An important consideration in oncology is to maximize efficacy while minimizing toxicity. These data suggest that RSV is effective in reducing viability of GBM cells, without adversely effecting normal cells. Down-regulation of ganglioside synthetic enzymes, specifically GD3 synthase suggests it may be an effective complementary intervention in GBM treatment. Co-recruitment of immune factors may provide both additional anti-tumor assistance and cytoprotective support for healthy cells.

Disclosures: **D.M. Byers:** None. **B.G. Jeter:** None.

Poster

367. Glioblastoma

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 367.08

Topic: B.11. Neuro-Oncology

Support: C22CR4151

Title: Role of CPEB3 ribozyme in glioblastoma

Authors: *C. CHEN[1], A. BARHOOSH[1], M. NIKAN[2], C. BENAVENTE[1], A. LUPTAK[1]; [1]Univ. of California, Irvine, Irvine, CA; [2]Ionis Pharmaceuticals, Carlsbad, CA

Abstract: Regulation of mRNA translation plays an essential role in cellular differentiation and proliferation. Dysregulation of post-transcriptional control and translational machinery have been implicated in malignant tumor development. One of the mechanisms to govern translation is cytoplasmic polyadenylation, and recent evidence suggests that this process modulates gene reprogramming associated with cancer progression. Cytoplasmic polyadenylation element binding proteins (CPEB1-CPEB4) are sequence-specific RNA-binding proteins that act as a translational regulator to control poly(A) tail elongation of target mRNAs, contributing to phenotypic changes in cancer cells. Among CPEBs, aberrant expression of CPEB3 has been shown in several types of cancers. The *CPEB3* pre-mRNA harbors a self-cleaving ribozyme was identified in the second intron. We have previously uncovered the role of the CPEB3 ribozyme in memory formation in mice, but the impact of the ribozyme on cancer cells remains unexplored. Given that CPEB3 acts as a tumor suppressor gene and downregulation of CPEB3 promotes cancer progression, we hypothesize that inhibition of the ribozyme might regulate CPEB3 protein expression and modulate tumor progression. Our preliminary studies from the Cancer Genome Atlas (TCGA) analysis revealed that CPEB3 expression is down-regulated in the glioblastoma (GBM) cohort. In the low-grade glioma cohort, patients with low CPEB3 expression have poor overall survival (OS), suggesting that low CPEB3 expression might contribute to a worse prognosis. To elucidate whether the CPEB3 ribozyme plays a role in tumor development and progression in malignant glioma, we used antisense oligonucleotides (ASOs) to inhibit ribozyme activity. Results suggest that treatment of ribozyme ASO led to an increase in CPEB3 mRNA and protein expression in GBM cells. In addition, inhibition of the ribozyme resulted in a decrease in cell migration and proliferation, suggesting ASO restores CPEB3 protein levels and reestablish its tumor suppressor function in GBM cells. Together, our study expands our understanding of the role of the CPEB3 ribozyme in glioblastoma and further investigates therapeutic strategies targeting CPEB3 in cancer.

Disclosures: C. Chen: None. **A. Barhoosh:** None. **M. Nikan:** None. **C. Benavente:** None. **A. Luptak:** None.

Poster

367. Glioblastoma

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 367.09

Topic: B.11. Neuro-Oncology

Title: Repurposing Neuroactive Drugs for Glioblastoma

Authors: *S. LEE[1], T. WEISS[2], M. BUEHLER[2], J. MENA[1], R. WEGMANN[1], Z. LOTTENBACH[1], M. BIHL[2], S. GOETZE[1], A. VAN DROGEN[1], E. RUSHING[2], B.

WOLLSCHEID[1], M. WELLER[2], B. SNIJDER[1];
[1]ETH Zurich, Zurich, Switzerland; [2]Univ. Hosp. and Univ. of Zurich, Zurich, Switzerland

Abstract: Glioblastoma (GBM) represents the most aggressive and malignant form of brain cancer, with median survival of 15 months. With only one available 1st-line treatment, development of new therapies remains an urgent clinical need. Significant challenges in GBM treatment include drug delivery across the blood-brain barrier (BBB), a complex tumor microenvironment (TME), and the lack of predictive cancer models. Here, we present the results of a clinically predictive drug-testing platform that systematically addresses these therapeutic roadblocks. By image-based screening of 69 BBB-permeable neuroactive drugs across 27 GBM surgical patient samples ex vivo, we identify a set of repurposable drugs with unexpected anti-glioma activity. These neuroactive drugs span a broad range of agents approved for other psychiatric disorders and neurodegenerative diseases such as depression, schizophrenia, and Parkinson's disease. Single-cell transcriptional profiling of GBM patient samples and functional genetics revealed novel glioma-dependencies on the expression of specific neurological ion channels and receptors. Furthermore, a drug-target network enrichment analysis uncovered a AP-1/TP53/BTG2 gene signature associated with the anti-glioma activity of neuroactive drugs. In silico screening of over 1 million compounds for this common gene signature identified additional drug hits that could be validated in patient samples with 90% accuracy. Multiplexed transcriptomics revealed rapid AP-1 transcription factor family activation to be the common underlying feature of neuroactive drugs with anti-glioma activity. Among the most promising candidate drugs, we identified the atypical antidepressant Vortioxetine as the strongest inducer of this gene signature and confirmed its efficacy in vivo across multiple mouse models. Together, this study provides a clinically predictive drug-testing platform and elucidates anti-tumorigenic pathways underlying repurposable neuroactive drugs.

Disclosures: S. Lee: None. **T. Weiss:** None. **M. Buehler:** None. **J. Mena:** None. **R. Wegmann:** None. **Z. Lottenbach:** None. **M. Bihl:** None. **S. Goetze:** None. **A. van Drogen:** None. **E. Rushing:** None. **B. Wollscheid:** None. **M. Weller:** None. **B. Snijder:** None.

Poster

367. Glioblastoma

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Program #/Poster #: 367.10

Topic: B.11. Neuro-Oncology

Support: 5R01CA203861-05

Title: Resting State Functional Architecture in Patients with Glioblastoma

Authors: *K. PARK[1], J. S. SHIMONY[2], S. CHAKRABARTY[5], A. B. TANENBAUM[3], K. DONOVAN[1], P. LUCKETT[1], C. D. HACKER[4], M. MILCHENKO[2], E. C. LEUTHARDT[1], A. Z. SNYDER[2];

[1]Div. of Neurotechnology, [2]Mallinckrodt Inst. of Radiology, [3]Dept. of Neurol., [4]Dept. of Neurolog. Surgery, Washington Univ. Sch. of Med., SAINT LOUIS, MO; [5]Dept. of Electrical and Systems Engin., Washington Univ. in St. Louis, SAINT LOUIS, MO

Abstract: Background: Presurgical resting-state fMRI is becoming increasingly used for functional mapping and to study the effects of tumors on the organization of functional networks. This is a challenge in patients with large tumors owing to mass effect and potentially altered representation of function, i.e., remapping. The best approach to spatial normalization remains uncertain. In this work, we investigated the effects of alternative approaches to atlas registration on resting-state fMRI functional connectivity in patients with glioblastoma (GBM) and studied the impact of GBM on functional brain organization using the optimal registration option. **Methods**: Data representing 59 patients with GBM and 161 age-matched healthy controls were analyzed. We systemically investigated affine vs. non-linear atlas registration and two associated masking options. Outcomes were assessed both in terms of structural data match to an atlas template as well as functional connectivity (FC) match to a reference dataset. Next, we evaluated the impact of GBMs on the organization of brain networks accounting for the hierarchical organization of resting state networks. Finally, we identified functionally altered brain regions in patients with GBM using a novel aberrancy mapping procedure. **Results**: The greatest similarity for both structural normalization and FC were achieved using non-linear atlas registration and tumor masking. FC averaged over GBM patients was remarkably normal. FC abnormalities in individual patients were not apparent when evaluated at coarse parcellations. FC abnormalities in individual patients evaluated at fine parcellations were co-extensive with the tumor but also frequently occurred in parts of the brain distant from the tumor. **Conclusions**: Our results demonstrate that non-linear atlas registration with tumor masking optimizes both structural normalization and FC mapping. The aberrancy map results, obtained with fine parcellations, are consistent with prior findings showing that FC abnormalities induced by focal lesions are widely distributed. However, the results obtained with coarse parcellations indicate that functional architecture generally is grossly normal in tumor patients.

Disclosures: **K. Park:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sora Neuroscience LLC. **J.S. Shimony:** None. **S. Chakrabarty:** None. **A.B. Tanenbaum:** None. **K. Donovan:** None. **P. Luckett:** None. **C.D. Hacker:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sora Neuroscience. **M. Milchenko:** None. **E.C. Leuthardt:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sora Neuroscience LLC. **A.Z. Snyder:** F. Consulting Fees (e.g., advisory boards); Sora Neuroscience LLC.

Poster

367. Glioblastoma

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 367.11

Topic: B.11. Neuro-Oncology

Title: Induction of senescence in human glioblastoma cells supplemented with endocannabinoids.

Authors: *L. ZAPI-COLÍN[1], I. CONTRERAS[2], J. A. ESTRADA[3];
[1]Univ. Autónoma del Estado de México, Toluca, Mexico; [2]Univ. Autonoma del Estado De México, Toluca, Mexico; [3]Univ. Autonoma Del Estado De Mexico, Toluca, Mexico

Abstract: Glioblastoma multiforme is the most common malignant primary brain tumor, with an incidence of 3.19 cases per 100,000 and a remarkably poor prognosis, showing 5-year survival rates of around 5%. Despite the progress made in surgical resection techniques, radiation therapy, and chemotherapeutic strategies, current patients' overall median survival time is 15 months. This is attributed to treatment limitations, as well as a typically elderly age of onset, the diffuse nature of glioblastomas, and an incomplete understanding of tumor pathophysiology. Endocannabinoid receptor CB1R is the most widely-expressed receptor protein from the GPCR family in the brain, and arachidonoylethanolamide is one of its main agonists. Cannabinoids/endocannabinoids have shown significant anti-inflammatory, anti-proliferative, anti-invasive, anti-metastatic, and pro-apoptotic properties in different types of cancer, both *in vitro* and *in vivo*. These molecules modulate multiple signaling pathways and the activity of molecules like p53, and p21 waf1/cip1, to induce cell cycle arrest, autophagy, and apoptosis in tumor cells. P21, in particular, has been used as a reliable marker for cellular senescence. Cellular senescence is a proliferation arrest caused by different stress-inducing factors and is characterized by alterations in cell morphology, gene expression, heterochromatin formation, and metabolic activity. The current understanding of senescence has derived mainly from studies in non-transformed fibroblasts and primary epithelial cells. In this study, we stimulated U87MG cells with three different concentrations of arachidonoylethanolamide (1, 5, or 10 μM) and incubated for 24, 48, or 72 hours after that evaluated the presence of p21 protein by western blot and immunofluorescence and the induction of a senescent state in tumor cells through the b-galactosidase production. The results showed an increase in the concentration of p21 in a doses dependent fashion, however, the time of incubation did not significantly affect the expression of the protein. Although the treatment with AEA reduced the confluency of the cells there were no differences found in the b-galactosidase assay, in this case, controversial data have been reported about the suitability of SA-β-GAL as a marker of senescence, particularly in the nervous system. In conclusion treatment with AEA can increase the expression of p21 which is indicative of cell arrest. Further clinical studies are urgently required to determine the true potential of these intriguing, low-toxicity compounds in cancer therapy. Particularly given their synergistic effects with chemotherapeutic agents.

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Poster

367. Glioblastoma

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Program #/Poster #: 367.12

Topic: B.11. Neuro-Oncology
NSF EFRI CEE 2129617

Title: Reprogramming of glioblastoma metabolic activity stimulated by increasing ECM stiffness

Authors: *C. PAYNE[1], P. VILLARREAL[1], M. SOWERS[1], S. BOSSMANN[2], M. MOTAMEDI[1], B. SZCZESNY[1], G. VARGAS[1];
[1]Univ. of Texas Med. Br., Univ. of Texas Med. Br., Galveston, TX; [2]Univ. of Kansas Med. Ctr., Kansas City, KS

Abstract: Glioblastoma (GBM) is a highly invasive and lethal form of brain cancer. The 5-year survival of those diagnosed with GBM is less than 10% and on average, patients have 12-15 months of survival following final diagnosis. Regardless of the treatment, GBM remains a quick advancing and fatal disease. In an effort to develop more effective therapeutics, research has begun looking at the mechanobiology of GBM. Specific to mechanobiology are the biochemical and biophysical cues encoded in the tumor microenvironment that promote cell invasion. The extracellular matrix (ECM) is a significant component of the microenvironment and stiffening of the GBM ECM has been shown to enhance GBM cell migration and proliferation. Research has postulated that to support the increased invasion of the GBM cells due to the stiffening of the GBM ECM, reprogramming of the cells' metabolism must occur. Our project aims to show that increasing stiffening of the ECM results in more glycolytically active GBM cells thereby providing the energy needed to promote the invasion of GBM cells. To study the effect ECM stiffness has on the metabolic activity of GBM cells, we seeded U87MG cells on premanufactured collagen coated hydrogels of 100Pa, 2kPa, and 4kPa stiffness. Stiffnesses were selected based on the stiffness of normal brain tissue (100Pa) and the tumor stiffness seen in the GBM ECM (4kPa). We measured lactate secretion, a well-studied glycolytic endpoint, using a colorimetric assay and changes in REDOX ratio (NADH/FAD) through the autofluorescence intensity of NADH and FAD using two photon microscopy. Our **s**tudies revealed that lactate secretion of U87MG cells linearly increased with higher stiffness, with a 20% increase in lactate secretion between the 100PA plate to the 4kPa plate. Two-photon imaging of U87MG cells seeded on the hydrogel plates, identified a decrease in FAD and an increase of NADH autofluorescence as ECM stiffness increased. Overall, this resulted in a doubling of the REDOX ratio from the least stiff plate to the stiffest plate. Together, these results show that the U87 MG cells are more glycolytically active as the ECM stiffness increases. These findings indicate that ECM stiffness plays a role in biophysical/biochemical signaling for the reprogramming of GBM metabolism providing further insight into how this cancer's mechanobiology promotes the reprogramming of the cells' metabolism to support GBM's aggressive and lethal progression.

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Poster

367. Glioblastoma

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 367.13

Topic: B.11. Neuro-Oncology

Support: NIH Grant 5R01CA247290-02

Title: Evaluating the safety, feasibility, and efficacy of intracranial convection-enhanced delivery of laponite magnetic iron-oxide nanoparticles for the potential treatment of glioblastoma

Authors: *J. Y. ZHANG[1], W. F. MAYS[2], D. RIVERA[1], M. ANASTASIADOU[1], A. BOURAS[1], T. CHANENCHUK[1], C. G. HADJIPANAYIS[1];
[1]Icahn Sch. of Med. at Mount Sinai, Icahn Sch. of Med. at Mount Sinai, New York, NY;
[2]Oakwood Univ., Huntsville, AL

Abstract: Glioblastoma (GBM) is the most common primary brain cancer in adults and is universally lethal. Magnetic hyperthermia therapy (MHT) is a nanotechnology-based treatment that consists of local heat generation in a tumor region through direct delivery of magnetic iron-oxide nanoparticles (MIONPs), which are then activated by exposure to an external alternating magnetic field (AMF). The local hyperthermia generated can potentially enhance the anti-tumor effects of radiation therapy (RT) and chemotherapy (temozolomide, TMZ), making MHT in combination with RT and TMZ a promising therapeutic approach for GBM patients. Convection-enhanced delivery (CED) is a well-established technique used to deliver therapeutic agents into the brain and bypass the blood-brain barrier (BBB). CED allows for the delivery of robust MIONP concentrations directly into the tumor bulk and surrounding infiltrating tumor cells. Laponite is a synthetic clay matrix used as a MIONP delivery system. Stable dispersions of laponite are especially important as they complement the bulk flow properties of CED.
In this study, we seek to evaluate the safety and feasibility of intracranial CED as a method of laponite MIONP delivery, as well as to investigate the heating efficacy of the laponite MIONPs. Our preliminary data have confirmed the safety and feasibility of intracranial laponite MIONP CED. Healthy immunocompetent C57BL/6 mice (n=5) were anesthetized and placed on a stereotactic frame, after which intracranial laponite MIONP CED was performed at a flow rate of 0.5 μL/min, delivering a total MIONP volume of 10 μL. No MIONP leak-back was observed. Mice were allowed to recover after CED, and were monitored daily for short- and long-term adverse effects. No signs of toxicity were observed during a 4-week observation period. Additionally, MIONPs were directly visualizable in fresh brain tissue immediately post-CED, and on brain magnetic resonance imaging (MRI) 24 hours post-CED. The MIONPs were also detected in frozen brain sections via Prussian Blue staining. Currently, temperature measurements of stable laponite MIONP dispersions following exposure to an external AMF are being conducted in vitro using different AMF and laponite MIONP solution parameters. Based on the data from this arm, the intracranial heating efficacy of laponite MIONPs after CED and AMF exposure will be determined in vivo, using healthy immunocompetent C57BL/6 mice. Short-term toxicity of laponite MIONPs post-treatment will also be assessed in these mice. Results will inform further animal studies investigating the anti-tumor efficacy of combination laponite MIONP MHT with RT and TMZ.

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Poster

367. Glioblastoma

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Program #/Poster #: 367.14

Topic: B.11. Neuro-Oncology

Support: PhRMA Foundation Postdoctoral Fellowship
NIH Grant K08-NS110919-02

Title: Neuronal origin influences spontaneous network activity in glioblastoma-neuron cocultures

Authors: ***A. G. S. DANIEL**, S. KRISHNA, G. POPOVA, J. KAUR, S. HERVEY-JUMPER; Neurolog. Surgery, UC San Francisco, San Francisco, CA

Abstract: Neuronal activity is emerging as a driver of cancer initiation and proliferation. Several models have been developed to recapitulate cancer-neuron interactions within the tumor microenvironment; however, there is substantial diversity of neuronal subtypes within the murine and human cortex. Differences in the neuronal microenvironment may influence glioma cell behavior and fail to recapitulate *in vivo* mechanisms. Here, we investigate the electrophysiological properties of cortical neurons and activity-regulated paracrine signaling across co-culture conditions using microelectrode arrays (MEA). Cortical neurons were obtained from three conditions, (1) human prenatal tissue, (2) murine embyronic tissue (E18), and (3) murine postnatal tissue (P1.5), then co-cultured with primary patient-derived glioblastoma cultures. Neuronal activity was assessed using weighted mean firing rate (WMFR; spike rate multiplied by number of active electrodes) and network burst synchrony index. Characterization of the activity-dependent paracrine signaling was performed using proteomics. Initiation of spontaneous firing activity differed across neuronal conditions, with activity occurring earlier in postnatal P1.5 cultures (day in vitro [DIV] 7), whereas E18 and human prenatal cultures demonstrated delayed patterns of activity (DIV 14 and DIV 21, respectively). Glioblastoma cells were added on the first day of consistent spiking. All neuronal co-culture conditions demonstrated glioma-induced hyper-activity (increased WMFR), however in different patterns. E18 co-cultures exhibited glioma-induced hyperexcitability within 24 hours with a subsequent decrease in activity over 14 days with minimal synchrony. P1.5 demonstrated no glioma-induced hyper-excitability at 24 hours; however, WMFR peaked by 7 days and remained elevated through co-culture day 14 (including concurrent increase in synchrony). Human postnatal neurons demonstrated a steady and consistent increase in WMFR as early as 24 hours post co-culture and remained active through day 14 with no synchrony change. Our data suggest that careful consideration should go into selecting neuronal co-culture conditions for mechanistic studies.

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Poster

367. Glioblastoma

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Program #/Poster #: 367.15

Topic: B.11. Neuro-Oncology

Support: ARTN; 2T32 AA14127-12
K22 NS092767
NCI P30CA118100
ACSIRG# 131567
UNM Grant Pilot Project #1541

Title: Neurodevelopmental Transcription Factors Promote Proliferation and Play Opposing Roles in Cellular Migration in Glioblastoma

Authors: *B. L. MYERS[1], K. J. BRAYER[2], T. Y. VUE[1];
[1]Univ. of New Mexico Sch. of Med. Dept. of Neurosciences, Albuquerque, NM; [2]Univ. of New Mexico Comprehensive Cancer Ctr., Albuquerque, NM

Abstract: Glioblastoma (GBM) makes up about 50% of primary brain tumors and survival has not increased over the last 30 years, with less than 5% of patients surviving past 5 years of diagnosis. The lethality of GBM is likely due to high degree of inter- and intratumoral heterogeneity and the presence of basic-helix-loop-helix (bHLH) transcription factors such as ASCL1 and OLIG2, which have been shown to be present in the majority of GBMs. Previously, we showed in patient-derived GBM xenograft (PDX-GBM) that ASCL1 binds to promoter and enhancer regions of cell cycle, mitotic, and chromatin organization genes, as well as neurodevelopmental transcription factors including that of OLIG2. Similarly, in this study we showed that OLIG2 binding directly overlaps with the majority (~90%) of ASCL1 binding sites, including at promoter and/or enhancer regions of both ASCL1 and OLIG1/2 loci, illustrating a potential redundant and/or feed-forward function between these two bHLHs in GBM. It has been proposed that the presence of ASCL1 and OLIG2 contribute to the neural stem cell-like properties of these cancer cells, which may be responsible for the increased treatment resistivity and high recurrence rate of GBMs in patients. Using an immune competent glioma mouse model, we are able to efficiently induce tumors from glial progenitors surrounding the lateral ventricle while altering the level of ASCL1 and OLIG2 to assess their combinatory roles in GBM progression. Remarkably, we found that both ASCL1 and OLIG2 are required for tumor initiation, whereas the loss of only ASCL1 resulted in reduced cellular migration from the tumor bulk while the loss of OLIG2 promotes a highly migratory phenotype along white matter tracts and into the surrounding parenchyma. In contrast, elevating the levels of ASCL1 increased both tumor cell proliferation and a highly migratory phenotype similar to the loss of OLIG2. Using

single cell RNA-sequencing, we demonstrate that tumor cells of the glioma mouse model are highly heterogeneous, comprising of multiple cell types and GBM subtypes similar to human GBMs. Interestingly, we found that tumor cells which express high levels of ASCL1 exhibit neural stem cell and astrocytic gene signatures, which further support ASCL1's role as a marker of glioma-stem-cells. Collectively, these research findings illustrate an important role for ASCL1 and OLIG2 in regulating GBM initiation, proliferation, and migration in the brain where ASCL1 may directly be responsible for the highly invasive and proliferative phenotype of GBMs.

Disclosures: B.L. Myers: None. **K.J. Brayer:** None. **T.Y. Vue:** None.

Poster

367. Glioblastoma

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Topic: B.11. Neuro-Oncology

Support: UCLA Health - Nuclear Medicine
UCLA School of Medicine
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CIHR Grant FDN-148413

Title: Development of Peptide-Based Apelin Theranostic Probes for Glioblastoma

Authors: *F. ALBANAA[1], P. JEANJEAN[1], S. KWOCK[1], C. TSE[1], J. FOURNIER[4], J.-M. LONGPRÉ[4], P. SARRET[4], T. F. CLOUGHESY[1,2,3,5], D. A. NATHANSON[1,5], J. CZERNIN[1,5], G. CARLUCCI[1,5], C. E. MONA[1,5], E. BESSERER-OFFROY[1,5,6];
[1]Mol. and Med. Pharmacol., [2]Neurol., [3]Neuro-Oncology Program, UCLA, Los Angeles, CA; [4]Pharmacology-Physiology, Univ. de Sherbrooke, Sherbrooke, QC, Canada; [5]UCLA Jonsson Comprehensive Cancer Ctr., Los Angeles, CA; [6]California NanoSystems Inst., Los Angeles, CA

Abstract: Glioblastoma (GBM) is the most aggressive and life-threatening form of brain cancer, with a 5- year survival rate of 4%. Despite numerous advances in research, a cure has remained elusive. Nonetheless, recent research efforts have identified the apelin receptor (APJ) as a promising therapeutic target for GBM. APJ is a class A G-protein coupled receptor that is highly expressed at the surface of GBM tumor cells and endothelial cells irrigating the tumor. Apelin, APJ's natural ligand, has been shown to promote GBM proliferation and increase tumor vascularization. Thus, the apelin/APJ axis plays a critical role in GBM progression, invasion, and tumor angiogenesis. Therefore, APJ represents a potential target for targeted radioligand therapy (RLT) and precision oncology. In this study, we designed, synthesized, and characterized new peptide-derived probes based on apelin-13 for imaging and therapy. RNA-Seq was used to

quantify expression of APJ in purified GBM patient samples from UCLA Health, as well as matching Patient-Direct Orthotopic Xenograft (PDOX) and derived gliomaspheres. Peptide-derived probes were synthesized using standard Fmoc-based solid-phase peptide synthesis. A DOTA was conjugated to the peptide sequence at its N-terminus using a proprietary linker. Different probes were developed, incorporating amino-acid substitutions in the apelin sequence. To increase probe retention into the blood stream, we also integrated an albumin-binder moiety based on Evan's blue into the probe sequence. Probes were then radiolabeled using 68Ga. NSG mice were implanted with U87MG, a human GBM cell line, engineered to overexpress APJ, for 68Ga-Apelin probes PET imaging. Images were acquired on a G8 microPET/CT scanner 30 min and 2 hours after IV injection. PET images were treated using OsiriX and SUVmax and SUVmean values were calculated. We found that APJ expression is heterogenous in patients and expression is conserved in PDOX. However, APJ expression is not carried through during gliomasphere generation. Our newly synthesized PET probes showed promise in PET imaging. 30 min after injection, the reported SUVmax is 2.2 with a tumor to background ratio of fifty-six. Apelin probes with an albumin-binding moiety showed an SUVmax of 2.5 after 30 min and 5.2 after 2 hours. These results suggest that apelin probes have the potential to image subcutaneous GBM tumors and to deliver targeted radionuclide therapies; however, studies in orthotopic models will be required to evaluate brain penetration of the probes.

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Poster

367. Glioblastoma

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 367.17

Topic: B.11. Neuro-Oncology

Support: UCLA Health - Nuclear Medicine
UCLA School of Medicine
UCLA Jonsson Comprehensive Cancer Center
NIH - National Center for Advancing Translational Sciences Grant UL1TR001881
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Title: Fap theranostics: opportunities and challenges in GBM

Authors: P. JEANJEAN[1], S. KWOCK[1], C. TSE[1], T. F. CLOUGHESY[1,2,3,4], J. CZERNIN[1,4], G. CARLUCCI[1,4], D. A. NATHANSON[1,4], C. E. MONA[1,4], E. BESSERER-OFFROY[1,4,5]; [1]Mol. and Med. Pharmacol., [2]Neurol., [3]Neuro-Oncology Program, UCLA, Los Angeles, CA; [4]UCLA Jonsson Comprehensive Cancer Ctr., Los Angeles, CA; [5]California NanoSystems Inst., Los Angeles, CA

Abstract: Glioblastomas (GBM) are grade IV gliomas and the most aggressive, invasive, and fatal type of brain cancer. GBM 2- and 5-year survival rates are 18% and 4%, respectively, after surgical resection combined with chemo- and radiotherapy. Despite intensive research efforts leading to various pharmacologic approaches, a cure has remained elusive. Recently, fibroblast activation protein (FAP) has emerged as a new therapeutic target. FAP is expressed by cancer-associated fibroblasts (CAFs) and tumors cells in GBM. Radiolabeled small molecules targeting FAP were recently investigated for their use as pan-cancer theranostics agents. This study aimed to define FAP expression in GBM using RNA-Seq and immunohistochemistry (IHC) as well as investigate the effects of FAP-targeted radioligand therapy (RLT) in GBM models.FAP expression was quantified using RNA-Seq and IHC on purified-GBM patient samples as well as matching patient-direct orthotopic xenograft (PDOX) and gliomaspheres (GS). We then used U87MG, a glioblastoma-derived cell line known to express FAP, as a xenograft model for RLT. NSG mice were subcutaneously implanted with U87MG cells. Tumor FAP expression was assessed by ^{68}Ga-FAPi-46 PET/CT when tumors reached ~ 100 mm^3. Mice were then randomized into the following groups: (*1*) vehicle, (*2*) 5 mg/kg temozolomide (TMZ), (*3*) 40 kBq ^{225}Ac-FAPi-46 and (*4*) 5 mg/kg TMZ + 40 kBq ^{225}Ac-FAPi-46.FAP showed heterogenous expression pattern across our purified-GBM samples that correlated with TCGA RNA-Seq data. Additionally, FAP expression was conserved in matching PDOX and GS. IHC staining for FAP in PDOX tissues showed an expression of FAP by tumor cells and pericytes at the periphery of blood vessels as previously shown. In our model, we confirmed that U87MG tumors expressed FAP by PET imaging (SUVmax=6.1 $\pm$ 1.2). Mice were then treated with a single injection of ^{225}Ac-FAPi-46 and showed a delay in tumor growth compared to vehicle-treated animals (median survival of 30 and 23 days, respectively). Treatment with TMZ induced a significant delay in tumor growth of 23 days. Finally, the combination of ^{225}Ac-FAPi-46 and TMZ show a significant improvement of survival rate compared to TMZ alone (median survival of 67 and 46 days, respectively).As FAP is expressed by both tumor and tumor microenvironment cells in GBM, FAP-targeted RLT can act on these 2 compartments. Our results suggest that ^{225}Ac-FAPi-46 has a real theranostic potential in GBM when combined with TMZ. We also confirmed that FAP expression is conserved from patient samples to PDOX and GS, allowing the use of these types of samples for preclinical evaluation of FAP RLT in orthotopic GBM models.

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Poster

367. Glioblastoma

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 367.18

Topic: B.11. Neuro-Oncology

Support: ANSEF 2367

Title: Glioblastoma patients blood serume enzymes activities as the potential diagnostic tool

Authors: *K. DANIELYAN[1], A. MANUKYAN[1], N. OHANYAN[1], S. CHAILYAN[1], L. HARUTYUNYAN[2], R. HARUTYUNYAN[2];
[1]H. Buniatian Inst. of Biochem., Yerevan, Armenia; [2]Natl. Ctr. of Oncology Named after V.A. Fanarjian, Yerevan, Armenia

Abstract: Background. Glioblastoma (GB) is the most common primary malignant brain tumor, affecting 16% of all primary brain and central nervous system neoplasms. From the other hand, XOR (Xanthine Oxidoreductase; EC 1.17.3.2) is the enzyme, elevated during oxidative stress, ADA-2 (Adenosine Deaminase; EC 3.5.4.4) - hallmark of over-activated immune response, whereas PRPS-1 (Phosphoribosylpyrophosphate Synthetase-1; EC 2.7.6.1) is the key regulative enzyme responsible for the syntheses of purine as well as pyrimidine nucleotides. We propose, PRPS-1 activation along with the other mentioned is evidencing about the cells pathological proliferation in GB and might serve as the diagnostic tool. Methods. We used colorimetric reactions for the detection of activities for ADA-2, XOR with the utility of Cary 60 spectrophotometer (Agilent, USA). PRPS-1activity was measured based on the created in our laboratory method (K.E. Danielyan, S.G. Chailyan. Diagnosis of Glioblastoma. Patent Certificate N 3357 A, Armenia, 2020). Results. We have noticed, elevation of XOR as well as ADA2 activities in the serum of the GB patients. For PRPS1 we have obtained two sets of the data. In the early stage of the GB development we have noticed the slight elevation of the enzymes activity, where as in the patients who were treated previously or carried the mature tumor, PRPS1 activity was diminished (although background activity was elevated). The specific activity of the PRPS-1 in GB was 0,81±0,1, 0,91±0,14 (with substrate). Activities of the XO for the glioblatoma were 2,54±0,05; 3,72±0,07 (substrate) and for ADA activities - 0,8±0,26-control; 1,86±0,39 (substrate). The results of PRPS1 activities for the control group was 6,34±1,65 and 6,95±1,76 in the presence of the substrate (in patients with mature tumor). Conclusion. Activities of ADA-2, XOR as well as PRPS-1 might be serving as the diagnostic tools for the detection of the dynamic development of GB as well as for delineation of necessity for the placement of the polymers (created in National Center of Oncology of RA and consisted from polyethylene glycol, polyvinyl butyral, polyvinyl acetate as well as polyvinyl alcohol) into the lumen of surgical cavity , containing methotrexate in basic layer.

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Poster

367. Glioblastoma

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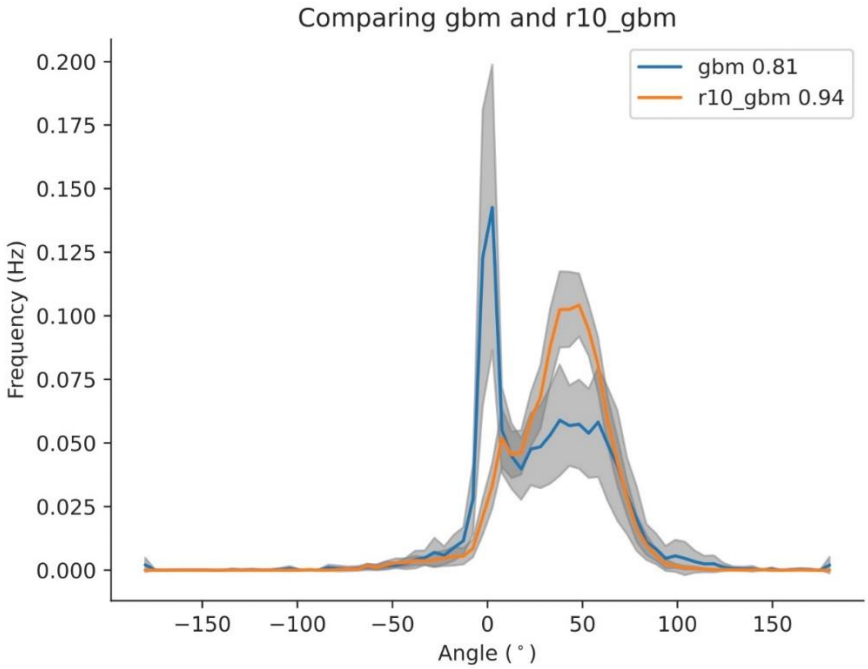
Program #/Poster #: 367.19

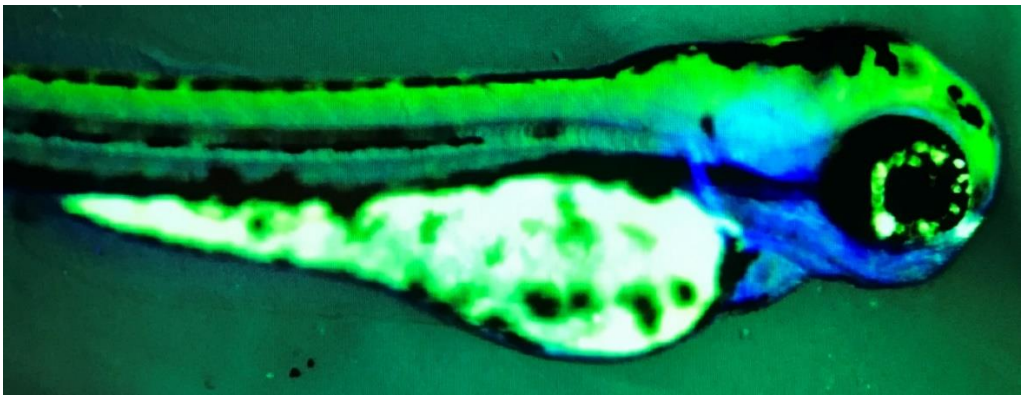
Topic: B.11. Neuro-Oncology

Title: Pharmacological suppression of neuronal activity can reduce glioblastoma burden in the zebrafish larvae model

Authors: *F. AKTER[1], K. KRISHNAN[3], C. REILLY[3], M. DUQUE RAMIREZ[1], P. PFLITSCH[1], Y. ISOE[2], F. ENGERT[3];
[1]Harvard Univ., cambridge, MA; [2]MCB, Harvard Univ., Cambridge, MA; [3]Harvard, Cambridge, MA

Abstract: Glioblastoma (GBM) is the most common malignant brain tumor in adults with a 5-year survival rate of 5.8%. Despite advances in our understanding of the disease, the biology of the tumor is incompletely understood. Objective: Xenotransplant human GBM cells into the hindbrain of the zebrafish larvae at 2 days post fertilization (dpf) to investigate the role of neuronal activity in GBM growth. Methods: Successful transplantation is confirmed using immunofluorescence and live confocal imaging followed by behavioral analysis at 7 dpf. Treatment groups include 1) GBM fish (n=24) (Figure 1); control fish treated with proliferative non-GBM cells (n=24), GBM fish treated with Riluzole to suppress neuronal activity (Nav 1.6 antagonist) daily at 2 dpf (n=24). Results: In vitro, Riluzole 10uM significantly reduces cell viability of GBM cells but not control cells. In vivo, GBM fish survive an average 8 dpf vs 18 dpf for control fish, with body axis malformation developing at 6 dpf vs 16 dpf, respectively. Interestingly, GBM fish treated with Riluzole daily, either did not develop tumors or displayed minimal growth. Behavioral analysis revealed rescue effect of the total bout rate and optomotor response in GBM fish treated with Riluzole (Figure 2). Conclusion: The zebrafish larvae xenotransplantation model is a reliable model for GBM. Riluzole, an FDA approved drug can reduce tumor burden and improve survival and therefore has potential for further exploration in human clinical trials.





Disclosures: **F. akter:** None. **K. Krishnan:** None. **C. Reilly:** None. **M. Duque Ramirez:** None. **P. Pflitsch:** None. **Y. Isoe:** None. **F. Engert:** A. Employment/Salary (full or part-time):; Harvard University.

Poster

368. Alzheimer Disease and Immune Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 368.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R44AG071386-01A1

Title: The Development of a Long-Term Potentiation Model for the Assessment of Drug-Induced Dementia

Authors: *K. AUTAR[1], V. SMITH[3], M. GRILLO[1], N. S. NARASIMHAN[3], W. BOGEN[3], M. JACKSON[3], C. LONG[3], X. GUO[2], J. J. HICKMAN[1];
[1]Univ. of Central Florida, Orlando, FL; [2]Univ. of Central Florida, Oviedo, FL; [3]Hesperos, Inc., Orlando, FL

Abstract: Alzheimer's disease (AD) is characterized by the formation of amyloid-beta (Aβ) plaques leading to synaptic deterioration and deficits in cognitive functioning. The cholinergic hypothesis of AD suggests that under physiological conditions, synaptic activity induces the release of the neurotransmitter acetylcholine (ACh), leading to subsequent modulation of acetylcholine receptors (AChR), enhancing synaptic plasticity and memory. However, in pathogenic conditions, abnormal accumulation of Aβ induces inhibition of ACh binding to and internalization of AChRs resulting in synaptic dysfunction and memory loss. In order to assess functional deficits induced by this "cholinergic burden", an organ-on-a-chip model was established to evaluate long-term potentiation (LTP) using healthy and AD patient-derived presenilin-1 (PSEN1) induced pluripotent stem cell-derived (iPSC) cortical neurons. The PSEN1 protein, located in the catalytic subunit of the gamma-secretase complex, is directly involved in the improper cleavage of $A\beta_{40}$, leading to excessive generation of $A\beta_{42}$. LTP was induced via a

high-frequency stimulation protocol, and was defined as enhanced, long-term neuronal activity correlated to synaptic plasticity. Drug-induced dementia was modeled by acutely dosing healthy iPSC-derived cortical neurons with a variety of anticholinergic drugs, including diphenhydramine, amitriptyline, and orphenadrine and evaluating its effect on the maintenance of LTP. The results indicated that each drug produced a dose-dependent inhibition of persistent LTP. The dementia-inducing effects of these drugs were further investigated in the AD cortical neuron model (PSEN1). This mutation has been known to cause improper glutamate reuptake, leading to excitotoxicity which, in combination with anticholinergic drugs resulted in a cholinergic burden with heightened functional deficits in persistent LTP. This serum-free model of drug-induced dementia demonstrates the ability of in-vitro models to assess cognitive deficits in mechanisms relating to AD.

Disclosures: **K. Autar:** None. **V. Smith:** None. **M. Grillo:** None. **N.S. Narasimhan:** None. **W. Bogen:** None. **M. Jackson:** None. **C. Long:** None. **X. Guo:** None. **J.J. Hickman:** None.

Poster

368. Alzheimer Disease and Immune Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 368.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Carney Institute Zimmerman Innovation Award in Brain Science
US Dept. of Veterans Affairs N2864-C
NIH Grant R21AG077697

Title: A hybrid three-dimensional cortical culture system to study human iPSC-derived microglia: toward deciphering the role of microglia in Alzheimer's disease

Authors: ***S. BROWN**[1,2], B. ASSETTA[3], M. LEHOUX[3], J. OMAR[1], X. YANG[3], Y.-W. HUANG[3,4], D. A. BORTON[1,4,5,2];
[1]Sch. of Engin., [2]Ctr. for Biomed. Engin., [3]Mol. Biology, Cell Biol. and Biochem., Brown Univ., Providence, RI; [4]Carney Inst. for Brain Sci., Providence, RI; [5]Providence Med. Center, Ctr. for Neurorestoration and Neurotechnology, DVA, Providence, RI

Abstract: As the most common form of neurodegenerative disease, Alzheimer Disease (AD) is estimated to contribute to nearly 60–70% of all cases of dementia globally. Currently, the path towards successful therapeutic intervention is obstructed by a limited understanding of the disease etiology and the myriad of underlying mechanisms. In recent years, a growing body of evidence indicating increased levels of inflammatory markers in AD patients, as well as immune-associated loci of several AD risk genes, suggest that neuroinflammation is a contributing factor to pathogenesis and disease progression. As microglia are dynamic mediators of neuroinflammation, determining their specific roles in AD pathology is essential to deciphering neuroinflammatory influence on underlying AD mechanisms. However, the intrinsic ability of

microglia to sense the surrounding neural environment and respond via diverse transcriptional programs, although key to their functionality, inherently makes them difficult to study ex vivo. Extensive in vitro characterization of both primary and iPSC-derived microglia (iMGs) has shown they can rapidly shift cell "states", marked by changes in form, function, and gene expression patterns in response to their culture environment. These responses to the ex vivo environment render unique cell states that do not mirror those found in the living brain. Interestingly, recent studies have demonstrated that xeno-engraftment of cultured human iMGs into rodent brains in vivo can effectively drive cell states that are more similar to homeostatic microglia in the human brain. Here, we present a hybrid in vitro model to study human iMGs in a three-dimensional cortical xenoculture system. We characterize dynamic morphofunctional profiles of human iMGs seeded in rodent-derived three-dimensional primary cortical cultures. Seeded iMGs embed into the three-dimensional cultures, interact with other neural cell types, and respond to neuroinflammatory stimuli. iMG behaviors were characterized by quantitative assessment of changes in morphology, phagocytic capacity, and cytokine secretion over time. We demonstrate use of this hybrid culture system to drive iMG cell states that better mimic those found in the living brain thus expanding the capability of in vitro tools to further probe the roles of human microglia in AD.

Disclosures: S. Brown: None. **B. Assetta:** None. **M. Lehoux:** None. **J. Omar:** None. **X. Yang:** None. **Y. Huang:** None. **D.A. Borton:** None.

Poster

368. Alzheimer Disease and Immune Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 368.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NASA Grant 80NSSC22M0027

Title: Tmem38b mrna is elevated in alzheimer's disease

Authors: K. LEGG[1], J. CONRAD[1], H. WHITE[1], O. S. SPICER[2], ***J. W. SHIM**[1];
[1]Biomed. engineering, Marshall Univ., Huntington, WV; [2]Natl. Inst. of Mental Hlth., Natl. Inst. of Hlth., Rockville, MD

Abstract: Symptoms of normal pressure hydrocephalus (NPH) and Alzheimer's disease (AD) are similar, and it is not uncommon to misdiagnose these two conditions. Although increasing evidence supports that neuroinflammation is detectable in humans with NPH and AD alike, which resident or infiltrating cell expressing mRNA markers mediates neuroinflammation remains poorly understood. Here, we hypothesize that NPH can be differentiated from AD with mRNA biomarkers of unvaried proximity to telomeres and that infiltrating T cells affect neurodegeneration. We examined human caudate nucleus tissue samples for the expression of transient receptor potential cation channel subfamily V member 4 (TRPV4) and transmembrane

protein 38B (TMEM38B). Using genome data viewer, we then analyzed the mutability of *TRPV4* and other genes in mice, rats, and humans through matching nucleotides of genes of interest with two factors associated with high mutation rate: (i) proximity to telomeres or (ii) high adenine and thymine (A+T) content. We found that *TRPV4* mRNA was elevated in NPH. Unlike NPH, mRNA expressions of microtubule associated protein tau (MAPT) and TMEM38B were elevated in AD. In mice, rats, and humans, the size of *TRPV4* was not varied, while in *many* other marker genes, the sizes were inconsistent among species. Our analyses reveal that *TRPV4* gene size and mutability is conserved across three species, suggesting that TRPV4 is a reproducible biomarker of NPH in mice, rats, and humans, while TMEM38B might be expressed by multiple cell types. Two mRNA markers in the caudate nucleus distinguish NPH from AD.

Disclosures: K. Legg: None. **J. Conrad:** None. **H. White:** None. **O.S. Spicer:** None. **J.W. Shim:** None.

Poster

368. Alzheimer Disease and Immune Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 368.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Modeling how the environment and genetic susceptibility interact to alter microglia response in disease contexts.

Authors: *M. THERRIEN, M.-J. DOLAN, S. JEREB, D. MEYER, N. WEIDER, T. KAMATH, T. ATKESON, A. GREKA, S. MCCARROLL, E. Z. MACOSKO, B. STEVENS; Broad Inst., Boston, MA

Abstract: Microglia, the brain's resident immune cells, respond readily to changes in the brain environment. Understanding how these immune cells react to changes in their environment and how genetic risks alter this response is essential to having a complete picture of disease mechanisms. Our goal is to connect insights from genetic association studies to new ways of functionally modeling the cellular and molecular causes of disease to enable predictive tracking and targeting of detrimental immune cell states in patients in the early stages of the disease. Single-cell transcriptomic studies reveal diverse microglial states in human and mouse brains, however, we lack models to track and manipulate these states. To address these knowledge gaps, our lab has developed a novel human-induced pluripotent stem cell (iPSC)-based platform (Dolan*, Therrien*et al. bioRxiv 2022). The platform allows us to generate iPSC-derived microglia (iMGLs) that take on diverse transcriptional signatures similar to those found in the human brain in response to exposure to brain-relevant substrates while avoiding culturing artifacts.
Building on this system, we have developed a toolset to study iMGL states and functions, including an optimized lentivirus protocol that allows for efficient genetic manipulation of microglia; reporters of microglia state to track the expression of key microglial genes, including

APOE; iPSC “villages”, or pooled cultures, that combine iPSC cell lines in the same dish, and xenograft mouse models to evaluate the influence of aging on human microglia states and functions in vivo. Using our new platform, we have characterized 50 iPSC-derived lines selected based on their polygenic risk score for Alzheimer's disease (AD) and APOE genotype. Using single-cell RNA-sequencing and functional assays, we identified features of microglia states and functions specific to common AD variants and others specific to the APOE genotype. Together, our data identified key environmental elements altering microglia and how AD risk genes affect states and functions.

Disclosures: M. Therrien: None. **M. Dolan:** None. **S. Jereb:** None. **D. Meyer:** None. **N. Weider:** None. **T. Kamath:** None. **T. Atkeson:** None. **A. Greka:** None. **S. McCarroll:** None. **E.Z. Macosko:** None. **B. Stevens:** None.

Poster

368. Alzheimer Disease and Immune Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 368.05

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: Cure Alzheimer's Fund

Title: Innate immunity protein IFITM3 is involved in Alzheimer’s disease-associated microglial response

Authors: *T. JAIN, G. FROST, J.-Y. HUR, L. STUDER, Y.-M. LI;
Sloan-Kettering Inst., New York, NY

Abstract: Alzheimer’s disease (AD) is the most common neurodegenerative disorder. It has complex neuropathology which includes an accumulation of amyloid beta (Aβ) plaques, hyper-phosphorylated tau tangles, and neuroinflammation. Our lab has identified that expression of the interferon response protein IFITM3 is upregulated in human AD brain as well as in 5xFAD mouse brain. We have shown that IFITM3 regulates γ-secretase activity in neurons and astrocytes, and a deficit in IFITM3 results in decreased γ-secretase cleavage of APP. Further, IFITM3 is highly expressed and inducible in microglia. Research has indicated that microglia play a critical role in AD progression. Our current focus is on investigating the function of IFITM3 in the phagocytic clearance of Aβ by microglia, its interplay with TREM2 and downstream signaling in the context of neuroinflammation. For this, we have generated 5xFAD;IFITM3$^{-/-}$;TREM2$^{-/-}$ mice and have established stem cell-derived IFITM3-/- microglial cells. With our studies, we hope to determine the role of IFITM3 in microglia, which has not been previously described before, using cellular assays and mouse models.

Disclosures: T. Jain: None. **G. Frost:** None. **J. Hur:** None. **L. Studer:** None. **Y. Li:** None.

Poster

368. Alzheimer Disease and Immune Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 368.06

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 1F32AG077881-01

Title: Exploring the effects of TREM2 and TYROBP on microglial homeostasis and activation

Authors: *G. E. FARIAS QUIPILDOR[1], R. BELFIORE[2], S. SALTON[3], M. E. EHRLICH[4], S. E. GANDY[5];
[1]Neurol. and Neurosci., [2]Neurol., [3]Neurosci. and Geriatrics, [4]Neurol. and Pediatrics, [5]Neurol. & Psychiatry, Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Microglia, the primary immune cell in the brain, have multiple activation phenotypes involved in broad functions in the brain, playing roles in neurotoxicity/neuroprotection – and in release of inflammatory and anti-inflammatory cytokines – as well as roles in cell survival, proliferation, and phagocytosis. TREM2 and TYROBP form a transmembrane complex in microglia that leads to intracellular signaling networks, and these proteins are important regulators of the transition from homeostatic microglia to its activation states. Recent findings have proposed a TYROBP-dependent and TREM2-independent molecular signature that is involved in the early transition step from homeostatic to disease-associated microglia (DAM). Interestingly, the sequential step of DAM activation is TREM2-dependent. However, the underlying mechanisms that determine how TREM2 or TYROBP regulate these downstream phenotypes are largely unknown. For this purpose, we have isolated primary microglia from C57BL/6 wild-type (WT) controls, *Trem2* knock-out (KO) and *Tyrobp* KO mice at post-natal day 0-3. Cells were stimulated with Alzheimer's disease (AD)-relevant provocations, such as amyloid beta (Aβ) oligomers, or 'inflammatory' stimuli, such as lipopolysaccharides (LPS). We explored protein and gene expression in the presence or absence of inhibitors within the TREM2/TYROBP downstream signaling pathway. Our results show that absence of either TREM2 or TYROBP is associated with increased basal levels of the phosphorylation state of ERK in primary microglia, when compared to WT controls. In addition, TREM2 KO and TYROBP KO cells show a less ramified cell morphology at baseline, as compared to WT microglia. Moreover, stimulating primary microglia with either Aβ oligomers or LPS leads to differential protein as well as gene expression outcomes in cells that lack either TREM2 or TYROBP. The dysregulated downstream signal transduction and morphology in the absence of TREM2 or TYROBP suggests their important roles not only in microglial homeostasis but also in the activation processes in response to different stimuli. Future goals include the investigation of the roles that these proteins play in the mechanisms underlying microglial activation in different contexts. We ultimately aim to identify potential therapeutic targets that could contribute to the delay or treatment of AD pathology.

Disclosures: G.E. Farias Quipildor: None. **R. Belfiore:** None. **S. Salton:** None. **M.E. Ehrlich:** None. **S.E. Gandy:** None.

Poster

368. Alzheimer Disease and Immune Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 368.07

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: National Research Foundation 2020R1A2C2010285
National Research Foundation I21SS7606036
Ministry of Health & Welfare and Ministry of Science and ICT HU22C0115
Korea Health Industry Development Institute
Korea Dementia Research Center

Title: Ifng-driven nrf2 downregulation in microglia exacerbates alzheimer's disease

Authors: *Y. KANG[1,2], S. HYEON[5], A. MCQUADE[6], J. LIM[7,8], S. BAEK[3], D.-G. JO[3], C. LEE[8], M. BLURTON-JONES[9,10,11], H. RYU[5], H. CHO[1,2,4];
[1]Inst. of Quantum Biophysics, [2]Dept. of Biophysics, [3]Sch. of Pharm., [4]Dept. of Intelligent Precision Healthcare Convergence, Sungkyunkwan Univ., Suwon-si, Korea, Republic of; [5]Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of; [6]Inst. for Neurodegenerative Dis., Univ. of California, San Francisco, CA; [7]IBS Sch., Univ. of Sci. and Technol., Daejeon, Korea, Republic of; [8]Ctr. for Cognition and Sociality, Inst. for Basic Sci., Daejeon, Korea, Republic of; [9]Neurobio. & Behavior, [10]Sue and Bill Gross Stem Cell Res. Ctr., [11]Inst. for Memory Impairments and Neurolog. Disorders, Univ. of California Irvine, Irvine, CA

Abstract: Recent reports reveal that microglia interacting with beta-amyloid plaques appears to be neuroprotective yet retaining proinflammatory phenotype often precedes neurodegeneration in AD. However, it remains unclear how microglial activity transits to the neurotoxic state in response to AD-associated environments, in part due to the lack of pathophysiological and accessible models of human AD brains. Here, we investigate the underlying mechanism of detrimental innate immunity in AD, particularly focusing on microglia, by employing our 3D human APP-mutated mini-brains including human induced pluripotent stem cells (iPSC)-derived microglia. We observe that reactive astrocytes initiate neuroinflammation by releasing interferon-gamma (IFNγ) and excessive oxidative stress (H_2O_2) under amyloid-beta (Abeta)-rich environments. Our results show that the astrocytic IFNγ downregulates microglial nuclear factor erythroid-2-related factor 2 (Nrf2) *via* Kelch-like ECH-associated Protein 1 (Keap1) upregulation. Interestingly, the downregulation of Nrf2 sensitizes microglia to the oxidative stress and induces the transition into the neurotoxic phenotype in AD. These proinflammatory microglia in turn produce neurotoxic nitric oxide and inflammatory mediators resulting in synaptic impairment, phosphorylated-tau accumulation, and discernable neuronal loss. We validate the reduction of Nrf2 in neurodegenerative microglia adjacent to aggregated phosphorylated-tau in IFNγ-expressing brain tissues of late-staged human AD patients and 5XFAD mice. Overall, our study concludes that IFNγ-driven Nrf2 downregulation in microglia as a key mediator of AD pathogenesis.

Disclosures: **Y. Kang:** None. **S. Hyeon:** None. **A. McQuade:** None. **J. Lim:** None. **S. Baek:** None. **D. Jo:** None. **C. Lee:** None. **M. Blurton-Jones:** None. **H. Ryu:** None. **H. Cho:** None.

Poster

368. Alzheimer Disease and Immune Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 368.08

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR
NSERC

Title: GM1 ganglioside alleviates LPS and beta-amyloid mediated microglia inflammation by increasing autophagy flux

Authors: *W. WANG, L. ZHAO, P. PITTOCK, S. WHITEHEAD;
Univ. of Western Ontario, London, ON, Canada

Abstract: Gangliosides are glycosphingolipids highly enriched in the central nervous system, making up 10% of the total lipid content and play a critical role in membrane organization and signal transduction. GM1, the most abundant ganglioside in the adult brain, is enriched in lipid rafts and serves a neuroprotective role. In contrast, the accumulation of its precursors GM2 and GM3 are associated with neurodegeneration. Ganglioside dysregulation is observed in neurodegenerative diseases, such as Alzheimer's Disease (AD). Microglia are resident macrophages of the CNS responsible for maintaining homeostasis and become activated in response to neuronal injury or toxicity. In the AD brain, microglia are responsible for clearance of beta-amyloid (Aβ) deposits, but chronic microglia activation promotes neuroinflammation that exacerbates AD progression. Importantly, GM1 ganglioside demonstrates an anti-inflammatory effect on microglia activation, yet the mechanism is unknown. This study interrogates the protective mechanism of GM1 on modulating the autophagy pathway in microglia in response to amyloid-mediated inflammation. We aim to reveal potential therapeutic benefits of restoring ganglioside balance to target neuroinflammation in AD.
Methods: BV2 microglia culture was activated by LPS and Aβ treatment. Autophagy modulation was achieved with the mTOR inhibitor rapamycin, and GM1. Inflammation was measured by gene expression of cytokines. Autophagy was measured by p62 and LC3 expression. Microglia ganglioside abundance was detected by electrospray-ionization mass spectrometry.
Results: LPS and Aβ aggregates induced a pro-inflammatory response in BV2 microglia. Elevated inflammatory cytokines correlated with suppressed autophagy and promoted GM1 ganglioside degradation in the lysosome. Exogenous GM1 decreased the inflammatory response to LPS and Aβ and restored autophagy flux by downregulating the Akt/mTOR pathway to promote autophagy initiation.
Conclusion: GM1 alleviated microglia inflammation by driving the autophagy pathway. Further

investigation is required to link autophagy flux with lysosomal clearance. GM1 upregulation of autophagy may be a promising therapeutic target to resolve neuroinflammation in AD.

Disclosures: **W. Wang:** None. **L. Zhao:** None. **P. Pittock:** None. **S. Whitehead:** None.

Poster

368. Alzheimer Disease and Immune Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 368.09

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Investigating the regulation of Sortilin by NSG1 and NSG2 using in vitro and in silico methods

Authors: J. A. LORENTSEN[1], M. OVERBY[2], B. SCHIØTT[1], H. K. MÜLLER[3], ***N. BERGLUND[4]**;
[1]Aarhus Univ. - Kemi, Aarhus, Denmark; [2]Clin. Med., Aarhus Univ., Aarhus C, Denmark; [3]Aarhus Univ. Psychiatric Hosp., Risskov, Denmark; [4]Univ. of Aarhus, Aarhus, Denmark

Abstract: Ectodomain shedding of the sorting receptor Sortilin (SORT1) is presumed to be related to the pathology of Alzheimer's disease (AD) and other dementias. Recent studies have shown that C-terminal fragments of SORT1 are present in the amyloid-β protein (Aβ) plaques found in the brain tissue of AD patients, which is believed to be the dominant hallmark of AD. The two neuron-specific proteins, called the neuronal-specific gene family members 1 and 2 (NSG1 and NSG2), are recently found to be novel interaction partners to SORT1. NSG1 and NSG2 are structurally similar, however, they affect ectodomain shedding of SORT1 in very different ways. NSG1 renders SORT1 more susceptible to ectodomain shedding upon interaction, while SORT1 is protected from ectodomain shedding when interacting with NSG2. The mechanisms for ectodomain shedding and the interaction of SORT1 with NSG1 and NSG2 are unknown, and it is not clear what causes the differences in the ectodomain shedding of SORT1.
Using molecular dynamics simulations and in vitro methods, we investigate how the interaction interface of SORT1-NSG1 and SORT1-NSG2 differ when simulating the proteins at different resolutions and in different membranes with and without the ectodomains of the proteins being present. Furthermore, we examine if any difference between NSG1 and NSG2, which could explain the differences found in the ectodomain shedding of SORT1, can be observed in the ectodomains or in the juxtamembrane region of SORT1.
We find that NSG1 and NSG2 bind to the same interface of SORT1 and provide atomistic insight into the interaction interface. Our results also indicate that NSG1 and NSG2 alone are unlikely to regulate shedding and that it is likely the interaction or lack of interaction with sheddase that regulates the ectodomain shedding.

Disclosures: **J.A. Lorentsen:** None. **M. Overby:** None. **B. Schiøtt:** None. **H.K. Müller:** None. **N. Berglund:** None.

Poster

368. Alzheimer Disease and Immune Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 368.10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG058063
QFASTR pilot award

Title: Cytoplasmic dsRNA - colocalized with TDP-43 inclusions in sporadic Alzheimer's disease - elicits innate immune-mediated death of human neural cells that is reversed by TYK2 kinase inhibitors

Authors: L. E. KÖNIG[1], A. CHUNG[1], A. ALEJANDRO-SOTO[1], S. RODRIGUEZ[2], *M. ALBERS[2];
[1]Harvard Med. Sch., Boston, MA; [2]Massachusetts Gen. Hosp., Boston, MA

Abstract: Background: Alzheimer's disease (AD) is a heterogeneous disorder without effective disease modifying therapies. Limbic-predominant age-related TAR Binding Protein (TDP)-43 encephalopathy with neuropathology changes (LATE-NC) occurs in nearly half of autopsy-proven cases of AD, and the presence of TDP-43 inclusions correlates with faster cognitive decline. We previously demonstrated that cytoplasmic double stranded RNA (cdsRNA), an established trigger of type I interferon (IFN-I) signaling, is coincident with cytoplasmic TDP-43 inclusions in brains of patients with ALS. CdsRNA drives neuroinflammation and neuronal death in multiple mouse lines.
Methods: We employed cyclic immunofluorescence, a multiplexed fluorescent imaging of human brain tissue, systems pharmacology, and a CRISPR-Cas9 whole genome screen of of differentiated human neural cells followed by validation by generating a dedicated TYK2 knockout in human neural cells.
Results: We demonstrate that cdsRNAs are coincident with cytoplasmic TDP-43 inclusions, but not neurofibrillary tangles, in autopsied brains of AD. We find a strong association of interferon induction in late stage AD brains relative to aged controls with no or very minor AD pathology. The FDA-approved JAK inhibitors ruxolitinib, tofacitinib, and baricitinib block inflammation and prevent neuronal death evoked by cdsRNA in an in vitro model of human neurons, oligodendrocytes, and astrocytes that lack microglia. A CRISPR-Cas9 screen identified IFNAR2 and TYK2, but not JAK1, JAK2, and JAK3, as hits that rescue human neural cell death. Validation of these hits was demonstrated by a blocking antibody of IFNAR2, an interferon receptor, and two independent knockout lines of TYK2. In addition, deucravacitinib, a TYK2-specific kinase inhibitor, rescued human neural cell death evoked by dsRNA in a dose-dependent manner.

Conclusion: Our findings suggest a subtype of sporadic AD or LATE-NC characterized by enhanced IFN-I signaling in setting of cdsRNA and TDP-43 cytoplasmic inclusions, which might be responsive to JAK inhibitors or TYK2 inhibitors. Together, the findings provide the rationale for a pilot drug repurposing clinical trial in MCI or AD patients with elevated interferon-related biomarkers in their CSF.

Disclosures: L.E. König: None. **A. Chung:** None. **A. Alejandro-Soto:** None. **S. Rodriguez:** A. Employment/Salary (full or part-time):; Sanofi. **M. Albers:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent through MGH, Shares in Aromha, Inc..

Poster

368. Alzheimer Disease and Immune Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 368.11

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R01AG063175

Title: Allele-specific open chromatin mapping in human iPSC-derived microglia identifies functional GWAS risk variants for Alzheimer's disease

Authors: *A. KOZLOVA[1], A. SUDWARTS[2,3], S. ZHANG[1,4], H. ZHANG[1], B. JAMISON[1], W. WOOD[1], S. SENGUPTA[1], A. R. SANDERS[1,4], G. THINAKARAN[2], J. DUAN[1,4];
[1]Home, NorthShore Univ. HealthSystem, Evanston, IL; [2]Byrd Alzheimer's Ctr. and Res. Inst., Tampa, FL; [3]Dept. of Mol. Med., Morsani Col. of Med., Tampa, FL; [4]Dept. of Psychiatry and Behavioral Neurosci., Univ. of Chicago, Chicago, IL

Abstract: Genome-wide association studies (GWAS) of Alzheimer's disease (AD) have identified 75 risk loci, providing an opportunity for understanding novel aspects of AD biology. However, each GWAS locus typically spans several genes and many equally associated genome-wide significant index/proxy single-nucleotide polymorphisms (SNPs); it remains challenging to identify which genes and risk SNPs are involved in AD risk. We have recently developed an approach to map putatively functional neuropsychiatric GWAS risk variants that affect chromatin accessibility and gene expression by comparing the quantitative measurements of open chromatin between the two alleles of a heterozygous SNP within the same sample, i.e., allele-specific open chromatin (ASoC) mapping. Here, by performing ASoC mapping with human induced pluripotent stem cell (hiPSC)-derived microglia (iMG), we identified ~250K open chromatin region (OCR) peaks from bulk ATAC-seq data of iMG, which showed strong enrichment of immune, hematopoietic, and migration-related Gene-Ontology terms. OCR peaks of iMG, but not hiPSC-derived neurons or astrocytes, showed strong enrichment for AD heritability. Our further ASoC mapping in iMGs of 28 donors identified 46,965 ASoC (FDR < 0.05) SNPs, of which 32 were AD GWAS risk SNPs at 19 AD risk loci. One of the ASoC SNPs,

rs10792832, was located inside an iMG-specific OCR peak ~87 kb upstream of PICALM (Phosphatidylinositol Binding Clathrin Assembly Protein), a gene whose function in microglia has not been well established. To tie the risk allele of rs10792832 with its effects on PICALM expression and microglia functioning, we performed CRISPR/Cas9 editing of rs10792832 on hiPSC lines (from homozygous G/G to A/A; G = risk allele). We found that PICALM mRNAs were decreased in isogenic iMGs carrying the risk allele under both baseline and LPS-stimulating conditions. Interestingly, the expression of the alternatively spliced iMG-specific transcript isoform was decreased in iMGs carrying risk allele only after 4 hours of LPS treatment. Consistent with the transcriptional effect of rs10792832 risk allele in iMG, we found a decrease of PICALM (including the iMG-specific isoform) in AD patients' brains. We next showed that iMGs carrying the risk allele of rs10792832 exhibited a compromised capability to phagocytose myelin, indicating that PICALM may play a role in AD by regulating myelin phagocytic activity of microglia. Our study provides a framework of prioritizing functional AD GWAS risk variants that affect chromatin accessibility in hiPSC-derived microglia, and sheds novel mechanistic insight on how an AD GWAS risk variant of PICALM confers disease risk in microglia.

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Poster

368. Alzheimer Disease and Immune Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 368.12

Topic: C.02. Alzheimer's Disease and Other Dementias

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Title: Microglial INPP5D limits plaque formation and glial reactivity in the PSAPP mouse model of Alzheimer's disease

Authors: *E. L. CASTRANIO[1], P. HASEL[5], J.-V. HAURE-MIRANDE[1], A. V. RAMIREZ JIMENEZ[1], B. W. HAMILTON[1], R. D. KIM[5], C. G. GLABE[9], M. WANG[2], B. ZHANG[2], S. E. GANDY[1,3,10], S. A. LIDDELOW[5,6,7,8], M. E. EHRLICH[1,2,4];
[1]Neurol., [2]Genet. and Genomic Sci., [3]Psychiatry, [4]Pediatrics, Icahn Sch. of Med. at Mount Sinai, New York, NY; [5]Neurosci. Inst., [6]Neurosci. and Physiol., [7]Ophthalmology, [8]Parekh Ctr. for

Interdisciplinary Neurol., NYU Grossman Sch. of Med., New York, NY; [9]Mol. Biol. and Biochem., Univ. California Irvine, Irvine, CA; [10]James J Peters VA Med. Ctr., Bronx, NY

Abstract: Late-onset Alzheimer's disease (AD) is the most common form of dementia, characterized by progressive memory decline, leading to loss of cerebrocortical function and eventual death. Inositol polyphosphate-5-phosphatase D (*INPP5D*) has been highlighted by multiple approaches as an AD risk gene, and its expression in brain is restricted to microglia. The role that *INPP5D* plays in either early or late disease, and the mechanism remain unknown. We therefore knocked down *Inpp5d* conditionally in microglia using *Inpp5d*^{flox} mice crossed with an inducible *Cx3cr1*$^{CreER/+}$ to determine early effects as amyloid pathology develops in *APP*$^{KM670/671NL}$/*PSEN1*$^{\Delta exon9}$ (*PSAPP*) mice at a time correlating to a preclinical level. To induce recombination, 3-month-old (mo) *Inpp5d*$^{fl/fl}$/*Cx3cr1*$^{CreER/+}$ mice, with and without the *PSAPP* transgenes, were injected for 5 consecutive days with either tamoxifen (TAM) or corn oil (CO). At age 6 mo, we utilized immunohistochemistry to characterize plaque pathology and microgliosis. We observed that *Inpp5d* knockdown in TAM-treated *PSAPP/Inpp5d*$^{fl/fl}$/*Cx3cr1*$^{CreER/+}$ mice significantly increased the percent area of 6E10^{+} deposits in the hippocampi by over 50% compared to CO-treated controls. Additionally, overall area and diameter of individual amyloid plaques were increased. We also found a significant spatially-constrained increase in microglia associated with Aβ-plaques due to *Inpp5d* knockdown, but there was no overall change in microglia number or astrocyte number in the hippocampi. We observed no changes in synaptic markers PSD95 and synaptophysin. There was a ~50% increase in the levels of antibody A11-positive prefibrillar Aβ oligomers in TAM-treated *PSAPP/Inpp5d*$^{fl/fl}$/*Cx3cr1*$^{CreER/+}$ mice. We performed spatial transcriptomics to analyze brain region-resolved and peri-plaque gene expression patterns. Our spatial transcriptomics analysis identified a plaque-specific expression profile, Cluster 26, that was extensively altered by the knockdown of *Inpp5d*. Finally, we projected the differential gene expression profiles of the *Inpp5d* knockdown mouse onto the *INPP5D* regulatory network from human AD and control brains in the Mount Sinai Brain Bank cohort. This analysis revealed significant overlap between our Cluster 26 signature and that of human AD gene networks. These results demonstrate that conditional *Inpp5d* downregulation in the *PSAPP* mouse increases plaque burden and microglial recruitment to plaques. Our spatial transcriptomics analysis highlighted an extended DEG signature associated with plaques and identified a potentially highly specific marker of plaques in the AD brain.

Disclosures: E.L. Castranio: None. **P. Hasel:** None. **J. Haure-Mirande:** None. **A.V. Ramirez Jimenez:** None. **B.W. Hamilton:** None. **R.D. Kim:** None. **C.G. Glabe:** None. **M. Wang:** None. **B. Zhang:** None. **S.E. Gandy:** None. **S.A. Liddelow:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AstronauTx Ltd. **M.E. Ehrlich:** None.

Poster

368. Alzheimer Disease and Immune Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 368.13

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Assessing and resolving the enigmatic role Complement Receptor 1 (CR1) plays in Alzheimer's Disease

Authors: *S. KRAUSE[1,2], J. A. BRANDON[1,2], K. A. NATIONS[1,2], P. H. DOYLE[1,2], E. D. HUCKVALE[1,2], E. L. VANCE[1,2], M. L. PAGE[1,2], M. HODGMAN[1,2], B. AGUZZOLI HEBERLE[1,2], T. D. JENSEN[3], J. B. MILLER[1,2,4], J. D. FRYER[6,7], M. T. W. EBBERT[1,2,5]; [1]Neurosci., Univ. of Kentucky, Lexington, KY; [2]Sanders Brown Ctr. on Aging, Lexington, KY; [3]Genet., Stanford Univ., Stanford, CA; [4]Div. of Biomed. Informatic, Intrnl. Med., [5]Div. of Biomed. Informatics, Intrnl. Mdicine, Univ. of Kentucky Col. of Med., Lexington, KY; [6]Neurosci., Mayo Clin., Scottsdale, AZ; [7]Neurosci. Grad. Program, Mayo Clin. Grad. Sch. of Biomed. Sci., Scottsdale, AZ

Abstract: Complement receptor 1 (CR1) is a top-five Alzheimer's disease (AD) gene, yet the exact mechanism for CR1's involvement in AD remains an enigma. CR1 encodes for a transmembrane receptor that binds several proteins in the immune system's complement cascade. These proteins, C3b, C4b, and C1q, are suggested to have a role in AD pathogenesis. Amyloid beta (AB) is known to activate the complement cascade by binding to C3b and C1q, thus inducing phagocytosis. Additionally, all the CR1 binding proteins have been found in AB plaques. Complement is also shown to be involved in synaptic elimination and pruning. Despite the clear link between complement proteins and AD, CR1 is the only complement protein that has been implemented with AD through GWAS, with several isoforms being linked to a higher risk of AD. Additionally, most understanding of CR1 comes from short-read sequencing. However, CR1 has a camouflaged region which is a duplicated genomic region that cannot be adequately assembled or aligned using standard short-read sequencing. There likely unidentified CR1 mutations and RNA isoforms driving human health and disease. Using long-read sequencing, these areas can be resolved and those mutations and isoforms can be identified. The proposed project is a focused and concerted plan to resolve this problem and better understand CR1's role in AD through long-read sequencing and molecular biology and electrophysiology approaches. The central hypothesis is that CR1 has unidentified DNA haplotypes and RNA isoforms driving disease. Additionally, we further hypothesize CR1 is essential for clearing out AB, maintain synaptic strength, and promoting synaptic plasticity.

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Poster

368. Alzheimer Disease and Immune Mechanisms

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Program #/Poster #: 368.14

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: GOED Grant GR09234

Title: Evaluating proteomic and metabolomic differences from glial cell cultures in neurodegenerative disease mouse models

Authors: *E. N. STROM, A. M. LEISGANG OSSE, A. A. ORTIZ, T. L. JONES-LEPP, S. KURZYNIEC, J. W. KINNEY;
Univ. of Nevada, Las Vegas, Las Vegas, NV

Abstract: Alzheimer's Disease (AD) is a neurodegenerative disease that causes progressive memory loss, cognitive impairment and brain atrophy. Three hallmarks of the disease are β-amyloid plaques, neurofibrillary tangles and chronic neuroinflammation. Proliferation and activation of microglia in the brain is a prominent feature in the pathogenesis of AD. Microglia, the resident immune cells of the brain, are one of the first responders to insult or pathology in neurodegenerative disease in the central nervous system. Evidence suggests that microglia activation has a protective effect in AD early on, however chronic activation of microglia, associated with its altered function, promotes AD pathology. A number of mechanisms are involved in the regulation of glial function, including receptors for classical neurotransmitters. The principle inhibitory neurotransmitter, gamma amino butyric acid (GABA), including the metabotropic receptor GABAB is expressed on microglia and has been shown to modulate the immune response. The loss of this receptor could play an important role in exacerbating the disease. We developed a novel mouse model (GAB/CX3ert) with the loss of the GABAB receptor restricted to glia that exhibits changes in amyloid processing and network function relevant to AD. With rapidly emerging proteomic techniques such as mass spectrometry, this opens the possibility for identifying differentially expressed proteins in neurodegenerative mouse models. Investigating proteomic and metabolomic differences relevant to AD disease will help elucidate the mechanisms regulating glial function and AD pathology. In this study, we evaluated both proteomic alterations and metabolomic differences between young GAB/CX3ert, Tau P301S and wildtype mice strains using several Shimadzu mass spectrometry platforms. Using both targeted and untargeted approaches allow for identifying modifications in novel targets along with comparing the whole mouse proteome between strains. We identified several specific peptide alterations and metabolomic differences between strains that may contribute to AD pathology.

Disclosures: E.N. Strom: A. Employment/Salary (full or part-time):; University of Nevada, Las Vegas. **A.M. Leisgang Osse:** None. **A.A. Ortiz:** None. **T.L. Jones-Lepp:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; University of Nevada, Las Vegas. **S. Kurzyniec:** A. Employment/Salary (full or part-time):; Shimadzu. **J.W. Kinney:** A. Employment/Salary (full or part-time):; University of Nevada, Las Vegas.

Poster

368. Alzheimer Disease and Immune Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 368.15

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: 3RF1AG059717-01S1

Title: Genetics of microglial INPP5D isoform expression in Alzheimer's disease

Authors: *D. ZAJAC[1], J. SIMPSON[1], J. M. MORGANTI[2], S. ESTUS[3];
[2]Neurosci., [3]Physiol., [1]Univ. of Kentucky, Lexington, KY

Abstract: Genetics of microglial INPP5D isoform expression in Alzheimer's diseaseD. ZAJAC, J. SIMPSON, J. MORGANTI, S. ESTUS
INPP5D (Phosphatidylinositol-3,4,5-trisphosphate 5-phosphatase 1), the gene encoding SHIP1, contains single nucleotide polymorphisms (SNPs) that are strongly associated with Alzheimer's Disease (AD) risk. In the brain, *INPP5D* is expressed as several isoforms, mostly in microglia. Full-length SHIP1 is encoded by 27 exons that encode an amino-terminal SH2 domain followed by the phosphatase domain. Truncated isoforms lacking the SH2 domain begin from internal transcription start sites. To better understand the function of *INPP5D* in the human brain, we investigated *INPP5D* isoform expression as a function of AD status, and AD-associated SNPs. Single cell RNA-seq was performed on APP/PS1 mice to understand the relationship between *INPP5D* expression and microglial activation. The expression of microglial *INPP5D* isoforms was analyzed by using qPCR on RNA from AD and non-AD anterior cingulate human brain samples. Samples were genotyped for the AD-associated SNPs rs35349669 and rs10933431 using TaqMan SNP kits (Thermo). Isoform expression results were analyzed as a function of microglial gene expression (ITGAM and AIF1), total *INPP5D* expression, AD status, and SNP status. In addition, reporter SNPs in the 5' UTR region were used to detect unequal allelic expression of *INPP5D* as a function of AD SNP status. APP/PS1 mice showed an increased in disease-associated microglia compared to controls. *INPP5D* was expressed at equivalent levels in both homeostatic and disease-associated microglia. Expression of each *INPP5D* isoform was strongly correlated with microglial gene expression and showed an increase with AD neuropathology but did not show a significant association with AD SNPs. Sequencing of samples heterozygous for both AD SNPs provided preliminary evidence for unequal allele expression modulated by SNP status. We used paired-indexing primers and next generation sequencing to quantify amplicons targeting the allelic expression reporter SNPs in both cDNA and genomic DNA. We then used proxy SNPs to genotype each allele and found that the rs35349669 SNP correlates with allelic expression imbalance. In summary, *INPP5D* expression is uniform in both disease-associated and homeostatic microglia. Expression of *INPP5D* isoforms is increased with AD neuropathology. The mechanisms whereby AD genetics influence *INPP5D* expression is not clear, but preliminary evidence supports the hypothesis that the rs35349669 allele that increases AD risk contributes to allelic expression imbalance in both AD and non-AD human samples.

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Poster

369. Metabolism Alterations in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 369.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: The underlying cellular mechanism of intermittent fasting treatment in high sucrose diet AD pathology in APP/PS1 transgenic mice

Authors: *H.-H. YAO[1], H.-J. TSAY[2];
[1]Neurosci., Natl. Yang-Ming Chiao Tung Univ., Taipei City, Taiwan; [2]Neurosci., Natl. Yang Ming Chiao Tung university, Taipei, Taiwan

Abstract: Alzheimer's disease (AD) contains cognitive and non-cognitive symptoms, but the importance of non-cognitive symptoms is often overlooked. Non-cognitive symptoms including weight loss, feeding dysfunction and alternated metabolic index can occur before the onset of cognitive symptoms and worsen with AD progression. In addition, AD also has high comorbidity rate with diabetes type II. With the western diet which is common use of high fat and high fructose corn syrup in modern human diet, the epidemic of obesity and metabolic disorders becomes more severe. High fat diet is proven to induce metabolic stress and AD central pathogenesis accelerates cognitive symptoms. In our past research, we reveal the high sucrose diet (HSD) elevates neuroinflammation reflected by Amyloid beta-induced GFAP and peripheral inflammation in APP/PS1 mice. Thus, the western diet is a risk factor of AD. Intermittent fasting (IF) can improve metabolism, inflammation, and some studies have pointed out that it may have the effect on improving the cognitive function of AD patients. Our study uses IF to try slowing the AD pathology in HSD APP/PS1 mice and spatial transcriptomics is used to reveal the mechanism of IF. The result figures out that 8-week IF reduces AD pathology and improve the cognitive function in APP/PS1 transgenic mice. The altered transcripts profiles in AD-related pathway by HSD can be altered by IF.

Disclosures: H. Yao: None. **H. Tsay:** None.

Poster

369. Metabolism Alterations in Alzheimer's Disease

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Program #/Poster #: 369.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: 1R01AG071782
2P01AG012411

Title: Amyloid β-peptide impacts on glucose regulation are dependent on apolipoprotein E genotype

Authors: *S. W. BARGER, J. H. SUNG, Y. OU;
Geriatrics, Univ. of Arkansas for Med. Sci., Little Rock, AR

Abstract: The apolipoprotein E gene (*APOE*) constitutes the greatest genetic risk factor for Alzheimer's disease, wherein the ε4 allele confers a dramatically elevated risk compared to the more common ε3 allele. Biological mechanisms that differ across these alleles have been explored in mouse models wherein the murine *Apoe* gene has undergone targeted replacement with sequences encoding human ApoE3 or -4 (ApoE-TR mice). Results with such models have indicated that the two variants of ApoE produce differential effects on energy metabolism, including metabolic syndrome. However, glucose regulation has not been compared in ApoE-TR mice with and without Aβ accumulation. We crossed ApoE3- and ApoE4-TR mice with a transgenic line that accumulates human $A\beta_{1-42}$. In male ApoE3-TR mice, introduction of Aβ caused aberrations in glucose tolerance, membrane translocation of astrocytic glucose transporter 1. Phosphorylation of Tau at AD-relevant sites was correlated with glucose intolerance. These effects appeared independent of insulin dysregulation and were not observed in females. In ApoE4-TR mice, the addition of Aβ had no significant effects due to a trend toward perturbation of the baselines. Metabolic changes may have a larger interaction with AD pathology and its consequences in individuals who do not carry an *APOE* ε4 allele. The fact that ApoE4 generally failed to exacerbate the effects of Aβ on glucose further highlights the growing distinction between the glycemic effects of Aβ versus those of peripheral insulin resistance.

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Poster

369. Metabolism Alterations in Alzheimer's Disease

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Program #/Poster #: 369.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R01AA027097
R56AG058849

Title: Imaging the effects of physical exercise on metabolism, immunity, and hemodynamics in an Alzheimer's Disease model

Authors: *A. CARDENAS-RIVERA, C. LIU, A. BIRMINGHAM, A. ALFADHEL, Z. LU, A. YASEEN;
Bioengineering, Northeastern Univ., Boston, MA

Abstract: Currently, 24 million people suffer from Alzheimer's disease, and the frequency is expected to double every 20 years. Recent results suggest that moderate physical exercise

potentially mitigates the devastating effects of Alzheimer's Disease (AD) and other neurological diseases. Although the molecular mechanisms are not completely understood, it is widely believed that routine moderate exercise improves tissue perfusion and cell signaling while modulating immune responses. We used advanced in vivo microscopy technology to rigorously characterize how chronic exercise affects microvascular hemodynamics and oxygenation. APP/PS1 mice and wild-type littermates were subjected to moderate treadmill exercise (11 m/s for 30 min) five days per week from age 4 to 8 months. We used 2 photon microscopy and the phosphorescent oxygen sensitizer Oxyphor 2P to image microvascular pO2 and red blood cell flux through a cranial window over the somatosensory cortex at rest and during a whisker stimulation protocol (3 Hz). The cortex vascular density, the blood flow, and the dynamic change of vascular diameter under neural stimulation was monitored with systemic administration of FITC-Dextran (0.05 mL at 5%). The oxygen-sensitive phosphorescent tracer OxyPhor 2P was used to evaluate the change of the O2 under somatosensory stimulation. Our results indicate that vascular density is better preserved in the trained group (n=6) vs. the control (untrained, n = 6) and, also oxygen consumption is preserved in the control group. These findings suggest that the neurotoxic effect induced by the accumulation of the APP is reduced by the physical activity.

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Poster

369. Metabolism Alterations in Alzheimer's Disease

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Program #/Poster #: 369.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH DK41700
Molecular Basis of Disease Fellowship, Georgia State University

Title: Prodromal Alzheimer's disease increases susceptibility to obesity in a rodent model

Authors: *T. ANDERSON[1], H. LAIL[2], D. WANDERS[1,2], H. V. VU[1], K. WHITLEY[1], D. WEINSHENKER[4], M. B. PARENT[1,3];
[1]Georgia State Univ. Neurosci. Inst., Atlanta, GA; [2]Dept Nutr., [3]Dept Psychology, Georgia State Univ., Atlanta, GA; [4]Dept Human Genet., Emory Univ. Sch. of Med., Atlanta, GA

Abstract: Obesity and Alzheimer's disease (AD) are linked public health crises. Obesity and metabolic syndrome during middle age are associated with higher AD prevalence, earlier onset, and more severe AD pathology. This correlation is usually interpreted as obesity exacerbating AD, which animal research supports. However, emerging research suggests that early AD pathology may also dysregulate energy homeostasis to promote obesity. Although obesity in middle age is easy to see, AD develops over decades of amyloid beta (Aβ) accumulation that usually goes undetected. Experimentally, Aβ can bind insulin receptors and trigger their

endocytosis *in vitro*, and Aβ oligomers infused into cerebral ventricles acutely dysregulate central metabolic control in rodents and primates. Several AD models show insulin resistance, hyperphagia, and increased body weight and adiposity concurrent with memory impairment. To the best of our knowledge, however, the impact of prodromal AD on energy homeostasis has not been studied. We used TgF344-AD rats to investigate metabolic effects of prodromal AD. This model replicates AD pathology with progressive Aβ accumulation, endogenous tauopathy, memory dysfunction, and neuronal loss. We tested prodromal susceptibility to obesity by feeding wild-type (WT) and AD rats of both sexes either standard chow or a high-fat, high sugar Western diet (Research Diets D12451) starting at 4 months, before memory deficits appear. Energy homeostasis was assessed by recording all home-cage feeding with the BioDAQ system, weighing the rats weekly, and tracking body temperatures with Thermochron iButton sensors implanted intraperitoneally. After 11 or 22 weeks the rats were euthanized and brown adipose tissue (BAT) and retroperitoneal and gonadal white adipose tissues (rpWAT and gWAT) were collected. All experiments were performed blind to genotype, and blind to diet condition whenever possible. Before diet manipulation, 4-month-old AD rats already weighed more than WT rats. AD pups in replication cohorts significantly outweighed WT littermates from 8 weeks onward. AD rats ate more on either diet and gained more weight from the Western diet than WT rats. They had higher body temperatures than WT rats, suggesting that their increased weight was not from decreased physical activity. AD males on either diet and AD females on the Western diet disproportionately stored excess weight as proinflammatory gWAT, whereas WT rats on either diet had more anti-inflammatory BAT. These findings raise the possibility of a vicious cycle in which prodromal AD increases weight gain, diet-induced obesity, and inflammatory adiposity, which in turn promote AD development.

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Poster

369. Metabolism Alterations in Alzheimer's Disease

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Program #/Poster #: 369.05

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: New Jersey Health Foundation
Rowan University Foundation
Cure Canavan Fund

Title: Possible role for aspartoacylase in age-related metabolic decline

Authors: Q. NGUYEN, J. S. FRANCIS, V. MARKOV, V. S. CHAUHAN, A. J. K. REED, *P. LEONE;
Cell Biol. and Neurosci., RowanSOM, Stratford, NJ

Abstract: N-acetylaspartate (NAA) is an abundant amino acid derivative synthesized in neurons by the acetylation of mitochondrial-derived aspartate. Abnormally low NAA in the brain is a signature in neurodegenerative diseases across the clinical spectrum. Pathologically low NAA reflects ATP bioavailability because of the shared substrate requirements of NAA synthesis and mitochondrial energy metabolism. Currently, the only known functional role for NAA is the supply of acetyl groups for lipid synthesis during developmental myelination but the relevance of this to NAA in age-related neurodegenerative disease is unclear. The magnitude of NAA reduction increases as neurodegenerative disease pathology worsens, making NAA prognostic for disease severity. The synthesis and catabolism of NAA require the activities of two rate-limiting enzymes, Nat8L and aspartoacylase (ASPA), that are restricted to neurons and oligodendrocytes, respectively. Prior studies conducted in our laboratory in a mouse model of Alzheimer's Disease have documented increased *ASPA* expression and decreased *Nat8L* expression simultaneously with depleted ATP reserves immediately before cognitive decline, suggesting a role for Nat8L and ASPA transcriptional regulation in the response to early energetic crisis in age-related disease. The goal of this study was to investigate the transcriptional regulation of *Nat8L* and *ASPA* in different regions of the brains of aged mice. The hippocampus is highlighted as a region of significant downregulation of *Nat8L* with associated abnormalities in the expression of genes encoding for elements of mitochondrial oxidative phosphorylation. This downregulation of *Nat8L* was associated with a concurrent increase in *ASPA* expression, both in the absence of differences in numbers of neurons or oligodendroglia as a function of age. These results suggest NAA metabolism is regulated in response to oxidative stress and point to a novel role for white matter in defining a metabolic inflection point that is of relevance to risk for age-related neurodegenerative disease.

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Poster

369. Metabolism Alterations in Alzheimer's Disease

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Program #/Poster #: 369.06

Topic: C.02. Alzheimer’s Disease and Other Dementias

Title: The interaction of Western diet and pathologies of Alzheimer's disease on altering feeding behaviors and the appetite circuit

Authors: *H.-W. CHEN, H.-J. TSAY;
Neuroscience, Natl. Yang Ming Chiao Tung Univ., Taipei, Taiwan

Abstract: Western diets (WD) with high sugar and fat contents aggravate the pathology of Alzheimer’s disease (AD) including elevated neuroinflammation and amyloid burden. Although high fat diets induce overfeeding in rodent models, affected nuclei in the appetite control circuit remain unidentified. WT mice and APP/PS1 transgenic mice are subjected normal chew diet

(NCD) and high fat diet combined high fructose containing drinking water (HFHFrD), and the metabolic parameters and feeding behaviors of four groups are measured. The neuronal activation in the appetite control circuitry in response to refeeding is investigated by c-Fos immunohistochemistry. Although HFHFrD treatment does not induce the neuronal and peripheral inflammation and aggravates amyloid accumulation, the cognitive function of HFHFrD-fed mice is impaired. Furthermore, HFHFrD alters the daily caloric intake of APP/PS1 transgenic mice. Fasting-refeeding paradigm was used to synchronize feeding behaviors and neuronal activation. The caloric intake of HFHFrD group during the refeeding is attenuated compared with NCD group. At least two mechanisms underlying the reduced caloric intake of HFHFrD group during refeeding. One is HFHFrD impairs the appetite control circuitry. The other is the extent of energy deficit during 36-h fasting is different between NCD and HFHFrD-fed mice. The diet effect (NCD vs. HFHFrD) is not compared in this study. Therefore, the c-Fos induction in the appetite circuitry by refeeding of WT mice and APP/PS1 transgenic mice in NCD and HFHFrD separately is compared. Our findings reveal altered activation in appetite-related nuclei by the interaction of HFHFrD and AD pathology might contribute to the altered refeeding of HFHFrD-fed APP/PS1 transgenic mice.

Disclosures: H. Chen: None. **H. Tsay:** None.

Poster

369. Metabolism Alterations in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 369.07

Topic: C.02. Alzheimer’s Disease and Other Dementias

Title: Endoplasmic reticulum stress decreases the expression of doublecortin in the immature neurons of mice with long-term obesity

Authors: K. NAKAGAWA[1,2], S. ISLAM[1,3], M. UEDA[1,4], ***T. NAKAGAWA**[1];
[1]Gifu Univ. Grad Sch. of Med., Gifu, Japan; [2]Univ. of Tokyo Hlth. Sci., Tokyo, Japan; [3]BCSIR, Chattogram-4220, Bangladesh; [4]Aichi Med. Univ., Aichi, Japan

Abstract: Obesity in middle age is associated with dementia later years. The number of doublecortin (Dcx)-expressing immature neurons in the dentate gyrus (DG) during adult hippocampal neurogenesis (AHN) decreases during aging, particularly in the early stages of Alzheimer’s disease (AD), and is more reduced in advanced stages of AD. However, the underlying mechanisms by which obesity results in the development of dementia later in life and the relation to the reduction of AHN remain unknown. We previously show that the expression levels of ATF4 are increased in the hippocampus in the offspring of AD model mice crossed with Lepr$^{db/db}$ (*db/db*) mice through a process known as the endoplasmic reticulum (ER) stress. In this study, we used high fat diet (HFD)-induced mice with long-term obesity of APP23 mouse model of AD (HFD feeding for 41 weeks) and C57BL/6 (HFD feeding for 64 weeks), and *db/db* mice. We find that novel object location (NOL) and swimming capacities were compromised in

db/db and AD model mice with long-term obesity. Western blot analysis shows that the expression levels of CHOP, which is one of downstream target for ATF4, in the hippocampi of APP23 and C57BL6 mice with long-term obesity and *db/db* mice were increased compared with control mice. Although the number of Ki67-positive cells in the hippocampal DGs of *db/db* mice was not different from that in the DGs of control mice in aged groups, long processes in Dcx-expressing cells in the DGs of 45-week-old *db/db* mice and APP23 mice with long-term obesity mice were not observed, which coexpressed CHOP, by confocal microscopy, indicating that ER stress was activated in the Dcx-expressing immature neurons of mice with long-term obesity. We find that reduced Dcx expression in immature neurons through *Dcx* mRNA degradation in cultured neurospheres isolated from the mouse hippocampus. We also find that the expression levels of miR-148a-5p, miR-129b-3p (Dcx is predicted to be a target by miRDB), and miR-135a-2-3p were significantly increased [log2 fold change > 1, false discovery rate (FDR) < 0.05] in differentiating neurospheres under ER stress condition. We propose that loss of *Dcx* mRNA by ER stress during AHN may underlie the memory impairment that occurs later in the lives of obese subjects.

Disclosures: K. Nakagawa: None. **S. Islam:** None. **M. Ueda:** None. **T. Nakagawa:** None.

Poster

369. Metabolism Alterations in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 369.08

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: Margaretha av ugglas Foundation
NIH Grant 1R01AG065209-01A1

Title: Sex-dependent effects of CYP46A1 overexpression on cognitive function during aging

Authors: *M. LATORRE LEAL[1], P. RODRIGUEZ-RODRIGUEZ[1], L. FRANCHINI[3], M. DANIILIDOU[1], F. EROLI[1], B. WINBLAD[1], K. BLENNOW[4], H. ZETTERBERG[4], M. KIVIPELTO[1], M. PACCIARINI[5], Y. WANG[5], W. GRIFFITHS[5], I. BJÖRKHEM[2], P. MERINO-SERRAIS[6], A. CEDAZO-MINGUEZ[1], S. MAIOLI[1];
[1]Neurobiology, Care Sci. and Society, [2]Dept. of Lab. Med., Karolinska Institutet, Stockholm, Sweden; [3]Pharmacol. and Biomolecular Sci., Univ. degli Studi di Milano, Milan, Italy; [4]Dept. of Psychiatry and Neurochemistry, Univ. of Gothenburg, Gothenburg, Sweden; [5]Swansea Univ. Med. Sch., Swansea, United Kingdom; [6]Dept. of Functional and Systems Neurobio., Cajal Institute, CSIC, Madrid, Spain

Abstract: Up to date, compelling evidence has risen on the importance of CYP46A1 as a potential therapeutic target for Alzheimer’s Disease (AD) and other neurodegenerative diseases. Clinical studies report that AD is associated with markedly reduced levels of 24S-hydroxycholesterol (24OH) and expression of CYP46A1 in post-mortem human brains [1]. In

this matter, knock-out mice for CYP46A1 developed notable learning decline and memory loss while gene therapy activating hippocampal CYP46A1 reduces Aβ levels, and p-Tau accumulation and ultimately rescues spatial and non-spatial memory in AD female mice [2]. This work aims to investigate the effects of CYP46A1 activation in cognitive functions and neurodegeneration in a sex-specific manner. We focus on unravelling the biological mechanisms by which 24OH may promote neuroprotection in the brain via key-pathways like estrogen signalling. Our research was conducted on aged CYP46A1 overexpressing mice (Cyp46Tg), with high levels of 24OH. Additionally, young Cyp46Tg mice (both sexes) underwent surgical removal of gonads to assess the implication of peripherical hormonal production in our studies. All cohorts of mice were evaluated in behavioral, morphological, and molecular studies. Electrophysiological recordings were further performed in brain slices. In addition, hippocampal neurons from primary cultures were used to study molecular mechanisms. Finally, we measured 24OH and NFL levels in cerebrospinal fluid (CSF) from an AD clinic cohort. Cyp46Tg old females showed cognitive enhancement and increased levels of synaptic proteins in the hippocampus compared to control mice [3]. In contrast, aged male mice showed that CYP46A1 overexpression led to spatial memory impairment and anxiety like-behaviour. Supporting these results, sex-specific changes were further confirmed in the mouse mice' morphology of dendritic spines in the CA1 hippocampal region. Studies in vitro suggest that the sex-specific effects observed in mice could be due to the activation of estrogen signalling in neurons by 24OH. Our data analysis in CSF showed 24OH levels were negatively associated with markers of neurodegeneration in aged women but not in men. Our data demonstrate a beneficial role of CYP46A1 overexpression and 24OH in cognition during aging exclusively in female mice. To further elucidate the molecular mechanisms behind these outcomes can have a remarkable clinical impact and help to design new preventive/pharmacological targets for women at risk of developing AD.**[1] HL WANG ET AL., J ALZHEIMERS DIS 51 (1), 45 (2016).[2] E HUDRY ET AL. MOL THER 18 (1), 44 (2010).[3] S MAIOLI ET AL., PLOS ONE 8 (7), e68534 (2013).**

Disclosures: **M. Latorre Leal:** None. **P. Rodriguez-Rodriguez:** None. **L. Franchini:** None. **M. Daniilidou:** None. **F. Eroli:** None. **B. Winblad:** None. **K. Blennow:** None. **H. Zetterberg:** None. **M. Kivipelto:** None. **M. Pacciarini:** None. **Y. Wang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); CholesteniX Ltd. **W. Griffiths:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); CholesteniX Ltd. **I. Björkhem:** None. **P. Merino-Serrais:** None. **A. Cedazo-Minguez:** None. **S. Maioli:** None.

Poster

369. Metabolism Alterations in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 369.09

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: Alan and Janice Woll Family Foundation
Templeton Medical Research Foundation Grant

Title: Linking expression of neurosecretory proteins VGF and irisin across Alzheimer's disease progression

Authors: *K. A. BRETLAND[1], S. R. J. SALTON[2], C. M. DENGLER-CRISH[1];
[1]Pharmaceut. Sci., Northeast Ohio Med. Univ., Rootstown, OH; [2]Dept. of Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: The neurosecretory peptide VGF (non-acronymic) has been implicated as a major biomarker for Alzheimer's disease (AD) onset and progression. Clinical studies show that neural levels of VGF increase compensatorily in patients during prodromal dementia or mild cognitive impairment stages, but then become deficient by the time AD is diagnosed. In healthy individuals, VGF plays an important role in regulating energy balance, synaptic plasticity, and neurogenesis, and is known to be increased in the hippocampus as a result of exercise. As such, these characteristics are strikingly similar to those of the exercise-evoked hormone irisin, which is also shown to be dysregulated in AD. This is important because preclinical studies show that therapeutic restoration of either VGF or irisin may have neuroprotective effects against AD neuropathology. In this study, we investigated the regional and temporal expression of VGF and irisin in perfusion-fixed brain sections from transgenic AD model mice (3xtg and htau strains) across disease progression, and compared these results with normal age-related changes of these proteins in control mice. Antibody-based fluorescent labeling was used to identify irisin and VGF-positive cells in discrete regions of the hippocampus and adjacent layers of retrosplenial cortex; results were obtained via multichannel epifluorescent microscopy. We found that VGF was widely but sparsely distributed in cells throughout most areas of the hippocampus and within specific layers of the cortex. Intriguingly, we found extensive co-label between VGF and irisin within cells of the CA1, CA3, and dentate gyrus regions of the hippocampus. Overall, our preliminary findings indicated obvious differences in VGF distribution between mice of different pathological phenotypes/genotypes that further varied as a function of sex. These preliminary data are the first part of a larger project that will generate new information on the potential protective roles of VGF and irisin in normal aging and neurodegenerative disease.

Disclosures: K.A. Bretland: None. **S.R.J. Salton:** None. **C.M. Dengler-Crish:** None.

Poster

369. Metabolism Alterations in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 369.10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Florida Deptarment of Health 21A12

Title: Changes in the expression of the myokine, Irisin, over time following acute exercise in mice

Authors: *J. JUERGENSMEYER, C. DESAI, J. MURRAY, S. MORET, S. VILARINO, K. ALVIÑA;
Neurosci., Univ. of Florida, Gainesville, FL

Abstract: Alzheimer's disease (AD) is one of the most common types of neurodegenerative disorders and as our population ages, its prevalence is expected to increase. There are currently no effective treatments for AD, thus emphasizing the need to identify and develop therapeutic solutions. Physical activity promotes healthy aging and could be an effective non-pharmacological alternative to prevent or delay AD pathology. However, the mechanisms underlying such positive effects are not fully known. Irisin is a myokine that is secreted from skeletal muscle during exercise and has been associated with mechanisms that are dysfunctional in AD, such as glucose metabolism and hippocampal neurogenesis. Further, there have been contradicting reports on the changes in Irisin levels following exercise. While it is known that Irisin is degraded quickly when in circulation, its exact time course after release has not been fully determined. Contradictions might be a result of variability in the timing of tissue collection. For example, in humans, serum Irisin concentration has been reported to increase ~15% following exercise when blood is collected immediately or soon after the completion of exercise. However, it is not known how the elevation in serum Irisin concentration changes over longer periods of time. To answer this question, we performed an acute exercise experiment (swimming for 20min) in mice and measured post-exercise serum Irisin concentration and muscle protein expression at five different time points: immediately after, after 30, 60, 120 minutes, and 24 hours. Serum and muscle Irisin were quantified by ELISA and immunofluorescence, respectively. Our preliminary data showed a decrease in Irisin expression in muscle within 90 minutes of exercise, with no change in serum concentration. After completing all time points, we expect to see the highest serum Irisin immediately after exercise and progressively lower concentrations until returning to baseline. As Irisin is secreted from muscle during exercise, we expect to see low muscle expression with a progressive increase until baseline levels are restored. These results would suggest that consideration of the timing of tissue collection is necessary to detect changes in Irisin. This will also contribute to clarify previous contradicting reports. Further exploration of the activity of Irisin might yield new information that will potentially improve therapeutic strategies for AD.

Disclosures: J. Juergensmeyer: None. **C. Desai:** None. **J. Murray:** None. **S. Vilarino:** None. **K. Alviña:** None.

Poster

369. Metabolism Alterations in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 369.11

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: 2020R1A2C100660513

Title: Brain-pancreas connectivity study of habenular cholinergic neurons with transsynaptic retrograde tracer in AD and WT mice

Authors: S. CHUNG[1,3], S. LEE[1,3], M. SUH[1,4,2,3], *J. JEONG[4,2];
[1]Biomed. Engin., [2]Biomed. Inst. for Convergence at SKKU (BICS), Sungkyunkwan Univ., Suwon, Korea, Republic of; [3]Ctr. for Neurosci. Imaging Res. (CNIR), Inst. for Basic Sci. (IBS), SKKU, Suwon, Korea, Republic of; [4]IMNEWRUN Inc., Suwon, Korea, Republic of

Abstract: Degeneration of cholinergic circuit contributes to memory loss in Alzheimer's disease (AD) and disruption of glucose homeostasis mediated by an imbalance between energy intake and energy expenditure. Recent studies have shown that medial habenula (mHb) cholinergic neurons are connected to brain-pancreatic axis to regulate circulating glucagon and insulin levels (Duncan, A., Heyer, M.P., Ishikawa, M. *et al. Nature* **574,** 372-377 (2019). However, how mHb cholinergic neurons affect the cognitive function and learning and memory function has not yet fully understood. Here, single cell RNA sequencing data shows that the cholinergic neuronal circuit is impaired in AD mice compared with WT mice. Also, we observed impaired glucose homeostasis using oral glucose tolerance test (OGTT) in AD mice compared with WT mice. To investigate the role of cholinergic neurons on brain-pancreatic axis, we utilized transsynaptic retrograde tracer in WT and AD mice and studied the detailed brain connectivity of cholinergic neurons. As a result, we observed retrograde tracer labelled cholinergic neurons in the hippocampus, medial habenula and hypothalamus area. The fact that retrograde tracer labelled cholinergic neuron found in the hippocampus, indicates a possible link between cholinergic neurons and memory function. In addition, we found that glucagon-like peptide receptor 1 (GLP1R) positive neurons in the subiculum of hippocampus directly project to mHb cholinergic neurons. This suggests that GLP1R agonist, such as dulaglutide, may be involved in regulating not only overall glucose homeostasis but also the activity of cholinergic neurons in the brain. Based on these results, we suggest that modulating mHb cholinergic neurons may be effective in the treatment of AD, especially AD with hyperglycemia, insulin resistance and/or metabolic disorders.

Disclosures: S. Chung: None. **S. Lee:** None. **M. Suh:** None. **J. Jeong:** None.

Poster

369. Metabolism Alterations in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 369.12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR
Alzheimer Society
NSERC

Title: One gene to fatten them all: ATGL Knock Out drives lipid droplet accumulation in ependymal cells

Authors: *F. PRATESI[1], P. VALDERRAMA-CARMONA[2], P. E. M'BRA[1], M. BERNIER[3], A. AUMONT[1], K. FERNANDES[1];
[1]Neurosci., CDRV, Sherbrooke, QC, Canada; [2]Inst. of biology, Univ. de Antioquia, Medellin, Colombia; [3]Bishop Univ., Sherbrooke, QC, Canada

Abstract: Lipids are a complex family of biomolecules that serve as cellular building blocks, signaling molecules, and energy substrates. Lipid droplets are intracellular organelles that store neutral lipids such as fatty acids (in the form of triglycerides) and cholesterol. Interestingly, lipid droplets accumulate within aging ependymal cells at the brain's ventricular surface, and this age-related accumulation is markedly increased in Alzheimer's disease (AD). However, whether this ependymal lipid accumulation contributes to age- or AD-associated brain changes remains unknown. Here, we established a model for exploring this question by targeting adipose triglyceride lipase (ATGL), a lipid droplet-associated lipase that is essential for the release of fatty acids from lipid droplet triglycerides. Using a FoxJ1-CreERt2-driven transgenic model and floxed ATGL mice, we have created an inducible ependymal cell-specific knockout of ATGL. Tamoxifen-mediated deletion of ATGL in adult ependymal cells leads to a time-dependent accumulation of cytoplasmic lipid droplets at the brain's ventricular surface. Using this model, we are now testing whether forced ependymal lipid droplet accumulations is sufficient to mimic aspects of age- or AD-associated changes in brain structure and function.

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Poster

369. Metabolism Alterations in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 369.13

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: VPR award, Neuroscience Research Priority Area

Title: In vitro and ex vivo imaging of ATP:ADP ratio in neurons and astrocytes using the PercevalHR nanosensor

Authors: H. N. FRAZIER, S. L. CASE, R.-L. LIN, J. N. TROSPER, ***O. THIBAULT**;
Univ. Kentucky Med. Ctr., Univ. Kentucky Med. Ctr., Lexington, KY

Abstract: Aging and Alzheimer's disease (AD) are associated with disruptions in neuronal and astrocytic metabolic processes. As a proxy of metabolic activity, we characterized PercevalHR, a fluorescent nanosensor that reports on ATP:ADP ratios in neurons and astrocytes under both *in vitro* and *ex vivo* conditions. Briefly, *in vitro* PercevalHR expression was induced in mixed

hippocampal cultures of primary neurons and astrocytes using viral delivery (lentivirus with a human ubiquitin C promotor; FUGW-PercevalHR; $1.00x10^9$ IFU/mL; DIV 10). Cells were imaged using an epifluorescence microscope (excitation at 475 nm [ATP] or 427 nm [ADP]; emission at 535 nm; dichroic at 505 nm) at ~96 h post-infection in response to varying concentrations of glucose (0.1, 5.5, and 10 mM) as well as before and after acute depolarizations with application of glutamate (20 µM for 5 min) or KCl (50 mM for 2 min) using a cell perfusion system (2 mL/min; 37°C). For *ex vivo* measures, four mice received stereotaxic injections of an astrocyte-specific PercevalHR AAV (GFAP-PercevalHR; $5.54x10^{13}$ GC/mL) to both hippocampi (250 or 500 nL per side), followed by a 6-week recovery period. On the day of imaging, hippocampal slices (350 µm) were extracted and incubated in artificial cerebrospinal fluid in an oxygenated chamber at 37 °C for ~2 h. Slices were then relocated to a two-photon microscope and imaged (excitation at 950 [ATP] and 890 nm [isosbestic]) before and after acute exposure to glutamate (500 nM) or saline. Cultured neurons displayed some sensitivity to changes in glucose concentration, but much greater responses were detected during acute glutamate or KCl depolarizations. Cultured astrocytes within the same field of view displayed even less sensitivity to glucose manipulations, although responses to glutamate or KCl were still detectable. Astrocytes in hippocampal slices reported similar findings, where acute exposure to glutamate rapidly reduced ATP fluorescence. These results provide evidence that the PercevalHR nanosensor is a reliable reporter of ATP:ADP ratio and is applicable for studies of bioenergetic changes with relatively fast kinetics in both neurons and astrocytes in a variety of experimental settings. Future work will expand on these initial findings in order to characterize neuronal and astrocytic metabolic function *in vivo* during ambulation in animal models of aging and AD.

Disclosures: H.N. Frazier: None. **S.L. Case:** None. **R. Lin:** None. **J.N. Trosper:** None. **O. Thibault:** None.

Poster

369. Metabolism Alterations in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 369.14

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: 1R01AG062762-01
1R56AGO62762-01
5R01AG062762-02

Title: Age-dependent changes in a hyperglycemic mouse model relevant to Alzheimer’s disease

Authors: *A. A. ORTIZ[1], A. M. OSSE LEISGANG[1], K. HERNANDEZ[1], C. PINEDA[1], L. E. ROMERO[1], J. WANG[1], A. R. PLATT[3], M. GODINEZ CARDONA[1], E. N. STROM[1], A. M. SALAZAR[4], J. W. KINNEY[2];
[1]Brain Hlth., [2]Dept. of Brain Hlth., Univ. of Nevada Las Vegas, Las Vegas, NV; [3]Univ. of

Nevada, Las Vegas, Las Vegas, NV; [4]Dept. of Brain Hlth., Univ. of Nevada Las Vegas - Sch. of Integra, Las Vegas, NV

Abstract: Alzheimer's disease (AD) is a neurodegenerative disease that is characterized by progressive neuronal loss, learning and memory deficits, and cognitive decline. AD affects over 6.1 million Americans, and is the 6th leading cause of death in the US. Furthermore, by 2050, the US is projected to spend over $1.1 trillion on AD-related treatments. Pathological hallmarks of AD include beta-amyloid plaques, neurofibrillary tangles, and chronic neuroinflammation. AD is classified as early onset (EOAD) or late onset (LOAD). EOAD is associated with genetic mutations and accounts for 3-5% of all AD cases. In contrast, LOAD accounts for 95-97% of all AD cases with no genetic etiology, however, several genetic and/or other comorbidities confer increased risk for LOAD. Two major risk factors for LOAD include diabetes mellitus (DM) and aging. DM confers up to a 4-fold increased risk for developing AD; 81% of AD patients have type II diabetes and impairments in fasting glucose levels. Furthermore, over 25% of the population over the age of 65 are diabetic. As age is the largest risk factor for developing LOAD, DM and age may combine to impact AD onset and progression.Hyperglycemia – abnormal elevated blood glucose levels – is the primary characteristic of DM. We have previously shown that chronic hyperglycemia can initiate and promote neuroinflammation, as well as significant increases in hyperphosphorylated tau protein (pTau) and learning and memory deficits that are consistent with other AD models. To determine if hyperglycemia or the ensuing inflammatory response a mechanism driving AD related changes, we evaluated if lowering blood glucose in our hyperglycemia model would rescue pTau and inflammation. We induced hyperglycemia via a staggered and low dose of streptozotocin (STZ) in young and aged wild-type male mice. Following 6 weeks of sustained hyperglycemia, we treated animals with Phloridzin (PZ) to lower blood glucose levels, and then evaluated if the AD relevant changes were rescued.We observed age dependent changes in inflammatory signaling in the young vs old STZ-treated groups, as well as differences in the AD relevant changes following the PZ treatment. Additional differences were observed in inflammatory signaling between same age and different age groups.

Disclosures: **A.A. Ortiz:** None. **A.M. Osse Leisgang:** None. **K. Hernandez:** None. **C. Pineda:** None. **L.E. Romero:** None. **J. Wang:** None. **A.R. Platt:** None. **M. Godinez Cardona:** None. **E.N. Strom:** None. **A.M. Salazar:** None. **J.W. Kinney:** None.

Poster

369. Metabolism Alterations in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 369.15

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01AG061038
NIH Grant R01AG071682

Title: Brain mitochondria clusterin (mitoCLU) increases ATP production via regulation of ATP synthasome

Authors: *H.-J. MOON[1], S. K. HERRING[1], P. RAWAL[1], L. ZHAO[1,2];
[1]Pharmacol. and Toxicology, Sch. of Pharm., The Univ. of Kansas, Lawrence, US-KS, KS;
[2]Neurosci. Grad. Program, Univ. of Kansas, Lawrence, US-KS, KS

Abstract: Clusterin (CLU), or apolipoprotein J (ApoJ), is one of the predominant genetic risk factors for the development of late-onset Alzheimer's disease (LOAD). Several single nucleotide polymorphisms (SNPs) in CLU have been reported to modify LOAD risks; however, it is unclear how they affect CLU mRNA and protein isoform expression and function. We have found that the non-secreted, unglycosylated mitochondrial isoform of CLU protein (mitoCLU) is localized to the mitochondrial matrix and expressed in rodent and human neurons and astrocytes. To understand mitoCLU function in the brain, we performed mitoCLU coimmunoprecipitation and proteomic analysis using mitochondrial fractions isolated from human neuronal cell lines. We detected several potential mitoCLU-interacting proteins involved in the mitochondrial ATP synthasome supercomplex, including mitochondrial F1Fo-ATP synthase, ADP/ATP translocase, and inorganic phosphate carrier. A Mito stress test was performed to evaluate the impact of mitoCLU on mitochondrial respiration. MitoCLU significantly increased the mitochondrial spare respiratory capacity and total ATP production. In addition, RT^2 profiler PCR array revealed that *slc25a25*, ATP-Mg^{2+}/Pi inner mitochondrial membrane solute transporter, had a higher expression in human neuronal cells that overexpressed mitoCLU than in cells that overexpressed the secreted isoform of CLU. These findings suggest that mitoCLU may play an important role in regulating mitochondrial ATP production via facilitating the transport of substrates and the assembly of mitochondrial ATP synthasome, which may be reduced by CLU variants leading to the development of a deficient bioenergetic phenotype and LOAD risk.

Disclosures: H. Moon: None. **S.K. Herring:** None. **P. Rawal:** None. **L. Zhao:** None.

Poster

369. Metabolism Alterations in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 369.16

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R01AG061038
R01AG071682

Title: Glial expression and immunomodulatory role of Clusterin in the brain

Authors: *P. RAWAL, H. MOON, L. ZHAO;
Dept. of Pharmacol. and Toxicology, Sch. of Pharm., The Univ. of Kansas, Lawerence, KS

Abstract: Glial expression and immunomodulatory role of Clusterin in the brainAuthors*P. Rawal1, H.J. Moon1, and L. Zhao1,2;1Department of Pharmacology and Toxicology, School of Pharmacy, University of Kansas, Lawrence, KS 66045, USA2Neuroscience Graduate Program, University of Kansas, Lawrence, KS 66045, USA **DisclosuresP. Rawal**: None. **H.J. Moon**: None. **L. Zhao**: None.Clusterin (CLU), also known as apolipoprotein J, is one of the top three prominent genetic risk factors for the development of late-onset Alzheimer's disease (LOAD); however, how its genetic variants influence the risk of LOAD remains unknown. Apart from the well-characterized beta amyloid and tau pathologies, the presence of extensive neuroinflammation mediated by microglia, the brain-resident immune cells, has been emerging as a crucial player in the pathogenesis of LOAD. Therefore, modulation of microglial activation is essential for retaining microglial homeostasis and maintaining overall brain health. In this study, using mouse primary astrocytic and microglial cultures as well as a microglial cell line, we first analyzed the synthesis profile of CLU and found a positive CLU mRNA and protein expression in both astrocytes and microglia. While astrocytes can constitutively synthesize and secrete high levels of CLU, microglia relatively have very low levels of CLU expression that is increased in response to stimulation by lipopolysaccharide (LPS). We next investigated the functional role of CLU on microglial response to inflammatory challenge induced by LPS. We found that both astrocyte-secreted and recombinant CLU glycoprotein interacted with microglia and reduced the level of LPS-induced microglial inflammation. We have also identified a CLU-mediated crosstalk between astrocytes and microglia in which LPS induces microglial inflammation, which further induces activation of astrocytes leading to elevated astrocytic CLU expression and secretion possibly to act back on microglia and inhibit microglial hyperactivation. In addition to the immunomodulatory role of CLU in vitro, we found that the loss of CLU can lead to a chronic neuroinflammatory state indicative of AD risk in vivo. Furthermore, consistent with our in vitro studies, LPS stimulation resulted in an increased brain expression of CLU in WT mice. Overall, these findings indicate that CLU may play an important immunomodulatory and anti-inflammatory role in the brain.

Disclosures: P. Rawal: None. **H. Moon:** None. **L. Zhao:** None.

Poster

369. Metabolism Alterations in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 369.17

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01AG061038
NIH R01AG071682

Title: rhApoE2 improves neuronal glycolysis, synaptosomal activity, and spatial recognition memory in ApoE4 models in an age- and sex-dependent manner

Authors: *X. ZHANG[1], H.-J. MOON[1], T. SIAHAAN[2], L. ZHAO[1,3];
[1]Dept. of Pharmacol. & Toxicology, [2]Dept. of Pharmaceut. Chem., [3]Neurosci. Grad. Program, Univ. of Kansas, Lawrence, KS

Abstract: Continued clinical failures in the search for treating Alzheimer's disease (AD) raise questions about the validity of currently focused therapeutic targets, underscoring the importance of an alternative strategy that emphasizes on the neuroprotective mechanism to promote brain resilience against AD. We recently demonstrated that human ApoE genetic isoforms (ApoE2, ApoE3, ApoE4) differentially modulate glycolytic metabolism in neuronal aging with the ApoE2-expressing cells exhibiting the most robust while the ApoE4-expressing cells display the most deficient profile. These ApoE isoforms-mediated age-dependent glycolytic disparities directly correlated with markers of neuronal metabolic activity and overall cell health status. Our follow-up study revealed that exogenous expression of ApoE2 in ApoE4 cells induced a significant increase in hexokinase (HK) and glycolytic function. Recombinant human ApoE2 (rhApoE2) protein effectively protected primary neurons against H_2O_2 insult, potentially via the upregulation of HK. In the subsequent *in vivo* study, we delivered rhApoE2 protein using ADTC5 peptide as a blood-brain barrier modulator into the brains of human ApoE4 knock-in (hApoE4KI) mice via tail vein injection. rhApoE2 protein was then administrated once every week for 4 weeks in mid-aged hApoE4KI mice (short-term), or for 8 weeks in aged hApoE4KI mice (long-term). HK was significantly enhanced in the brains treated with rhApoE2 along with ADTC5 in both short-term and long-term studies, with no sign of neurotoxicity. In addition, synaptosomal activity was significantly increased in rhApoE2-treated mid-aged hApoE4KI mice, but not in aged mice. 2-month rhApoE2 treatment promoted synaptosomal glycolytic function in aged male hApoE4KI mice, although not in female mice. Moreover, rhApoE2 treatment significantly improved spatial recognition memory in aged male mice, however not in female mice. Of interest, rhApoE2 treatment reduced IL-1β expression in aged male mice spleen, suggesting its potential efficacy in ameliorating inflammatory process associated with ApoE4. Collectively, these data provide a proof of concept for the therapeutic opportunity of translating ApoE2-mediated neuroprotective mechanisms into a novel approach to bolster brain resilience in the prevention and early intervention of AD.

Disclosures: X. Zhang: None. **H. Moon:** None. **T. Siahaan:** None. **L. Zhao:** None.

Poster

369. Metabolism Alterations in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 369.18

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Orville Edward Egbert, M.D. Endowment fund
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RISE

Title: Mitochondria anomaly in the Scully mutant

Authors: *M. SOLIS, P. SABANDAL, K.-A. HAN;
Biol. Sci., Univ. of Texas at El Paso, El Paso, TX

Abstract: Dementia is the progressive decline in cognitive functions including memory and inhibitory control. It has complex etiology involving numerous genetic and non-genetic risk factors. Our understanding on how these risk factors interact to cause dementia remains largely incomplete. The overarching goal of this study is to address this knowledge gap in the model organism *Drosophila melanogaster*. We conducted a functional screen to identify novel dementia genes that interact with non-genetic risk factors (e.g. aging, sleep anomaly, social stress). We identified *Scully*, the fly homolog of 17-β-hydroxysteroid dehydrogenase 10 (17β-HSD10), encoding a multifunctional mitochondrial enzyme that binds to Aβ peptides. We found that the flies with heterozygous mutation in *Scully* (*Scu/+*) exhibited the accelerated loss of memory and inhibitory control in an aging-dependent manner. To determine the underlying mechanism, we examined the mitochondria in the wild-type and the *Scu/+* mutant. To assess mitochondrial dynamics, we expressed the MitoTimer reporter in the mushroom bodies, the key structure for high-order brain functions, at three different ages (4 days, 2 weeks, and 4 weeks old). We observed no differences in both immature and mature mitochondria between genotypes at 4 days old. However, in 4 weeks old, the *Scu/+* mutant showed significant reduction in both immature and mature mitochondria compared to the control. These observations indicate that the heterozygous *Scu* mutation leads to the decrease in mitochondria content in the mushroom body neurons with aging. The progress including assessments of mitochondrial dynamics and underlying mechanism will be presented. Our findings will provide novel insights into the mechanisms by which genetic and non-genetic risk factors interact for dementia.

Disclosures: **M. Solis:** None. **P. Sabandal:** None. **K. Han:** None.

Poster

369. Metabolism Alterations in Alzheimer's Disease

Location: SDCC Halls B-H

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Program #/Poster #: 369.19

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: P01-AG026572
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SAGA-17-419459

Title: Mitochondrial DNA transcript levels show distinct patterns from nuclear-encoded OXPHOS genes in the brain and reflect Alzheimer’s disease markers

Authors: *Y. SHANG[1], F. YIN[1,2], R. D. BRINTON[1,2,3];
[1]UAHS Brain Sci., [2]Dept. of Pharmacol., [3]Dept. of Neurol., Univ. of Arizona, Tucson, AZ

Abstract: Although mitochondrial function is altered in Alzheimer's disease, the crosstalk between mitochondrial DNA (mtDNA)- and nuclear DNA (nDNA)-encoded genes in the brains of aging individuals and those with Alzheimer's disease (AD) are largely unknown. Here, we analyzed the expression profiles of mtDNA- and nDNA-encoded oxidative phosphorylation (OXPHOS) genes using integrated datasets including transcriptome, proteome, and clinical biomarkers. Regression analyses using existing datasets (ROSMAP, Mayo, and MSBB) revealed that mtDNA- and nDNA-encoded OXPHOS protein levels were positively correlated while the corresponding transcripts were negatively correlated in both cognitively normal and AD brains. The transcriptional associations between these two sets of genes were reduced in the brains of individuals with AD. Further, using pathway analysis, we found that mtDNA transcripts correlated positively with Notch signaling but negatively with synapse-, bioenergetics-, translation-, and ubiquitin-related pathways in the brains of both cognitively normal and AD subjects. Cell-type deconvolution indicated that mtDNA transcripts were negatively correlated with neuronal cell fractions and positively correlated with the fraction of oligodendrocyte precursor cells. AD biomarkers recorded in the ROSMAP dataset such as β-amyloid, total tau, and neurofibrillary tangle (NFT) burden were also all positively correlated with mtDNA transcript levels. Our results suggest an overlooked correlation between bigenome-controlled mitochondrial gene expression and the risk of AD.

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Poster

369. Metabolism Alterations in Alzheimer's Disease

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 369.20

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: ABCA7 deficiency disturbs mitochondrial function in human ipsc-derived cortical organoids and neurons

Authors: *K. KAWATANI[1], M. L. HOLM[1], Y. A. MARTENS[1], J. ZHAO[2], Y. REN[3], P. JIANG[1], Z. LI[1], S. C. STARLING[1], T. PARSONS[4], R. B. PERKERSON[4], X. HAN[5], G. BU[2], T. KANEKIYO[2];
[1]Dept. of Neurosci., [2]Mayo Clin., [3]Hlth. Sci. Res., [4]Ctr. for Regenerative Med., Mayo Clin., Jacksonville, FL; [5]Univ. of Texas Hlth. Sci. Ctr. At San A, Univ. of Texas Hlth. Sci. Ctr. At San A, San Antonio, TX

Abstract: Background: ABCA7 is an ATP-binding cassette (ABC) transporter that regulates the distribution of lipids and other lipophilic molecules across cellular membranes. Loss of function variants in *ABCA7* have been shown to increase the risk of Alzheimer's disease (AD). However, the pathogenic mechanism caused by ABCA7 loss of function remains elusive. Of note, lipidomics in iPSC-derived cortical organoids revealed that ABCA7 deficiency altered the profiles of mitochondria predominant lipids. Since ABCA7 is abundantly expressed in neurons,

we investigated impacts of ABCA7 deficiency on mitochondrial function in human iPSC-derived cortical organoids and neurons. **Methods:** Using the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 system, we generated isogenic *ABCA7* knockout iPSCs as an ABCA7 loss of function model. We differentiated the iPSCs into cortical organoids and neurons, and comprehensively assessed mitochondrial functions. **Results:** We found that ABCA7 deficiency reduced the level and activity of ATP synthase and increased advanced oxidation protein products (AOPP) in iPSC-derived cortical organoids. Consistent with results, ATP production, basal respiration, maximum respiration, and spare respiratory capacity were reduced in ABCA7 deficient iPSC-derived cortical neurons compared to those form isogenic controls when analyzed using a Seahorse XFe96 extracellular flux analyzer. The number of reactive oxygen species (ROS) was also increased in the ABCA7 deficient iPSC-derived cortical neurons. Microelectrode array (MEA) revealed the repressed spike number and synchronized burst firing number by ABCA7 deficiency in iPSC-derived cortical neurons. **Conclusions:** We demonstrated that ABCA7 loss of function leads to mitochondrial dysfunctions accompanied with synaptic dysregulations in iPSC-derived neurons, suggesting that presynaptic mitochondrial function is predominantly compromised by altered lipid metabolism due to ABCA7 deficiency. These findings should provide novel mechanistic insight into how the loss of ABCA7 function in neurons impacts the risk for AD and has the potential to define novel targets for AD therapy.

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Poster

369. Metabolism Alterations in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 369.21

Topic: C.02. Alzheimer’s Disease and Other Dementias

Title: P52-zer6 (znf398) transcriptionally suppresses metallothionein-3, leading to increased ros in the mouse brain

Authors: *S. PARK, E. KWAG, J.-H. SHIN;
Sungkyunkwan Univ., Suwon-si, Korea, Republic of

Abstract: Krüppel-associated box-containing zinc finger proteins (KZFPs) are the largest family of transcriptional repressors in higher organisms. Among them, ZNF398/ZER6 is known to produce two isoforms of p52 and p71 by alternative splicing, and p52-ZER6 has been reported as a binding partner for estrogen receptor alpha (ERα). ERα is one of the two main types of estrogen receptors and shows the neuroprotective effects along with estrogen.ERα shows the neuroprotective effects along with estrogen. Metallothionein (MT) is a cysteine-rich, low molecular weight protein known to reduce oxidative stress, reactive oxygen species (ROS), and metal toxicity. There are four major MT isoforms, of which MT3 was identified as being the

most suppressed by p52-ZER6. We confirmed that MT3 is specifically expressed in the brain.To understand the physiological function of the relationship between p52-ZER6 and MT3, we investigated expressional changes of p52-ZER6 and MT3 under oxidative stress, demonstrating that the mRNA level of MT3 was increased in mouse dopaminergic neuron cells treated with H2O2, in a dose-dependent manner, whereas its level was significantly suppressed in p52-ZER6 overexpression.Since Alzheimer's disease (AD) is characterized by the abnormal deposition of amyloid-beta peptide (Aβ) and this phenomenon is mainly initiated by oxidative stress, we monitored the levels of MT3 and p52-ZER6 in the cortex and hippocampus of 5xFAD, an AD mouse model. Notably, an increase of p52-ZER6 and a decrease of MT3 was found in the cortex and hippocampus of 5xFAD mice as compared to WT mice. In this study, we identified MT3 as a target gene of p52-ZER6 and their expressional changes were involved in AD pathology. Thus, these results suggest the pathological mechanism underlying p52-ZER6-mediated neurotoxicity in AD and the possibility of ERα through how p52-ZER6 transcriptionally regulates MT3 in the neuroprotective effects of estrogen.

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Poster

370. Alzheimer's Disease: Ion Channels and Excitability

Location: SDCC Halls B-H

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Program #/Poster #: 370.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR project grant (FRN#PJT-156001)

Title: Sk channel inhibition improves depressive-like behavior and cognitive impairment in a transgenic mouse model of Alzheimer's disease

Authors: *S. HASAN, A. WILLIAMS, A. MICHAEL, M. WASEF, M. CORRIGAN, C. CLARKE, F. BAMBICO;
Mem. Univ. of Newfoundland, Mem. Univ. of Newfoundland, St.john's, NL, Canada

Abstract: Clinical studies suggest that depressive episodes in later life (late-life depression, LLD) could be a prodrome of Alzheimer's disease (AD). However, direct experimental evidence that establishes an LLD-AD link and potential prodromal biomarkers have not yet been identified. One putative molecular substrate involves functional changes in inhibitory small conductance calcium-activated potassium channels (SKCs). The subtype SK3 in the brain has been shown to increase with age and plays a crucial role in long-term potentiation (LTP)-related deficits, age-dependent cognitive impairment, hippocampal shrinkage, and known to underlie reduced dorsal raphe nucleus (DRN) serotonin (5-HT) neuron activity in an animal model of depression. We previously found that chronic activation of SKCs precipitated cognitive deficits and depressive-like behavior in an age-dependent manner. Here, we investigated if pharmacological inhibition of SKCs could improve the depressive-like behavior and cognitive

deficit in an age-dependent manner using the triple transgenic (3xTG) mouse model of AD. To achieve this, we administered NS8593 (3mg/kg, i.p.), a negative allosteric modulator of SKC, to 3xTG mice and C57BL/6 (wildtype, WT control) at 3 and 5 months of age for 15 days (30 minutes prior to each behavioral test). The age of the cohorts was selected based on the emergence of cognitive deficits in 3xTG mice, which is at 4 months of age. Behavioral tests were conducted to examine depressive-like behavior, cognitive ability, and motor function. Glucose utilization was recorded as a measure of metabolic activity of the DRN. NS8593 treatment significantly improved sucrose preference test (SPT) score ($p<0.01$) at 3 months old in both 3xTg ($p=0.036$) and WT mice ($p=0.04$) , but the improvement was diminished at 5 months ($p>0.05$), though there was an overall decrease of SPT score in 3xTG mice compared to WT mice. NS8593 treatment had no significant effect on immobility in the forced swim test ($p>0.05$) and on locomotor activity in the open field test at both ages ($p>0.05$). NS8593 significantly improved cognition in 3xTG mice at 5 months ($p=0.033$) but not at 3 months of age ($p>0.05$), nor in WT mice at any age. Behavioral tests at 12 months where extensive AD pathology is evident is currently in progress, along with immuno-histochemical study of SKC expression and glucose utilization data analysis. This study has thus presented compelling evidence indicating the age-dependent effect of SKC blockade and that depressive behavior may precede cognitive impairment seen in AD. It also provided experimental validity to target pharmacologically target SKCs for developing therapeutic interventions for AD.

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Poster

370. Alzheimer's Disease: Ion Channels and Excitability

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 370.02

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: NIH Grant No. T32AG0679
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Title: Pharmacologically Targeting the Nav1.6:GSK3β PPI Interface to Ameliorate Hippocampal Hyperexcitability in Early-Stage AD

Authors: *T. J. BAUMGARTNER, N. M. DVORAK, A. K. SINGH, P. A. WADSWORTH, F. LAEZZA;
Univ. of Texas Med. Br., UTMB, Galveston, TX

Abstract: Despite recent technological advancements and global initiatives to elucidate the pathophysiology of Alzheimer’s disease (AD), there remains a deficiency in disease-modifying therapeutic interventions. Multiple lines of evidence show a causal relationship between

hippocampal hyperexcitability and the onset of cognitive impairments during early-stage AD. Voltage-gated Na+ channels (Nav channels) are transmembrane proteins with critical regulatory roles in synaptic function and neuronal firing. Nav1.6 is the most densely expressed Nav channel isoform in the adult human brain. Importantly, Nav1.6 plays a critical role in action potential initiation due to its localization at the axon initial segment, and therefore serves as the primary target for modulation of neuronal excitability. The Nav1.6 channel is regulated through its interactions with various auxiliary proteins and signaling molecules. Recent studies from our laboratory have revealed that glycogen synthase kinase 3β (GSK3β) binds the Nav1.6 C-terminal tail and phosphorylates the T1938 residue of its C-terminal domain, indicating that GSK3β regulates the Nav1.6 channel via a dual-function mechanism including phosphorylation and complex formation. Functionally, genetic silencing of GSK3β suppresses Nav1.6-encoded currents, while increased phosphorylation of T1938 via GSK3β stimulates Nav1.6 activity and promotes maladaptive firing of neurons under vulnerable conditions. This evidence suggests that dysregulated GSK3β-mediated phosphorylation of Nav1.6 facilitates neuropathological phenotypes associated with the early-stage AD. Using the split-luciferase complementation assay, we have identified four chemical probes that significantly inhibit Nav1.6:GSK3β complex assembly compared to vehicle control (DMSO 0.5%, α=0.05, n=4). Of these four hits, compound 1063 displayed binding affinity for both Nav1.6 (K_d=13.4μM) and GSK3β (K_d=1.23μM) using surface plasmon resonance. Using whole-cell patch clamp electrophysiology in HEK293 cells stably expressing Nav1.6, it was observed that 1063 induced a significant decrease in peak I_{Na} density and tau of fast inactivation. Additionally, a depolarizing shift in the $V_{1/2}$ of activation and a hyperpolarizing shift in the $V_{1/2}$ of steady state inactivation of Nav1.6-mediated I_{Na} were observed compared to vehicle controls (DMSO 0.1%, α=0.05, n=7). These effects oppose those of GSK3β-mediated Nav1.6 regulation and are predicted to decrease neuronal activity. Cooperatively, these results implicate the Nav1.6:GSK3β PPI interface as a druggable target with potential to mitigate aberrant hyperexcitability in early-stage AD.

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Poster

370. Alzheimer's Disease: Ion Channels and Excitability

Location: SDCC Halls B-H

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Topic: C.02. Alzheimer’s Disease and Other Dementias

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Title: Katp channels are necessary for glucose dependent increases in amyloid-beta and alzheimer's-related pathology

Authors: J. GRIZZANTI[1], W. MORITZ[2], M. C. PAIT[5], M. STANLEY[7], S. D. KAYE[8], C. M. CARROLL[9], N. J. CONSTANTINO[6], L. J. DEITELZWEIG[8], J. A. SNIPES[10], D. KELLAR[8], E. E. CAESAR[3], N. NICOL[8], J. DHILLON[8], M. S. REMEDI[11], C. M. KARCH[12], C. G. NICHOLS[4], D. M. HOLTZMAN[13], ***S. L. MACAULEY**[8];
[1]Intrnl. Medicine-Gerontology, Wake Forest Baptist Hlth., Winston Salem, NC; [2]Washington Univ. Sch. of Med., St Louis, NC; [3]Washington Univ. Sch. of Med., St Louis, MO; [4]Washington Univ. Sch. of Med., Saint Louis, MO; [6]Physiol. and Pharmacol., [5]Wake Forest Univ. Sch. of Med., Winston Salem, NC; [7]Zoology, Univ. of British Columbia, Vancouver, BC, Canada; [9]Neurosci., [8]Wake Forest Sch. of Med., Winston Salem, NC; [10]Wake Forest Sch. of Med., Winston Salam, NC; [11]Dept. of Med., Washington Univ. In St. Louis, Saint Louis, MO; [12]Psychiatry, Washington Univ. In St Louis, Saint Louis, MO; [13]Dept Neurol., Washington Univ., Saint Louis, MO

Abstract: Elevated blood glucose levels, or hyperglycemia, is sufficient to increase brain excitability and Aβ release, offering a mechanistic link between type-2-diabetes and Alzheimer's disease (AD). Since the cellular mechanisms governing this relationship are poorly understood, we explored whether ATP-sensitive potassium (K_{ATP}) channels, which couple changes in energy availability with cellular excitability, play a role in AD pathogenesis. First, we demonstrate that K_{ATP} channel subunits, Kir6.2/*KCNJ11* and SUR1/*ABCC8* are expressed on excitatory and inhibitory neurons in the human brain and cortical expression of *KCNJ11* and *ABCC8* changes with Alzheimer's pathology in humans and mice. Next, we explored whether eliminating neuronal K_{ATP} channel activity uncoupled the relationship between metabolism, excitability, and Aβ pathology in a novel mouse model of cerebral amyloidosis and neuronal K_{ATP} channel ablation (e.g. APP/PS1, Kir6.2-/- mouse). Using both acute and chronic paradigms, we demonstrate that Kir6.2 containing-K_{ATP} channels are necessary for the hyperglycemic-dependent increases in interstitial fluid levels of amyloid-beta (Aβ), amyloidogenic processing of APP, and amyloid plaque formation, which may be dependent on lactate release. These studies identify a new role for Kir6.2-K_{ATP} channels in Alzheimer's disease pathology and suggest that pharmacological antagonism of Kir6.2-K_{ATP} channels might hold therapeutic promise in reducing Aβ pathology, in diabetic or prediabetic patients.

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Poster

370. Alzheimer's Disease: Ion Channels and Excitability

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 370.04

Topic: C.02. Alzheimer's Disease and Other Dementias

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BrightFocus Foundation (A20201775S)
Charleston Conference on Alzheimer's disease New Vision Award
Averill Foundation

Title: Atp-sensitive kir6.2-katp channels couple metabolism, excitability, and sleep/wake architecture

Authors: *N. J. CONSTANTINO[1], C. M. CARROLL[2], R. E. IRMEN[2], J. GRIZZANTI[4], J. A. SNIPES[2], R. W. GOULD[2], S. L. MACAULEY[3];
[1]Physiol. and Pharmacol., Wake Forest Univ. Sch. of Med., Winston Salem, NC; [2]Physiol. and Pharmacol., [3]Physiol. & Pharmacol., Wake Forest Sch. of Med., Winston Salem, NC; [4]Intrnl. Medicine-Gerontology, Wake Forest Baptist Hlth., Winston Salem, NC

Abstract: ATP-sensitive Kir6.2-KATP channels couple metabolism, excitability, and sleep/wake architecture
ATP sensitive inward rectifying potassium (KATP) channels modulate membrane potentials depending on the energy state of the cell, thus intrinsically linking metabolism with excitability. When there is a high ATP:ADP ratio, these channels are closed, depolarizing the cell, and biasing the cell toward hyperexcitability. Conversely, when the ATP:ADP ratio is low, the channels are opened, hyperpolarizing the cell, and decreasing excitability. Our lab demonstrated that in the CNS, KATP channels composed of the Kir6.2 subunit are expressed in high levels on both excitatory and inhibitory neurons in human brain tissue (Grizzanti et al, 2022). We also demonstrated that the relationship between glucose, lactate, and amyloid-beta (Aβ) is disrupted in mice lacking KATP channel activity (e.g. Kir6.2-/- mice). This study showed that the Kir6.2 subunit specifically is necessary for hyperglycemia induced increases in Aβ. In this current study, we are further exploring how KATP channels coordinate metabolic and neuronal activity using the Kir6.2-/- mice. Using intracranial biosensors, we have found that the relationship of interstitial fluid (ISF) glucose and lactate is uncoupled in Kir6.2-/- mice following metabolic challenges. Our work also demonstrated that following hyper- and hypoglycemic challenges, there is no alteration in sleep or wake changes measured by EEG in Kir6.2-/- mice, suggesting that these animals' sleep-wake transitions are uncoupled from peripheral and cerebral metabolism. We showed that the relationship between glucose and lactate during wake and sleep is lost in Kir6.2-/- animals, at baseline and following metabolic challenges. Additionally, we found that compared to wild-type (WT) controls, circadian fluctuations of glucose and lactate are lost in Kir6.2-/- mice under euglycemic conditions. Further demonstrating abnormal sleep-wake rhythms in Kir6.2-/- mice, we discovered that these mice have abnormal sleep-wake patterns compared to WT controls. Lastly, we found alterations in absolute EEG spectral power in Kir6.2-/- mice, suggesting changes in neuronal excitability in these animals. Using pharmacological interventions in combination with EEG recordings, we will determine how metabolism and excitability are disrupted in Kir6.2 knockout mice, and if this contributes to the sleep phenotypes observed in these mice.

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Poster

370. Alzheimer's Disease: Ion Channels and Excitability

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Program #/Poster #: 370.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: BrightFocus Foundation Grant A20201775S
NIA Grant 1K01AG050719

Title: Katp channel inhibition improves neurovascular coupling and reduces alzheimer's disease pathology

Authors: ***J. GRIZZANTI**[1], M. PAIT[1], M. ANWAR[1], R. IRMAN[1], S. D. KAYE[1], C. M. CARROLL[2], A. SNIPES[1], N. CRUZ-DIAZ[1], S. L. MACAULEY[3];
[2]Neurosci., [3]Physiol. & Pharmacol., [1]Wake Forest Sch. of Med., Winston Salem, NC

Abstract: The Alzheimer's disease (AD) brain is characterized by the pathological accumulation of amyloid-β (Aβ) derived neuritic plaques and hyperphosphorylated tau derived neurofibrillary tangles. Brain regions susceptible to the accumulation of AD pathology exemplify an array of pathophysiology, including aberrant neuronal excitability, poor vascular function, reduced metabolic tone, neuroinflammation, etc. Importantly, ATP-sensitive inwardly rectifying K^+ (K_{ATP}) channels modulate all these physiological functions by buffering the cellular excitability of neurons, pericytes, and vascular smooth muscle cells (VSMCs) based on energy availability. K_{ATP} channels present on pericytes and VSMCs are essential to physiological function. Furthermore, the expression of vascular K_{ATP} channels is reduced in the human AD brain and may contribute to vascular dysfunction and the accumulation of Aβ and p-tau. Human epidemiological data has also shown that diabetic patients taking the K_{ATP} inhibitor and sulfonylurea, glyburide, have a lower relative risk for developing AD than untreated controls. Importantly, glyburide does not cross the blood brain barrier and thus should not enter the brain to directly affect neurons. As such, we hypothesized that K_{ATP} channel blockade with the sulfonylurea, glyburide, would improve cerebral vasoreactivity and reduce AD-related pathology, including Aβ and tau aggregation. In order to test this hypothesis, we concurrently treated a rodent model of amyloidosis (APPswe/PSEN1dE9) and a rodent model of tauopathy (P301S PS19) with glyburide. All animals were treated for 3 months with a subcutaneous, low dose glyburide or placebo pellet. First, glyburide treatment reduced interstitial fluid (ISF) levels of Aβ by ~37% and ISF tau by ~35%. Additionally, treatment reduced Aβ plaque deposition via HJ3.4B staining by ~50% and tau aggregation by 57% via AT8 staining in APP/PS1 and P301S mice, respectively. Lastly, glyburide treatment improved neurovascular coupling by reducing arteriole stiffness and cerebral blood vessel tortuosity in APP/PS1 mice, as well as restoring cerebral vascular morphology towards that of wildtype controls. These data demonstrate that

peripheral, low dose glyburide treatment improves neurovascular function and subsequently reduces the accumulation of both Aβ and p-tau.

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Poster

370. Alzheimer's Disease: Ion Channels and Excitability

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG050431
NIH Grant AG069229

Title: Hippocampus Specific Knock-Down of PPARα Aggravates Plaque Pathology in 5xFAD Mice

Authors: *M. MCKAY[1], S. RAHA[2], K. PAHAN[3];
[1]Rush Univ. Grad. Col., Chicago, IL; [2]Neurosci., Rush Univ., Chicago, IL; [3]Dept Neurolog. Sci., Rush Univ. Med. Ctr., Chicago, IL

Abstract: Peroxisome proliferator-activated receptor alpha (PPARα) is a transcription factor that regulates fatty acid metabolism. Although, metabolically active organs like the liver are rich in PPARα, we and others have demonstrated that PPARα is constitutively expressed in hippocampal neurons. To delineate the role of PPARα in the hippocampus in relation to Alzheimer's disease (AD), we generated 5xFAD mice lacking PPARα in the hippocampus ($5xFAD^{\Delta Hippo}$) via complex breeding among $PPAR\alpha^{Flox/Flox}$, calcium/calmodulin-dependent protein kinase II alpha $(Camk2a)^{Cre}$ and 5xFAD mice. Although, phenotypically, $5xFAD^{\Delta Hippo}$ mice looked similar to 5xFAD mice, we observed aggravated amyloid plaque pathology and enhanced glial activation in the hippocampus of $5xFAD^{\Delta Hippo}$ mice as compared to 5xFAD mice. Accordingly, $5xFAD^{\Delta Hippo}$ mice also exhibited decreased spatial learning and memory in comparison to 5xFAD mice. These results suggest that hippocampal PPARα plays an important role in the manifestation of AD-related pathologies and symptoms, and that activation of hippocampal PPARα may be beneficial for AD. Supported by grants from National Institutes of Health (AG050431 and AG069229).

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Poster

370. Alzheimer's Disease: Ion Channels and Excitability

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 370.07

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: ANR-18-CE37-0014

Title: When local alterations meet collective oscillatory dynamics: On the causes of functional connectivity changes

Authors: *S. BENITEZ STULZ[1], S. CASTRO[2], B. S. GUTKIN[3], M. GILSON[1], D. BATTAGLIA[4];
[1]INS, Aix-Marseille Univ., Marseille, France; [2]LNCA, Univ. of Strasbourg, Strasbourg, France; [3]Group For Neural Theory, LNC INSERM U960, Ecole Normale Superieure, Group For Neural Theory, LNC INSERM U960, Ecole Normale Superieure, Paris, France; [4]INS, Univ. Aix-Marseille; LNCA, Univ. of Strasbourg, Strasbourg, France, Marseille, France

Abstract: Neuronal populations within local regions frequently undergo oscillatory modulations of their activity. The oscillations of distant populations coupled by long-range connections can lock in various patterns of stable phase differences and the flexible change of such phase locking patterns has been hypothesized to modulate communication and functional connectivity (FC) between them. It is therefore important to understand which factors can affect and control the established inter-regional phase relations (e.g., FC). Here, we emphasize that while the details of applied local alterations (stimulation or SC link changes) are important, we cannot neglect the global system's dynamics when predicting effects on FC. A tool which has proved useful to model and predict the behavior of coupled oscillating populations is the phase response curve (PRC). The PRC is a local transformation function that determines the phase-dependent response of an oscillator to any given external or internal input. Importantly, the PRC exclusively depends on parameters of the local regional microcircuit such as the relative strengths of recurrent excitation (E) and inhibition (I) but is ignorant of the oscillatory dynamics of the surrounding large-scale network or the inter-regional connectivity parameters. However, in a complex system of many interacting populations, local and global dynamics are non-trivially coupled and the PRC may be more dependent on them than previously assumed. Considering specific examples of multi-scale circuits, we first show that equivalent changes in FC can be induced by either modifying local connectivity parameters and thereby the PRC, or by modifying features of the long-range inter-regional SC. In this way, diffuse changes of local E and I strengths (induced e.g., by neuromodulation or pharmacological treatments) may be used to compensate for a disrupted connectome (due e.g., to neurodegeneration). Secondly, we show that the phase-shifting effects of local pulse perturbations do not depend uniquely on the phase at which perturbation is applied, as postulated by the PRC concept, but also on the collective configuration of dynamic FC which the system is transiently visiting. Thus, accounting for changes in collective dynamics beyond local dynamics and structure, is vital for understanding and predicting how the brain will react to internal or external perturbations.

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Poster

370. Alzheimer's Disease: Ion Channels and Excitability

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Program #/Poster #: 370.08

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01NS075487
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Title: Development of small-molecule Tau-SH3 interaction inhibitors that prevent amyloid-β toxicity and network hyperexcitability

Authors: *J. R. ROTH[1], T. RUSH[1], S. J. THOMPSON[1], A. R. ALDAHER[1], T. B. DUNN[1], J. S. MESINA[1], J. N. COCHRAN[1], N. R. BOYLE[1], H. B. DEAN[1], Z. YANG[2], V. PATHAK[4], P. RUIZ[4], J. J. DAY[3], J. R. BOSTWICK[4], M. J. SUTO[4], C. E. AUGELLIA-SZAFRAN[4], E. D. ROBERSON[1];
[1]Neurol., [2]Biochem. and Mol. Genet., [3]Neurobio., Univ. of Alabama at Birmingham, Birmingham, AL; [4]Chem. Dept., Southern Res., Birmingham, AL

Abstract: Alzheimer's disease (AD) is the leading cause of dementia and lacks highly effective treatments. Tau-based therapies are attractive and Tau reduction prevents amyloid-β (Aβ)-induced dysfunction in preclinical AD models. Tau reduction also prevents amyloid-independent dysfunction in diverse disease contexts, suggesting that strategies exploiting the mechanisms underlying Tau reduction may extend beyond AD. Tau binds several SH3 domain-containing proteins implicated in AD via its central proline-rich domain. We previously used a peptide inhibitor to demonstrate that blocking Tau interactions with SH3 domain-containing proteins ameliorates Aβ-induced dysfunction. Here, we describe a high-throughput screen for small molecules that inhibit Tau-FynSH3 interactions and optimization of a top hit with medicinal chemistry. The resulting lead compound is a potent cell-permeable Tau-SH3 interaction inhibitor that binds Tau and prevents Aβ-induced dysfunction, including network hyperexcitability. These data support the potential of using small molecule Tau-SH3 interaction inhibitors as a novel therapeutic approach to AD.

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Poster

370. Alzheimer's Disease: Ion Channels and Excitability

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 370.09

Title: WITHDRAWN

Poster

370. Alzheimer's Disease: Ion Channels and Excitability

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 370.10

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: 1K01AG050719
R01AG068330
BrightFocus Foundation (A20201775S)
Charleston Conference on Alzheimer’s disease New Vision Award
Averill Foundation

Title: Alterations in metabolism linked to sleep disruption in P301S PS19 mice, a model of tauopathy

Authors: *R. E. IRMEN[1], C. M. CARROLL[2], J. A. SNIPES[2], R. W. GOULD[2], S. L. MACAULEY[3];
[1]Physiol. and Pharmacol., Wake Forest Univ. Sch. of Med., Winston Salem, NC; [2]Physiol. and Pharmacol., [3]Physiol. & Pharmacol., Wake Forest Sch. of Med., Winston Salem, NC

Abstract: Two biomarkers for Alzheimer’s disease (AD) are amyloid-beta (Aβ) and tau aggregation. AD patients often experience comorbidities like metabolic dysfunction and/or sleep-wake cycle disruption, but it is unclear whether this can be directly linked to Aβ or tau aggregation. Previous research shows mouse models overexpressing Aβ also experience metabolic and sleep dysfunction, independent of comorbidities like type-2-diabetes. Our lab previously demonstrated that central and peripheral metabolic changes are linked. This bidirectional relationship is well described in mouse models of Aβ overexpression; however, this relationship is relatively unexplored in mouse models of tauopathy. Therefore, our lab is investigating how central and peripheral metabolism relate to sleep-wake disruption in the P301S PS19 mouse. To investigate changes in peripheral metabolism, body weights are measured and glucose tolerance tests are conducted on P301S PS19 and wild type female mice (n=6-10). Glucose levels are taken from a tail bleed after fasting for four hours. Next, the mice are given an ip dose of 2g/kg glucose followed by blood glucose measurements in 15-minute increments for

two hours. The TSE Phenomaster metabolic screening platform tracks indirect calorimetry, food intake, body weight, drinking, and activity throughout the circadian day, over the span of three days. Paired glucose and lactate biosensors placed within the mouse hippocampus are used to track second by second metabolic fluctuations over three circadian days. Along with the biosensors, EEG and EMG electrodes are positioned to record sleep-wake cycles during this period. All methods completed on 3-, 6-, and 9-month-old P301S and wild type female mice. While current experiments are ongoing, age-related changes in body weight occur in both P301S and WT mice. WT mice become glucose intolerant as they age, while P301S mice become glucose sensitive. Data from the TSE PhenoMaster suggest 3-month-old WT and P301S mice have normal diurnal rhythms in total energy expenditure, respiratory exchange ratio, and food/water intake. Early analysis suggests pathology and age-related differences in metabolism and sleep also develop over the circadian day. Our data suggests that tau aggregation affects peripheral glucose metabolism, in a manner different than Aβ pathology. We hypothesize that the peripheral metabolic changes caused by tau pathology are directly linked to disruptions in brain metabolism, circadian rhythms, and sleep. Our current research will be valuable in expanding knowledge of the relationship between metabolism, sleep, and tau in Alzheimer's disease.

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Poster

370. Alzheimer's Disease: Ion Channels and Excitability

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 370.11

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Intramural Program, NICHD, NIH

Title: Neurotrophic factor-alpha1(carboxypeptidase E) gene delivery in hippocampus prevents Alzheimer's Disease progression in a mouse model

Authors: *Y. LOH[1], L. XIAO[1], X. YANG[1], K. CAMPBELL[2], V. SHARMA[1], D. ABEBE[1], R. DALE[2];
[1]Section on Cell. Neurobio., [2]Bioinformatics and Scientific Programming Core, NICHD, NIH, Bethesda, MD

Abstract: Alzheimer's Disease (AD) is a prevalent neurodegenerative disease characterized by tau hyperphosphorylation, Aβ1-42 aggregation to form senile plaques, and cognitive dysfunction. Therapeutic agents directed at mitigating tau aggregation and clearing Aβ1-42 have been used to treat AD. Additionally, delivery of growth factor genes such as NGF, BDNF, FGF2, to the brain have ameliorated cognitive deficits, independent of amyloid expression. Thus far, these approaches have not prevented or stopped disease progression. Here we report that viral-

(AAV) delivery of a new trophic factor gene, Neurotrophic factor NF-α1(NF-α1) [1], also known as Carboxypeptidase E (CPE) in hippocampus at an early age of 2 months prevented later development of cognitive dysfunction at age 7-8 months in an AD mouse model, 3xTg-AD. Furthermore, MAP2 staining revealed neurodegeneration was prevented with AAV-NF-α1/CPE delivery in 3xTg-Ad mice. Tau hyperphosphorylation was mitigated, and elevated amyloid precursor protein (APP) expression was reduced to near non-AD (non-Tg) levels in the AAV-NF-α1/CPE treated versus non-treated animals. Expression of mitochondrial pro-survival protein, Bcl2 was up-regulated and pro-apoptotic protein Bax was down-regulated in AAV-NF-α1/CPE treated, compared to untreated AD mice. Thus, NF-α1/CPE gene therapy could offer a unique treatment strategy to prevent AD progression. [1] Xiao L, Sharma VK, Toulabi L, Yang X, Lee C, Abebe D, Peltekian A, Arnaoutova I, Lou H, Loh YP. Neurotrophic factor-alpha1, a novel tropin is critical for the prevention of stress-induced hippocampal CA3 cell death and cognitive dysfunction in mice: comparison to BDNF. *Transl Psychiatry.* 2021;11:24. doi: 10.1038/s41398-020-01112-w.

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Poster

370. Alzheimer's Disease: Ion Channels and Excitability

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 370.12

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: NIA U54 AG054345

Title: Characterization of aging X genetic X environment as precision disease models for evaluation of therapeutic interventions for the treatment of late-onset Alzheimer's disease

Authors: *M. SASNER[1], A. OBLAK[2], K. P. KOTREDES[1], D. GARCEAU[1], S.-P. G. WILLIAMS[4], S. DOOLEN[4], K. A. HAYNES[4], G. LITTLE[4], D. S. SANTOS[4], C. INGRAHAM[2], C. LLOYD[2], B. PERKINS[2], P. R. TERRITO[5], B. T. LAMB[3], G. W. CARTER[1], G. HOWELL[1], S. J. SUKOFF RIZZO[4];
[1]The Jackson Lab., Bar Harbor, ME; [2]STARK Neurosci. Res. Inst., [3]Stark Neurosciences Res. Inst., Stark Neurosciences Res. Inst., Indianapolis, IN; [4]Univ. of Pittsburgh Sch. of Med. Aging Inst., Univ. of Pittsburgh, Pittsburgh, PA; [5]Indiana Univ., Indinanapolis, IN

Abstract: The development of therapeutics for Alzheimer’s disease is severely hampered by poor translational studies in animal models. Almost all mouse models express familial AD mutations, but the vast majority of patients have late-onset AD (LOAD). In the MODEL-AD program, our approach is to improve recapitulating AD by the combination of improved mouse models expressing genetic risk variants associated with LOAD combined with environmental risk factors and aging to enable improved translation. Here we highlight findings from studies in

mice expressing humanized APOE4 and Aβ along with the R47H risk variant in the Trem2 gene ("LOAD2") and aged in the presence of a high-fat, high-sugar diet ("HFD").
Evaluation of LOAD2 mice exposed to HFD from adolescence (beginning at 2 mo. of age= "aHFD") or at middle age (beginning at 6 mo. of age = "mHFD") revealed increases in insoluble Aβ42 in brain, increased Aβ42: Aβ40 in plasma and increased proinflammatory cytokines by 12 months of age, with higher levels in LOAD2+aHFD. In LOAD2+aHFD mice we also observed an increase in CSF NfL at 12 months and a reduction in neurons in hippocampus at 18 months of age. Vascular and perfusion changes were also noted at 12 months of age in aHFD animals. These converging phenotypes indicate a model of neurodegeneration in the absence of dense core neuritic plaques, which were not detected up to 24 months of age. Interestingly, metabolomics analysis revealed novel age-dependent serum changes that model metabolomics signatures of AD patients in LOAD2 +aHFD mice (18 months), which may be a more translationally relevant signature.
This model may therefore better recapitulate the earliest stages of LOAD prior to significant amyloid deposition, given the age-related neurodegeneration coupled with genetic, aging and environmental risk factors of AD. Furthermore, this model may also be well positioned to evaluate potential therapeutics as prophylactic treatment for LOAD in the earliest stages of amyloid seeding, with a focus on the neurodegenerative and metabolomics signatures. Ongoing studies include comprehensive behavioral phenotyping and cognitive assessments using the translational touchscreen assays, including evaluation of reversal learning and pattern separation assays.

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Poster

370. Alzheimer's Disease: Ion Channels and Excitability

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 370.13

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Prosaposin gene therapy improves features of Gaucher disease in preclinical studies but does not reverse the neuropathology in mouse models of Alzheimer's Disease and Amyotrophic Lateral Sclerosis

Authors: *G. PESCI[1], A. TEWARI[1], P. BIEZONSKY[2], D. BIEZONSKY[2], L. WONG[2], J. HALLER[2], S. NELSON[2], S. KAMALAKARAN[2], Z. YANG[2], J. HENDRICKS[2], R. RAGHUNATHAN[2], H.-N. LIN[2], Z. ZHAOSTEIN[2], P. RAUT[2], Y. WANG[2], T. MCGATHEY[2], Z. YANG[2], M. HAYASHI[2], A. ABELIOVICH[2], F. HEFTI[2];
[1]Preclinical R&D, [2]Eli Lilly and Co., New York, NY

Abstract: Prosaposin is a highly conserved glycoprotein and the precursor for four lysosomal activator proteins called saposins. These saposins have distinct roles in regulating lysosomal hydrolysis of sphingolipids. Dysfunction of saposins results in an assortment of lysosomal storage disorders (LSD), such as Gaucher Disease (GD) with severe neuropathology. Mutations in the *PSAP* gene can cause a deficiency of either full-length prosaposin or any of the individual saposins, resulting in the accumulation of nondegradable, toxic glycolipids. Secreted full-length prosaposin has been shown to exert neuroprotective and glioprotective effects in cell culture systems but its in vivo application has not been examined. We hypothesized that increasing prosaposin levels might be useful therapeutically for LSDs and neurodegenerative disorders. Using a gene therapy approach, we administered AAV-hPSAP to two established mouse models of GD, the CBE and 4L/PS-NA mouse models via intracerebroventricular (ICV) injections. AAV-hPSAP improved neuroinflammation, and glycolipid levels in the brain with a trend towards improved motor function. To examine the neuroprotective role of prosaposin, we used two mouse models of Alzheimer's disease (AD), APP-PS1 and rTg4510 and a mouse model of Amyotrophic Lateral Sclerosis, TAR6/6. ICV delivery of AAV-hPSAP had no effect on memory dysfunction, phospho-Tau or neurofilament light chain (Nf-L) levels in AD mouse models. In the TAR6/6 model, ICV delivery of AAV-hPSAP did not improve motor dysfunction, Nf-L or TDP-43 levels. In summary, we demonstrate the therapeutic potential of prosaposin in LSD but further work is warranted to understand whether the protective effect of prosaposin can be extended to other neurodegenerative disorders.

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Poster

370. Alzheimer's Disease: Ion Channels and Excitability

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 370.14

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Swedish Research Council (K2015-61X-22051-04-4)
Swedish Brain Foundation (FO2019-0324)
Swedish Alzheimer Foundation (AF-930978)
CONACYT 514592

Title: Low dose of levetiracetam counteracts Aβ-induced alterations of hippocampal network states by restoring fast-spiking interneuron activity

Authors: *A. G. ISLA[1,2], H. BALLEZA-TAPIA[3], F. CHU[4], A. FISAHN[3];
[1]Dept. of Biosci., Inst. Tecnológico y de Estudios Superiores de Monterrey, Mexico City,

Mexico; [2]Nvs, Karolinska Institutet, Solna, Sweden; [3]NVS, Karolinska Inst., Solna, Sweden; [4]Neurosci. Center, Dept. of Neurol., The First Hosp. of Jilin Univ., Changchun, China

Abstract: ABSTRACT Background: Alzheimer's disease (AD) is characterized at an early stage by memory alterations that worsen during the development of the disease. Hippocampal neuronal network activity, including gamma oscillations and sharp waves, is essential for the generation and maintenance of memory processes, but this has been largely overlooked in searching for biomarkers for AD. Several clinical trials in phase 3 have failed despite being able to counteract classical AD-related alterations. One explanation behind this failure could be the lack of recovery of the regular neuronal network activity essential for memory. Nowadays, Levetiracetam (LEV), an SV2A modulator approved for epilepsy, is being used in trials with AD patients without further support for neurophysiological relevant effects on restoring the normal function of hippocampal neuronal network activity. **Methods:** We perform concomitant recordings of local field potential and patch-clamp on hippocampal slices of WT and App^{NL-G-F} AD animal model. **Results:** Levetiracetam has a dose-dependent effect on hippocampal neuronal network activity and counteracts Aβ-induced effects in the early prodromal stage of the disease in App^{NL-G-F} mice by re-activating silenced FSN. **Conclusions:** The precise modulation of neuronal circuits with Levetiracetam is a promising strategy to counteract early-stage alterations in hippocampal activity by modulating FSN in different memory-relevant neuronal network states.

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Poster

371. Alzheimer's Disease: Pre-Clinical Approaches

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 371.01

Topic: C.02. Alzheimer’s Disease and Other Dementias

Title: Glyphosate accelerates amyloid-beta production in the APP/PS1 mouse model of Alzheimer’s disease

Authors: *J. K. WINSTONE[1,2,3], W. WINSLOW[1], R. VELAZQUEZ, Jr.[1,2,3];
[1]ASU-Banner Neurodegenerative Dis. Res. Ctr., The Biodesign Inst. at Arizona State Univ., Tempe, AZ; [2]Sch. of Life Sci., Arizona State Univ., Tempe, AZ; [3]Arizona Alzheimer's Consortium, Phoenix, AZ

Abstract: Glyphosate is the active ingredient in many widely used commercial herbicides and weed killers. Recent work from our lab has shown that 2 weeks of oral glyphosate exposure (125, 250, and 500 mg/kg/day) in C57bl6 mice is sufficient to induce a neuroinflammatory response evident by increased tumor necrosis factor alpha (TNFα). We have also shown that *in vitro* application of glyphosate to primary cortical neurons derived from APP/PS1 mice elevates the production of soluble amyloid-beta (Aβ) 40 and 42. Here, we examine the effects of chronic

daily oral exposure to a vehicle, low dose (50mg/kg/day) relevant to human exposure, or high-dose (500 mg/kg/day) glyphosate in both male and female APP/PS1 mice and their littermate controls. Mice were orally gavaged daily starting at 4 months of age and were sacrificed at 8 months of age after receiving continuous daily exposure for 4 months. Mice were weighed weekly and there were no significant changes in bodyweight amongst groups. Post-mortem analysis of hippocampal tissue via enzyme-linked immunoassay (ELISA) showed that low and high doses of glyphosate are capable of elevating soluble Aβ 40 and 42 in both sexes of APP/PS1 mice, with females showing higher levels than males. Insoluble Aβ 40 was only elevated in the high dosed females. Interestingly, insoluble Aβ 42 was elevated in the high dose in males and the low dose in females while Aβ oligomers showed no significant differences in either sex. Analysis of cortical tissue via ELISA confirmed the previously reported elevation in TNFα in the brain following glyphosate exposure. Analysis of Aβ species in the cortex mirrored the soluble Aβ results seen in the hippocampus, but showed significant differences in insoluble Aβ 40 in males exposed to high dose and females exposed to low and high dose glyphosate. Insoluble Aβ 42 was significantly elevated in the cortex following high and low dose glyphosate exposure in both males and females. Collectively, these results indicate that Aβ production is accelerated following glyphosate exposure, and that female mice are particularly susceptible to high levels. This novel data highlights a particular concern surrounding environmental exposure to glyphosate and its ability to exacerbate Alzheimer's disease in a sex-dependent manner.

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Poster

371. Alzheimer's Disease: Pre-Clinical Approaches

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Program #/Poster #: 371.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Edson Initiative Seed Grant
R01NS109422

Title: Validating the efficacy of a potent DYRK1a Inhibitor (DYR533) in the 3xTg- mouse model of Alzheimer's Disease

Authors: *S. BARTHOLOMEW[1,2], W. WINSLOW[1], Y. SHAW[3], S. ROKEY[4], C. FOLEY[4], C. HULME[3,4], T. DUNCKLEY[1,5], R. VELAZQUEZ[1,2,5];
[1]Neurodegenerative Dis. Res. Ctr., Arizona State Univ., Tempe, AZ; [2]Sch. of Life Sciences, Arizona State Univ., Tempe, AZ; [3]Div. Drug Discovery and Development, Dept. of Pharmacol. & Toxicology, [4]Dept. of Chem. and Biochemistry, Col. of Sci., The Univ. of Arizona, Tucson, AZ; [5]Arizona Alzheimer's Consortium, Phoenix, AZ

Abstract: Currently, there are no effective therapies to ameliorate the pathological progression and associated cognitive deficits in Alzheimer's disease (AD), a rapidly progressing

neurodegenerative disorder characterized by the formation of dense amyloid-β (Aβ) plaques and neurofibrillary tangles. The Dual-specificity tyrosine phosphorylation-regulated kinase-1a (DYRK1a) is known to phosphorylate both the tau and amyloid precursor protein (APP). Previous work has shown that DYRK1a is upregulated in postmortem brain tissue of patients with AD, and this increased activity has been associated with cognitive deficits. We have previously shown both reduced Aβ plaque deposition and decreased phosphorylated tau in the brains of the 3xTg-AD mouse model of AD when using a DYRK1a inhibitor termed DYR219. Here, we utilized a more potent DYRK1a inhibitor, DYR533, which has a four-hour half-life, compared to the 15-minute half-life of DYR219, along with 100% bioavailability. Eight-month-old female 3xTg-AD and control mice were given daily intraperitoneal injections at either a 1.0 mg/kg, 2.5 mg/kg, 5.0 mg/kg or a vehicle control dosage for two months. At 10 months of age, mice were euthanized, and hippocampal and cortical tissue was harvested for neuropathological assessment. Enzyme-linked immunoassay showed that soluble cortical $A\beta_{42}$ levels were significantly reduced in the 1.0 mg/kg and 5.0 mg/kg 3xTg-AD DYR533 dosed groups compared to the vehicle group. Additionally, DYR533 decreased soluble and insoluble fractions of phosphorylated tau at serine 396 in hippocampal and cortical tissue of the 3xTg-AD mouse in a dose dependent manner. Notably, we found a reduction in phosphorylated tau at threonine 217, which was recently shown to be the earliest marker associated with AD progression. Collectively, we demonstrate that the more potent, selective, and orally bioavailable DYRK1a inhibitor (DYR533) reduces Aβ pathology and tau phosphorylation, ultimately paving the way for further clinical development of this candidate AD therapeutic.

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Poster

371. Alzheimer's Disease: Pre-Clinical Approaches

Location: SDCC Halls B-H

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Program #/Poster #: 371.03

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: NIA Grant R01AG062500
NIA Grant R01AG059627

Title: Dietary choline deficiency induces system-wide cellular and molecular dysfunction across several pathogenic axes associated with Alzheimer’s disease

Authors: *J. M. JUDD[1], N. DAVE[1], A. K. DECKER[1], W. WINSLOW[1], P. SARETTE[1], O. V. ESPINOSA[1], S. TALLINO[1,2], J. SANDLER[3], A. S. BILAL[4], I. MCDONOUGH[1], J.

WINSTONE[1,2], E. A. BLACKWOOD[4], C. GLEMBOTSKI[4], T. L. KARR[3,1], R. VELAZQUEZ, Jr.[1,2,5];
[1]Neurodegenerative Dis. Res. Ctr., Biodesign Inst. at Arizona State Univ., Tempe, AZ; [2]Sch. of Life Sci., [3]Biosci. Mass Spectrometry Facility at Biodesign Inst., Arizona State Univ., Tempe, AZ; [4]Translational Cardiovasc. Res. Ctr., Univ. of Arizona, Phoenix, AZ; [5]Arizona Alzheimer's Consortium, Phoenix, AZ

Abstract: Alzheimer's disease (AD) is a neurodegenerative disease that is characterized by the clinical symptoms of memory loss and cognitive deficits and the accumulation of Aβ plaques and neurofibrillary tau tangles. AD is an increasingly prevalent and costly health burden, creating an urgent need for mechanistic insight into modifiable environmental risk factors, such as diet, to offset disease occurrence. Choline is a primary dietary source of methyl groups and has been shown to play a key role in healthy body and brain functions. While some choline is produced endogenously, it is not sufficient for healthy metabolic function without dietary intake. Approximately 90% of Americans do not meet the adequate intake threshold for dietary choline consumption, making it imperative to determine whether deficiency increases disease outcomes. To determine the impact of dietary choline deficiency on body- and brain-related functions, we placed 3xTg-AD, a mouse model of AD, and non-transgenic (NonTg) control mice on either an adequate choline (ChN) diet or a choline-deficient (Ch-) diet from 3 (early adulthood) to 12 (late adulthood) months of age. A Ch- diet drastically increased weight and impaired glucose metabolism compared to the ChN diet. Behavioral phenotyping revealed motor deficits in Ch- mice. Subsequent tissue analysis showed pathological cardiac remodeling, non-alcoholic fatty liver disease and steatohepatitis, and elevated markers of AD pathology, including elevated Aβ oligomers, insoluble $A\beta_{40-42}$, and phosphorylated tau at serines 181 and 396 in the hippocampus (Hp) and cortex of Ch- mice. To gain mechanistic insight, we performed unbiased proteomics of Hp and plasma samples. A Ch- diet altered Hp networks associated with postsynaptic receptor regulation, long-term potentiation, amyloidogenic processing of amyloid precursor protein, microtubule stabilization, and neuronal development. In the plasma proteomic analysis, we found alterations in networks associated with insulin metabolism, mitochondrial function, immune response, and inflammation. Collectively, these data highlight how the current trend of inadequate choline intake may induce system-wide cellular and molecular dysfunction and increase the risk of AD across several pathogenic axes, highlighting the important need for adequate dietary choline intake throughout life.

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Poster

371. Alzheimer's Disease: Pre-Clinical Approaches

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 371.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH 5R01AG059627

Title: Temporal and regional-specific elevations of soluble Amyloid-β_{40-42}in the Ts65Dn mouse model of Down syndrome

Authors: *W. WINSLOW[1], S. TALLINO[1,2], S. BARTHOLOMEW[1,2], R. VELAZQUEZ[1,2,3]; [1]Neurodegenerative Dis. Res. Ctr., Biodesign Inst. at Arizona State Univ., Tempe, AZ; [2]Sch. of Life Sci., Arizona State Univ., Tempe, AZ; [3]Arizona Alzheimer's Consortium, Phoenix, AZ

Abstract: Down syndrome (DS) is a leading cause of intellectual disability that also results in hallmark Alzheimer's disease (AD) pathologies such as amyloid-beta (Aβ) plaques and hyperphosphorylated tau. The Ts65Dn mouse model is commonly used to study DS, as trisomic Ts65Dn mice carry 2/3 of the triplicated gene homologues as occur in human DS. The Ts65Dn strain also allows investigation of mechanisms common to DS and AD pathology, with many of these triplicated genes implicated in AD. For example, trisomic Ts65Dn mice overproduce amyloid precursor protein (APP), which is then processed into soluble Aβ 40-42 fragments. Notably, Ts65Dn mice show alterations to the basal forebrain, which parallels the loss of function in this region observed in DS and AD patients early on in disease progression. However, a complete picture of soluble Aβ 40-42 accumulation in a region-, age-, and sex-specific manner has not yet been characterized in the Ts65Dn model. Here, we show that trisomic mice (Jackson Laboratory Strain #005252) accumulate Aβ 40-42 in the basal forebrain, frontal cortex, hippocampus, and cerebellum in an age-specific manner, with elevation in the frontal cortex and hippocampus as early as 4 months of age. Furthermore, we detected sex-differences in the accumulation of Aβ 40-42 within the basal forebrain, with females having significantly higher Aβ 40-42 at 7-8 months of age. Finally, we show that APP expression in the basal forebrain and hippocampus inversely correlates with Aβ 40-42 levels. This temporal and spatial characterization of Aβ 40-42 in the Ts65Dn model sets the stage for exploring soluble Aβ 40-42 's role in the pathology of these key regions.

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Poster

371. Alzheimer's Disease: Pre-Clinical Approaches

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 371.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: 5R01AG059627

Title: Adulthood choline supplementation in the Ts65Dn mouse model of Down syndrome

Authors: *S. TALLINO[1,2], S. BARTHOLOMEW[1,2], I. SEPULVEDA[1], J. WINSTONE[1,2], R. VELAZQUEZ[1,2,3];
[1]Neurodegenerative Dis. Res. Ctr., Biodesign Inst. at Arizona State Univ., Tempe, AZ; [2]Sch. of Life Sci., Arizona State Univ., Tempe, AZ; [3]Arizona Alzheimer's Consortium, Phoenix, AZ

Abstract: Choline metabolism lies at the heart of multiple cognition-relevant pathways, with choline sourced both from the diet and from endogenous metabolic pathways. Adequate choline is particularly important for cognition in Down syndrome (DS) in humans and in Ts65Dn mice, the most commonly used DS rodent model. Past work has shown that maternal choline supplementation improves hippocampal-dependent cognitive outcomes for Ts65Dn offspring, an effect which persists during aging even after supplementation has ceased. However, whether adult initiation of choline supplementation can improve cognition has yet to be determined. Here, we placed trisomic (3N) and disomic (2N) Ts65Dn mice (n = 16-18 per diet per genotype, balanced by sex; Jackson Laboratory Strain #005252) on diets containing either 1.1 mg/kg (ChN) or 5 mg/kg (Ch+) choline chloride from 4.5 months until endpoint at 14 months old, a total of 38 weeks of diet regimens. By 32 weeks, we observed a highly significant main effect of sex on percent weight change, with females gaining markedly more weight than males overall, and a modest main effect of diet, with animals on Ch+ diets gaining less weight; these changes were independent of food intake, which was not significantly different between groups. Blood was collected at baseline and at endpoint, and enzyme-linked immunosorbent assay showed a significant decrease in serum choline as a function of age, suggesting Ch+ diets did not prevent age-related decline in circulating choline. Glucose tolerance measured at endpoint revealed a significant effect of Ch+ diet lowering fasting glucose, but no changes to recovery from glucose challenge. Animals were assessed by rotarod (for motor function) and radial arm water maze (RAWM; for spatial memory) at 12.5 mo. Behavioral analysis revealed no significant difference in locomotion. RAWM data revealed a significant effect of genotype on latency to platform and correct/total arm entry ratio, but no effects of choline diet. In conclusion, adulthood choline supplementation does not appear to ameliorate hippocampal-dependent cognitive deficits observed in trisomic mice, emphasizing the importance of prenatal and early-life dietary intervention. However, supplementation did ameliorate age-associated weight gain in female mice and improved fasting blood glucose, suggesting modest impact of a Ch+ diet on risk factors associated with Alzheimer’s disease.

Disclosures: S. Tallino: None. **S. Bartholomew:** None. **I. Sepulveda:** None. **J. Winstone:** None. **R. Velazquez:** None.

Poster

371. Alzheimer's Disease: Pre-Clinical Approaches

Location: SDCC Halls B-H

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Program #/Poster #: 371.06

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: AG014449
AG017617
AG072599
AG074004

Title: Perinatal maternal choline supplementation (MCS) rescues early endosome pathology and protects vulnerable cholinergic and GABAergic septohippocampal neurons from degeneration in an aged trisomic mouse model of Down syndrome and Alzheimer's disease

Authors: *M. K. GAUTIER[1,3,4], C. M. KELLEY[8,9], S. LEE[2,5], E. J. MUFSON[10,11], S. D. GINSBERG[1,4,6,7];
[1]Ctr. for Dementia Res., [2]Ctr. for Biomed. Imaging, Nathan Kline Inst., Orangeburg, NY; [3]Pathobiology and Translational Med., [4]NYU Neurosci. Inst., [5]Child and Adolescent Psychiatry, [6]Psychiatry, [7]Neurosci. and Physiol., New York Univ. Grossman Sch. of Med., New York, NY; [8]Complex Adaptive Systems Initiative, Arizona State Univ., Tempe, AZ; [9]The Inst. for Future Hlth., Scottsdale, AZ; [10]Neurobio., [11]Neurol., Barrow Neurolog. Inst., Phoenix, AZ

Abstract: Down syndrome (DS) is a genetic disorder caused by the triplication of chromosome 21. By middle-age, individuals with DS develop Alzheimer's disease (AD) neuropathology including amyloid-β plaques and neurofibrillary tangles, abnormal early endosomes, and degeneration of cholinergic basal forebrain neurons. The trisomic Ts65Dn mouse recapitulates key aspects of DS/AD pathology, including cognitive dysfunction, dysregulation of the endosomal-lysosomal (EL) system, and loss of basal forebrain cholinergic neurons (BFCNs). Degeneration of BFCNs in the medial septal nucleus/vertical limb of the diagonal band (MSN/VDB) is associated with deficient neurotrophic support due to a loss of septohippocampal endosomal transport. Maternal choline supplementation (MCS), a well-tolerated treatment modality, rescues BFCNs from degeneration in young-adult Ts65Dn mice and alters aberrant gene expression networks, including those related to neurotrophic signaling and EL system functionality. Quantitative analysis of Rab5-immunoreactive (ir) early endosome phenotype in choline acetyltransferase (ChAT)-ir BFCNs using 3D reconstructed *z*-stacks revealed an age- and genotype-dependent increase in early endosome number and size in ~11-month-old (MO) Ts65Dn mice and disomic (2N) littermates compared to ~4 MO counterparts. Perinatal MCS prevented significant increases in early endosomal size in aged Ts65Dn offspring and attenuated increases in early endosome number in offspring independent of genotype. Microarray analysis of BFCNs from ~6 MO offspring revealed connectivity level changes in genes associated with early endosome homeostasis including Rab4, Rab5, and EEA1. Morphometric analysis of vulnerable MSN/VDB neuronal populations using the custom-designed stereology macro COANSI revealed significant age- and genotype-associated decreases in cholinergic and GABAergic MSN/VDB neuronal number and density in Ts65Dn mice. MCS significantly increased both ChAT- and parvalbumin-ir neuron number and density and prevented age-associated increases in BFCN cross-sectional area in aged supplemented Ts65Dn offspring. The regional area of the MSN/VDB occupied by ChAT-ir neuropil was significantly increased in ~4 MO Ts65Dn and 2N MCS offspring, suggesting early life choline delivery has permanent organizational effects on neurodevelopment. Taken together, these data suggest perinatal MCS confers long-lasting neuroprotective benefits on diverse neuronal populations within the septohippocampal circuit of Ts65Dn mice, potentially via the amelioration of early endosome pathology, which has implications for the treatment of DS and AD.

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Poster

371. Alzheimer's Disease: Pre-Clinical Approaches

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

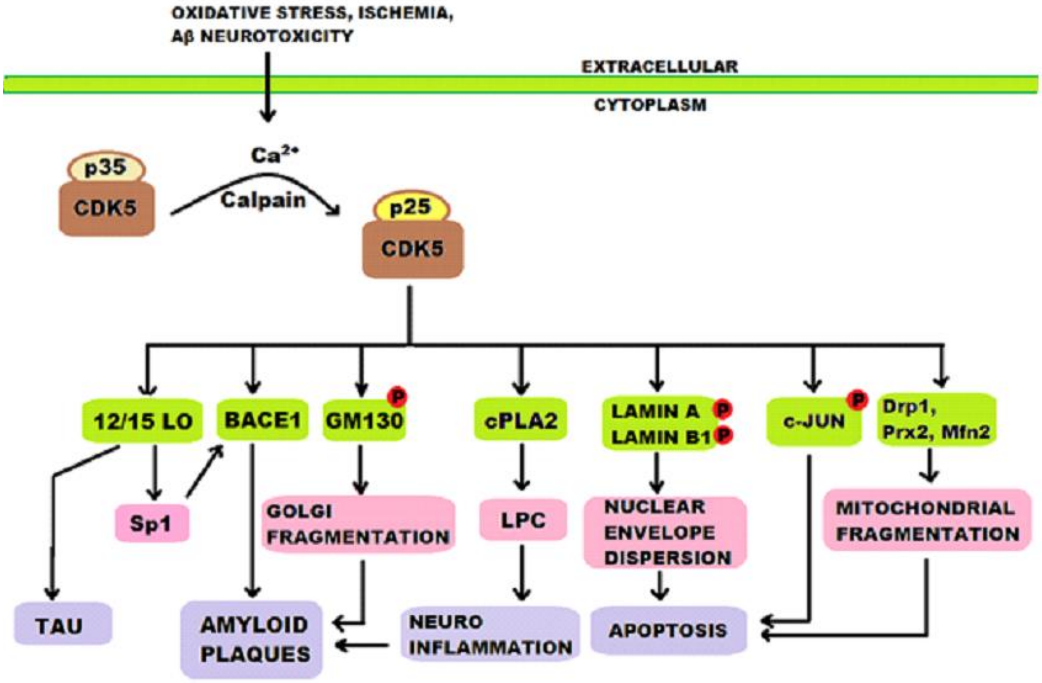
Program #/Poster #: 371.07

Topic: C.02. Alzheimer’s Disease and Other Dementias

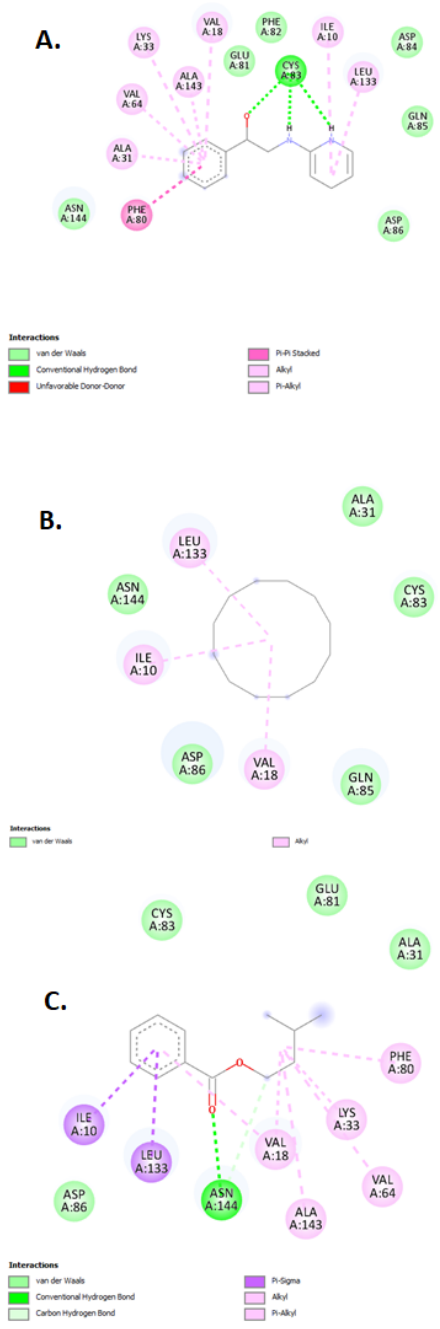
Title: Identification And In-silico Study of Date Palm Phytochemicals Against CDK5/p25 Activation In The Treatment of Alzheimer's Disease

Authors: *M. K. AJENIKOKO[1], A. O. AJAGBE[2], A. A. OKESINA[3], O. ADESANYA[4], L. A. J. SHITTU[5], A. B. TIJANI-ADEKILEKUN[4];
[1]Kampala Intl. Univ., Kampala Intl. University, Western Campus, Ishaka, Uganda; [2]Anat., Niles Univ. of Nigeria, Abuja, Nigeria; [3]Anat., Univ. of Rwanda, Kigali, Rwanda; [4]Anat., Kampala Intl. Univ., Ishaka, Uganda; [5]Dept. of Res. and Develop., Jireh Labs. Intl. LLC, Alief, TX

Abstract: There is increasing concern about the rising prevalence of Alzheimer's disease over the past decades around the world due to the incapacity of current medicines to slow down degradation. A unique feature seen in Alzheimer's disease is the formation of neurofibrillary tangles from hyperphosphorylation of tau, linked with cyclin-dependent kinase-5 (Cdk5)/p25 activation; thus, tau inhibition has the potential to be a promising therapeutic target. Using bioinformatic tools-molecular docking and virtual screening, we investigated the effectiveness of Date phytochemicals on Cdk5/p25 activation. Moreover, we used the PreADMET web server to predict the ADME, Toxicity, and Druglikeness properties of the screened compounds. Date compounds/ligands retrieved from the Pubchem database were docked with Cdk5 protein (PDB ID- 1UNL) to identify compounds with high binding affinity using PyRx tool. We found that Verbanol, cyclododecane, and isoamyl benzoate have high binding affinities, which suggest them as good drug candidates for slowing down degenerative processes caused by hyperphosphorylated tau. In addition, verbanol and isoamyl benzoate in the top three hit compounds have the potential to cross the blood-brain barrier. Hence, Date phytochemicals or their derivatives can be employed further in in-vitro and in-vivo research to develop natural medications for Alzheimer's disease.



Original image from: Bhounsule, A. S., Bhatt, L. K., Prabhavalkar, K. S., & Oza, M. (2017). Cyclin dependent kinase 5: A novel avenue for Alzheimer's disease. *Brain Research Bulletin*, *132*, 28–38. https://doi.org/10.1016/j.brainresbull.2017.05.006



Receptor-Ligand interactions on a 2D diagram showing the interaction between 1UNL and: A. Verbanol B. Cyclododecane C. Isoamyl benzoate

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Poster

371. Alzheimer's Disease: Pre-Clinical Approaches

Location: SDCC Halls B-H

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Program #/Poster #: 371.08

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant NS113233

Title: Therapeutic antisense oligonucleotides in mouse models of neurological disease

Authors: *M. P. STRATTON, J. L. CENTA, M. L. HASTINGS;
Rosalind Franklin Univ. of Med. and Sci., North Chicago, IL

Abstract: Antisense oligonucleotides (ASOs) have proven to be an effective therapeutic platform for the treatment of disease. These short, single-stranded, modified nucleotides function by base-pairing with the complementary sequence of an RNA and modulating gene expression in a manner that is dependent on ASO design and targeting site. We have devised a number of approaches to alter splicing with ASOs to correct or improve gene expression and pathology in neurological diseases such as Parkinson's and Alzheimer's disease. One of our approaches is being developed for the treatment of CLN3 Batten disease, a fatal, pediatric lysosomal storage disease caused by mutations in a gene encoding the lysosomal membrane protein CLN3. The most common mutation associated with CLN3 Batten is a deletion of exons 7 and 8 ($CLN3^{\Delta ex7/8}$), which disrupts the open reading frame. We devised a therapeutic strategy for treating CLN3 Batten disease using an ASO that alters *CLN3* splicing to correct the open reading frame of the mutated transcript. A mouse model that genetically recapitulates the reading frame correction has an attenuated disease phenotype, validating the ASO treatment approach. Treatment of $Cln3^{\Delta ex7/8}$ neonatal mice with this ASO resulted in the desired splicing effect throughout the central nervous system, improved motor coordination, reduced histopathological features of the disease in the brain, and extended life in a severe mouse model of the disease. Our results demonstrate that ASO-mediated reading frame correction is a promising therapeutic approach for CLN3 Batten disease and has important implications for the treatment of other neurological diseases.

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Poster

371. Alzheimer's Disease: Pre-Clinical Approaches

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 371.09

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Repetitive anodal transcranial direct current stimulation hastens emergence and recovery following isoflurane anesthesia in rat model of Alzheimer's disease

Authors: *M. MANSOURI[1], M. GRAVES[1], P. S. GARCIA[2];
[1]Columbia Univ., New York, NY; [2]Anesthesiol., Columbia Univ. Med. Ctr., New York, NY

Abstract: Introduction: Rapid and smooth recovery from general anesthesia is a goal for clinical anesthesia since peri-operative neurocognitive disorders such as delirium are known to accelerate dementia. Patients with Alzheimer's disease (AD) appear to be particularly at risk of cognitive deterioration following anesthesia, and some studies suggest that exposure to anesthetics may increase the risk of developing AD. Recent interests in the neural mechanisms involved in emergence from unconsciousness, has not been fully understood and thus far there has been no effective clinical strategy proven to improve recovery from surgical anesthesia in patients with vulnerable brains. One promising approach is the pre-habilitative use of non-invasive brain stimulation techniques such as, transcranial direct current stimulation (tDCS) to stabilize neural activity around a physiologic setpoint. To date, this technique has shown to be efficacious as treatment for neuropsychiatric disorders and enhancement of cognitive performance after stroke. In this study, we hypothesized that repetitive anodal tDCS can aid in the recovery from general anesthesia in a rodent model used to study AD. **Methods:** The study was performed on four groups of 11-12-month TgF344-AD (AD) and wild-type (WT) rats (n=32; 450-545 g). Four days after the tDCS socket implantation over the right motor cortex (AP: +1.5mm, ML: +2mm), repetitive anodal direct electrical current of 0.2 mA intensity applied for 15 min/day for 10 consecutive days in tDCS groups, while sham stimulation was applied to sham groups. Isoflurane sensitivity, emergence and recovery behaviors following a 2-hour isoflurane challenge (1.5%) was evaluated 24 hours after the last tDCS session. **Results:** Suppression of movement to tail clamp occurred at higher isoflurane concentrations in TgF344-AD animals irrespective of tDCS application (two-way ANOVA, $p < 0.05$). Although Isoflurane sensitivity was not affected by tDCS in either TgF344-AD animals or the WT control animals (two-way ANOVA, $p > 0.05$), time to appearance of behavioral markers indicating the start of recovery, return of righting reflex (RORR), was hastened by tDCS in both Tg-F334-AD rats and wild-type rats (two-way ANOVA, $p < 0.01$). **Conclusion:** Our findings confirm previous work demonstrating a mild hyperexcitability in brains of TgF344-AD animals at this age. Our work also suggests that anodal tDCS over the motor cortex might be a therapeutic candidate to hasten recovery from inhaled anesthesia in patients with or without AD.

Disclosures: M. Mansouri: None. **M. Graves:** None. **P.S. Garcia:** None.

Poster

371. Alzheimer's Disease: Pre-Clinical Approaches

Location: SDCC Halls B-H

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Program #/Poster #: 371.10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: PHS grant R21-MH121723-S1

Title: Retinal Regulation of Locus Coeruleus: A Chemogenetic Approach to Treat Neurodegenerative Disorders.

Authors: *S. DELCOURTE, H. E. BOWREY, G. CROZIER, Y. RAKHOLIA, G. S. ASTON-JONES;
Brain Hlth. Inst., Rutgers Univ., Piscataway, NJ

Abstract: Alzheimer's Disease (AD) is the most prevalent form of dementia. Clinical studies show that abnormal accumulation of tau protein in the locus coeruleus (LC) may play an early role in AD progression. Using an animal model of AD with this early LC tau pathology, the TG-F344 AD rat, Rorabaugh et al. (Brain 2017) showed that specific chemogenetic activation of LC rescued impaired reversal learning observed in this model. Given its deep location in the brainstem, LC is difficult to access in humans, limiting this approach for clinical application. Suprachiasmatic nucleus (SCN) provides an indirect input to LC via a relay in dorsomedial hypothalamus (DMH) (Aston-Jones et al., Nat. Neurosci. 2001). SCN is therefore in a key position to integrate light information with LC, via a circuit we denote as the Photic Regulation of Arousal and Mood (PRAM) pathway: retina-SCN-DMH-LC (Bowrey & Aston-Jones, Anxiety Depress. 2017). Methods: 3 month-old Tg-F344 or WT rats received intravitreal injections of an AAV encoding a Gq DREADD (AAV2-hSyn-hM3D(Gq)-mCherry) or control virus (AAV2-hSyn-EGFP). 6 months later, we assessed the effects of retinal DREADD stimulation on learning and memory in Tg-F344 rats using the Morris Swim Maze (MWM). Rats learned the location of the platform over 6 sessions, and were then subjected to a Referral (extinction) session and 4 reversal sessions (new platform location). Injections of the DREADD agonist clozapine-N-oxide (CNO; 2mg/kg, ip) were given 30min before the Referral and each Reversal session.Results: Electrophysiological and Fos analyses showed that Gq DREADD retinal stimulation increased RGC, SCN, DMH and LC activities. Tg-F344-AD rats showed poor initial as well as reversal learning. Retinal DREADD stimulation decreased reversal deficits in the Tg-F344 rats without affecting WT performance.Conclusion: Dysregulation of the noradrenergic LC, which is associated with behavioral deficits in Alzheimer disease, can be attenuated by PRAM-induced activation of LC. The PRAM pathway is a novel circuit for a relatively non-invasive approach to treating multiple neuropsychiatric disorders linked to LC.

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Poster

371. Alzheimer's Disease: Pre-Clinical Approaches

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Program #/Poster #: 371.11

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: A grant-in-aid for Practical Research Projects for Rare/Intractable Diseases from AMED (ID 21ek0109424h0002)
Grant-in-Aid for Challenging Research (Exploratory) from the Japan Society for the Promotion of Science (ID 21K19442)

Title: Aberrant immunity caused by RVCL-mutant TREX1 depends on nuclear localization enzyme activity

Authors: *S. KITAHARA[1], S. ANDO[2], T. KATO[3], H. NOZAKI[4], K. KASAHARA[5], O. ONODERA[5];
[1]Dept. of Neurol., Brain Res. institute, Niigata Univ., Niigata, Japan; [2]Dept. of Neurol., Brain Res. institute, Niigata Univ., Niigata city, Japan; [3]Dept. of Mol. neuroscience, Brain Res. Institute, Niigata Univ., Niigata, Japan; [4]Dept. of Neurol., Niigata City Gen. Hosp., NIIGATA, Japan; [5]Dept. of Neurol., Brain Res. Institute, Niig, Niigata, Japan

Abstract: Retinal vasculopathy with cerebral leukoencephalopathy (RVCL) is one of the hereditary small vessel diseases. TREX1, the causative gene of RVCL, is a DNA exonuclease. *TREX1* mutations with loss of enzyme activity cause autoimmune diseases distinct from RVCL. In contrast, TREX1 with RVCL-associated mutations have conserved enzymatic activity, and its effect on the immune system is not clear. The aim of our study is to elucidate the relevance of RVCL-associated mutant TREX1 and the immune dysregulation. We established IMR-90 cells with doxycycline-inducible expression by Retro-X Tet-On 3G System (TaKaRa BIO). The analyzed *TREX1* mutation included wild type (*WT*), *p.Val235fsGlyfsTer6*:RVCL mutation (*V235fs*) and *V235fs* with nuclear-export-signal (*NES*) or *Arg62Ala (R62A)* mutation which abolishes the nuclease activity of TREX1. Total RNA was extracted with Direct-zol RNA Kit (ZYMO Research) followed by cDNA production with VILO SuperScript IV (Invitrogen). QX200 Droplet Digital PCR system (BIO-RAD) was applied for measurement of the inflammatory cytokines and Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an endogenous control. The expression levels of inflammatory cytokines were standardized by taking the ratio to that of GAPDH. The statistical analyses were performed using the Mann-Whitney or one-way ANOVA followed by and Bonferroni's multiple comparison post hoc test. $P < 0.05$ was considered to indicate significance. In cells expressing *V235fs*-TREX1 (n=11), interleukin-6 (IL-6) and IL-8 expression levels were elevated compared with those of *WT*-TREX1 expressing cells (n=10) (IL-6: 1.3 ± 0.4 ng/ml in WT, 3.0 ± 0.8 ng/ml, $P < 0.001$; IL-8: 1.2 ± 0.4 ng/ml in WT, 3.6 ± 2.0 ng/ml, $P < 0.001$). The addition of *R62A* mutation to *V235fs* (n=11) attenuated the upregulated IL-6 (1.0 ± 0.1 vs. 3.0 ± 0.8 ng/ml, $P < 0.001$) and IL-8 (1.1 ± 0.2 vs. 3.6 ± 2.0 ng/ml, $P < 0.001$) expression levels by *V235fs*-TREX1. In addition, the addition of the *NES* (n=11) mutation attenuated the IL-6 (1.2 ± 0.4 vs. 3.0 ± 0.8 ng/ml, $P < 0.001$) and IL-8 (1.0 ± 0.8 vs. 3.6 ± 2.0 ng/ml, $P < 0.001$) expression levels similarly. These results indicate that RVCL-related mutant TREX1 brought the immune dysregulation depending on its nuclear localization and exonuclease activity. The aberrant immunity might be involved in the pathogenesis of RVCL.

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Poster

371. Alzheimer's Disease: Pre-Clinical Approaches

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 371.12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: OmicScouts

Title: Protein half-lives in human iPSC neurons, which are used as models for CNS drug discovery

Authors: *A. K. E. KOPKE[1], L. REICHART[2], A. SCHROEDER[3], J. MUNTEL[4], G. HAGEMANN[2], R. BERGER[2], H. HAHNE[5];
[1]bioExpert, Nieder-Olm, Germany; [2]Cell Biol. & Biochem., [3]Computat. Biol., [4]Mass spectrometry-based proteomics, [5]CEO, OmicScouts, Freising, Germany

Abstract: Pharmacologically-induced reduction of target protein levels is becoming widely used to develop novel therapeutic options. New treatments such as PROTACs (Proteolysis targeting chimera), immunomodulatory drugs, and degrons result in the degradation of target proteins; whereas RNAi and similar approaches downregulate gene expression, resulting in reduced target synthesis rates. Consequently, knowledge on target protein half-lives and monitoring of treatment effects on protein synthesis and degradation has become more important. Studying protein half-lives in iPSC derived human neurons is essential to devise and monitor suitable strategies for modulating target protein abundance, as well as to identify unwanted secondary targets. We have so far investigated the following human neurons: glutamatergic cortical, NGN2, GABAergic, and sensory neurons and evaluated turnover of between 6,000 and 10,000 proteins per cell line. We have tested the effects of treatment candidates on proteostasis in these neurons; monitoring protein degradation and re-synthesis at the same time. We have also established an affordable novel screening tool, quantifying target abundance and treatment-induced change for more than 8000 proteins for the price of a Western blot.Our results document that different iPSC derived human neurons show different median half-life over all proteins, and also different half-lives (t½) for the same protein. Some proteins, like the amyloid precursor protein (APP), show very fast t½ of a few hours, whereas other proteins had very long t½ of weeks. We produced databases of protein turnover, which are a valuable resource for determining potential target turnover, as well as turnover of upstream and downstream targets and regulators.
PROTACs are molecular chimeras of: (1) a target specific small molecule and (2) a moiety that recruits an E3 ligase, which are combined by a linker. Testing PROTACs for collaborators, we have found that many exhibit non-selective protein degradation or translational suppression, whereas few were truly selective and induced only the degradation of their intended target. These off-target activities were difficult to observe in Western Blots, even when a control protein was taken along in the analysis. Obviously, activity and selectivity are dose dependent and our technology lends itself for the investigation of dose and time dependent dynamic protein changes.

Disclosures: **A.K.E. Kopke:** 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.; OmicScouts. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Owner bioExpert. F. Consulting Fees (e.g., advisory boards); OmicScouts. **L. Reichart:** A. Employment/Salary (full or part-time):; OmicScouts. **A. Schroeder:** A. Employment/Salary (full or part-time):; OmicScouts. **J. Muntel:** A. Employment/Salary (full or part-time):; OmicScouts. **G. Hagemann:** A. Employment/Salary (full or part-time):; OmicScouts. **R. Berger:** A. Employment/Salary (full or part-time):; OmicScouts. **H. Hahne:** A. Employment/Salary (full or part-time):; OmicScouts. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-Owner OmicScouts.

Poster

371. Alzheimer's Disease: Pre-Clinical Approaches

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 371.13

Topic: C.02. Alzheimer’s Disease and Other Dementias

Title: Alzheimer's disease preclinical efficacy database: optimizing the scientific rigor and predictive value of preclinical therapeutic studies in alzheimer's disease animal models

Authors: *S. CHAKROBORTY, J. VISWANATHAN, M. LANFRANCO GALLOFRE, Z. MARTIN, J. YUAN, S. PETANCESKA, L. REFOLO;
Div. of Neurosci., Natl. Inst. on Aging, Bethesda, MD

Abstract: Assessments of preclinical efficacy studies in Alzheimer’s disease (AD) animal models highlight poor methodological rigor and inadequate reporting practices as contributive to the preclinical to clinical translation gap in AD therapy development. The Alzheimer’s Disease Preclinical Efficacy Database (AlzPED), a searchable and publicly available knowledgebase developed by the National Institute on Aging aims to address poor scientific rigor and reporting practices of preclinical efficacy testing studies for researchers, funding agencies, and the public. The database is missioned to improve transparency in reporting, increase awareness of the need for greater rigor in study design, and identify critical experimental design elements and methodology missing from studies that make them susceptible to over-interpretation and reduce their reproducibility and translational value. Using key word-driven literature searches published studies are acquired and curated by two experts for bibliographic details, funding source, study goals and principal findings, data on relevant translational criteria like therapy type, therapeutic agent, therapeutic target, animal models, and AD-related outcome measures, prior to publication in the database. Rigor in study design and methodology is evaluated with a Rigor Report Card consisting of a standardized set of study design elements recommended for animal studies.AlzPED hosts curated summaries from nearly 1300 published preclinical therapeutic studies in AD animal models, and data related to 251 therapeutic targets, 1123 therapeutic

agents, 195 animal models, more than 2000 AD-related outcome measures, and thousands of principal findings. Evaluation of Rigor Report Cards from each study demonstrates significant under-reporting of critical elements of methodology such as power/sample size calculation, blinding, randomization, balancing for sex, animal genetic background, and inclusion/exclusion criteria, these being reported by fewer than 35% of the nearly 1300 curated studies. Our analysis of curated studies demonstrates serious deficiencies in reporting critical elements of methodology. These deficiencies diminish the scientific rigor, reproducibility, and translational value of the preclinical studies. Adopting a standardized set of best practices like those proposed by AlzPED can improve the predictive power of preclinical studies in AD animal models and promote the effective translation of preclinical drug testing data to the clinic.

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Poster

371. Alzheimer's Disease: Pre-Clinical Approaches

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 371.14

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: DFG Po732

Title: Serotonin receptors as a novel target in the treatment of neurodegenerative disorders

Authors: *E. G. PONIMASKIN[1], J. LABUS[1], K. ROEHRS[1], K. JAHREIS[1], W. SUN[2], A. DITYATEV[2], S. JIA[2], Y. K. KIM[3], S. LIM[3];
[1]Hannover Med. School,, Hannover Med. School,, Hannover, Germany; [2]DZNE, Magdeburg, Germany; [3]Korea Inst. of Sci. & Technol. (KIST), Seoul, Korea, Republic of

Abstract: Aggregation of the microtubule-associated protein, Tau, leads to the development multiple diseases called tauopathies, with Alzheimer’s disease (AD) and frontotemporal dementia (FTD) as the most prominent members. During the last decade, the serotonergic system regained attention as a potential target for the treatment of neurodegenerative diseases, in particular AD, although the role of defined serotonin receptors in pathological Tau aggregation remained an enigma. Our study uncovered a causal link between constitutive activity of serotonin receptor 5-HT7 (5-HT7R) and pathological Tau hyperphosphorylation and the formation of neurofibrillary tangles in primary neuronal cultures and in cortical neurons in vivo. We elucidated the underlying molecular machinery by demonstrating a physical interaction between 5-HT7R and the Tau kinase, CDK5, leading to a G protein independent activation of CDK5. We also defined the structural requirements for the 5-HT7R and CDK5 interaction and deciphered 5-HT7R/CDK5 interaction interface. The therapeutic potential of the 5-HT7R/CDK5 pathway was demonstrated by showing that the selective knockdown of the 5-HT7R in the prefrontal cortex (PFC) of mice abrogated the deleterious effects of Tau[R406W] overexpression

in this region on synaptic plasticity and cognition. Using structural and functional screenings, we identified several clinically approved drugs to possess a high inverse agonism towards the 5-HT7R. Treatment with these drugs ameliorated various aspects of Tau pathology, including abrogation of hyperphosphorylation, tangle formation, apoptosis, and memory deficits in a mouse model of tauopathy. Moreover, a prospective cohort study carried out using a comprehensive health insurance database revealed that patients treated with such drugs had reduced risk of dementia.

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Poster

371. Alzheimer's Disease: Pre-Clinical Approaches

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 371.15

Topic: C.02. Alzheimer’s Disease and Other Dementias

Title: L-methionine treatment improves cognition through normalization of hippocampal DNA methylation in 5xFAD mouse model

Authors: *Y. LIU[1], C. POON[1], J. ROY[1], L. TSE[1], B. TONG[2], Y. CHAO[1], H. STEINBUSCH[3], K. YAO[1], K. CHEUNG[2], M. FUNG[1], L. LIM[1];
[1]Sch. of Biomed. Sciences, Li Ka Shing Fac. of Med., Hong Kong Univ., Hong Kong, Hong Kong; [2]Sch. of Chinese Med., Hong Kong Baptist Univ., Hong Kong, Hong Kong; [3]Dept. of Neurosci., Maastricht Univ., Maastricht, Netherlands

Abstract: Alzheimer’s disease (AD) has become a global disease burden and currently no effective treatment is available for AD. Decline in brain DNA methylation has been reported in AD patients and animal models, and accumulating evidence suggests that DNA methylation processes are pivotal for normal learning and memory functions. In this study, we investigated the therapeutic effects of L-methionine (MET) and its potential mechanisms in a 5xFAD mouse model. We demonstrated significant reduction of hippocampal-dependent memory functions and locomotion in 5xFAD mice, and these behavioral impairments were rescued by MET treatment. Such rescue was accompanied by remarkable reduction of Aβ burden, decreased microglial activation, and enhanced neurogenesis in the hippocampus of 5xFAD mice. Interestingly, we found DNA hypomethylation and reduction of several key methylation regulators *Dnmt3a*, *TET1* and *Gadd45b* in the hippocampus of 5xFAD mice, and these methylation deficits were normalized by MET treatment. Furthermore, MET treatment also restored the levels of memory-related genes such as *Arc*, *Reelin* and *CaN*, that involve the MeCP2-CREB-BDNF and CaN-Akt-GSK3β pathways in 5xFAD mice. Intra-hippocampal overexpression of *Dnmt3a2* resulted in spatial memory impairments, while knock-down of *Dnmt3a2* abolished the memory enhancing effects of MET treatment in the Morris water maze test. Field electrophysiology on hippocampal slices prepared from *Dnmt3a2* overexpression or knock-down mice was conducted to further

investigate synaptic mechanisms at the CA3-CA1 synapse. We found that *Dnmt3a2* overexpression resulted in decreased paired-pulse facilitation and theta-burst stimulation-induced long-term potentiation in 5xFAD mice, while combining MET with *Dnmt3a2* knockdown produced enhanced theta-burst stimulation-induced long-term potentiation in 5xFAD mice. Together, our results suggest that modulating DNA methylation by MET supplementation may have promising therapeutic potential for AD.

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Poster

371. Alzheimer's Disease: Pre-Clinical Approaches

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 371.16

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant NS095799

Title: Novel small-molecule activators of Tip60 for epigenetic treatment of Alzheimer's Disease

Authors: *A. BHATNAGAR[1], M. CHEN[1], S. KORTAGERE[2], F. ELEFANT[1];
[1]Biol., Drexel Univ., Philadelphia, PA; [2]Microbiology and Immunol., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Reduced histone acetylation in the brain causes transcriptional dysregulation and cognitive impairment that are key initial steps in Alzheimer's disease (AD) etiology. Accordingly, small molecular compounds designed to increase histone acetylation are a research hotspot for developing AD cognitive enhancing drugs. Pharmacological treatments are primarily centered on histone deacetylase inhibitors (HDACi) that while promising in reversing AD cognitive impairment, unfortunately also exhibit side effects due to non-specific global hyperacetylation. Alternatively, enhancing activity of specific histone acetyltransferases (HATs) with non-redundant functions in promoting cognition associated histone acetylation serves as an exciting new therapeutic strategy that remains to be fully explored. To this end, our lab identified a neuroprotective role by the HAT Tip60 in AD. Increasing Tip60 activity in the *Drosophila* AD brain reduces inappropriate repressor HDAC2 chromatin binding that reinstates Tip60 mediated histone acetylation, synaptic gene expression and cognitive function. We have designed and synthesized novel small molecule compounds to pharmacologically stimulate Tip60's acetyltransferase activity. Using *in silico* protein-ligand GOLD docking algorithm, we predicted two general HAT activators, CTB and CTPB, to dock with Tip60's acetyltransferase domain. These compounds were confirmed to physically bind with Tip60 protein *in vitro* and they rescued functional behavioral defects caused by Tip60 knockdown *in vivo*. We proceeded with our top performer CTB as lead compound to further optimize for robust specificity towards

Tip60 and blood-brain barrier penetration. We carried out a ligand-based virtual drug screening using the ZINC15 chemical database with over 15 million compounds to select 100 compounds with similar sub-structure as CTB and additional beneficial chemical modifications. Top 10 compounds with highest docking scores and favorable pharmacokinetics were synthesized to assess therapeutic effectiveness in our *Drosophila* AD model *in vivo*. We propose our first-in-class small molecule Tip60 HAT activators will serve as powerful novel chemical entities for development of specific acetylation-based cognition enhancing drugs for AD.

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Poster

371. Alzheimer's Disease: Pre-Clinical Approaches

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Program #/Poster #: 371.17

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Hunter RISE grant #5R25GM060665
NIA R01AG057555
City University of New York (Neuroscience Collaborative program, The Graduate Center

Title: Inhibition of histone deacetylase with RG2833 mitigates spatial memory deficits and disease associated neuroinflammation in a transgenic rat model of Alzheimer's Disease

Authors: *K. NDUKWE[1], L. XIE[2], P. A. SERRANO[3], P. ROCKWELL[1], M. E. FIGUEIREDO-PEREIRA[1];
[1]Dept. of Biol. Sci., [2]Computer Sci. Dep, Hunter Col. CUNY, New York, NY; [3]Dept of Psychology, Hunter College, CUNY, New York, NY

Abstract: Alzheimer's disease (AD) is one of the most common causes of dementia. In the United States, AD affects about 5.8 million Americans and is projected to reach 13.8 million individuals by 2050. Epigenetic alterations such as histone-modifications play a role in memory function, and disruption in the epigenetic processes is linked to the pathogenesis of neurodegenerative and neuropsychiatric diseases. Currently, there are no effective therapies for AD, and therapies targeting epigenetic mechanisms such as pharmacologic inhibitors of histone deacetylases (HDACs), are effective in improving cognitive performance in animal models of AD. The effects of histone deacetylase inhibitors on AD pathology and brain immune resident cells and neurons in an AD brain are not well understood. In this study, we treated Fisher transgenic 344-AD (TgF344-AD) rats, a model of AD, with the brain-penetrant inhibitor of histone deacetylase RG2833. Oral treatment with RG2833 (30mg/kg of body weight) lasted for 5 months starting at the age of 6 months, and rats were evaluated for spatial memory performance and AD pathology. Using an active place avoidance test we identified a significant hippocampal-dependent spatial memory performance deficit in TgF344-AD rats compared to wild type

littermates at 11 months of age. Notably, this deficit was mitigated in the RG2833-treated transgenic rats. Furthermore, RNAseq analysis of hippocampal tissue from TgF344-AD rats, revealed that treatment with RG2833 upregulated the expression of immediate early genes Arc and c-Fos, which are involved in synaptic plasticity and memory consolidation. We also found that RG2833 treatment failed to decrease amyloid beta or tau paired helical filament accumulation. However, RG2833 treated TgF344-AD rats exhibited a decrease in gliosis manifested by fewer amoeboid and ramified microglia in the hippocampal CA1 region compared to the untreated TgF344-AD littermate controls. These data indicate that histone modifying therapies can improve cognitive behavior by improving the expression of neuroprotective genes and by modulating the activation of immune cells and possibly dampening the inflammatory response. Based on our data, we propose that RG2833 could be an effective therapeutic to treat disorders such as AD.

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Poster

371. Alzheimer's Disease: Pre-Clinical Approaches

Location: SDCC Halls B-H

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Program #/Poster #: 371.18

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: R01-AG057522

Title: Apoe-targeted epigenome therapy for alzheimer’s disease: proof of concept studies

Authors: *O. CHIBA-FALEK[1], B. KANTOR[2];
[1]Neurol., [2]Neurobio., Duke Univ., Durham, NC

Abstract: Background: There is an urgent need to refocus Alzheimer’s disease (AD) drug discovery on new targets and shifting the paradigm of AD drug development towards precision medicine. Apolipoprotein E gene (*APOE*) is the strongest and most reproducible genetic risk factor for late-onset Alzheimer’s disease (LOAD), and thus holds promise as a potential therapeutics target for LOAD. In this study we developed an epigenome therapy platform to reduce *APOE* expression generally and *APOE*e4 specifically by targeted modification of the epigenome landscape within *APOE* locus. **Methods:** We developed epigenome therapy strategy based on CRISPR/deactivated (d)Cas9 editing technology fused with an effector molecule and delivered by viral vehicle. We designed a set of gRNAs to target regulatory elements in the *APOE* promoter and within exon 4 overlapping the SNP that defines the *APOE*e4 allele. We evaluated our epigenome therapy platform *in vitro* using human hiPSC-derived models and *in vivo* by stereotactic injection of reporter gene into the hippocampus of mice. **Results:** The viral delivered dCas9-repressor vector showed decreased *APOE*-mRNA and protein overall levels in hiPSC-derived neuronal model. To specifically target the *APOE*e4 allele we utilized the VRER-

dCas9 variant protein. Evaluation of the system specificity showed a reduction in *APOE*-mRNA levels in the hiPSC-derived models with the e4 allele while there was no effect in the *isogeneic* hiPSC-derived models homozygous for the e3 allele. Moving onto *in vivo* studies in mice administration of AAV dCas9-repressor vector and the GFP reporter gene into the hippocampus showed 50-70% decrease in GFP expression demonstrating promising preliminary data. Collectively, our results provided *in vitro* and *in vivo proof-of-concept* for the utility and efficacy of the *APOE*-targeted epigenome therapy. **Conclusions:** Our epigenome therapy strategy for fine-tuning of *APOE* expression based on dCas9 technology is *translational* toward the development of a therapeutics approach to prevent and/or delay LOAD onset. Furthermore, the technology offers the opportunity to refine the platform for the development of gene-specific and even allele- and cell-type- specific therapies, and by that enables the advancement of strategies for precision medicine in LOAD.

Disclosures: O. Chiba-Falek: 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.; CLAIRIgene. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); CLAIRIgene. **B. Kantor:** 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.; CLAIRIgene. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); CLAIRIgene.

Poster

371. Alzheimer's Disease: Pre-Clinical Approaches

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 371.19

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: NIH R21 NS118223

Title: Paternally-mediated inheritance of epigenetically-induced resilience to vascular cognitive impairment and dementia

Authors: E. E. BROYLES, D. H. CORELL, ***J. M. GIDDAY**;
LSU Hlth. Sci. Ctr., New Orleans, LA

Abstract: Vascular cognitive impairment and dementia (VCID) represents a significant public health burden for which no treatment is available. Using a mouse model of VCID, we recently showed in C57BL/6J mice that two months of repetitive hypoxic conditioning (RHC) prior to a 3-month period of carotid microcoil-induced chronic cerebral hypoperfusion (CCH) epigenetically induced resilience to loss of reference memory in the treated mice, as well as in

untreated, first-generation (F1) adult offspring conceived after RHC treatment of both parents (Belmonte et al., 2022). The present study was undertaken in the same model to determine if RHC treatment of the father, or mother, or both, was required for intergenerational inheritance of VCID protection. Five groups of adult F1 male mice were studied: Groups 1-4 were subjected to CCH for 3 months prior to reference memory assessment using the Novel Object Recognition (NOR) test; Group 5 was the corresponding non-disease control. Group 1 mice were derived from parents that were both treated with RHC prior to mating. Group 2 and 3 mice were derived from parents wherein only the father, or mother, respectively, was RHC-treated, and Group 4 mice were derived from parents without RHC treatment. The time spent exploring both the familiar and novel object presented during the NOR test was quantified by video playback, by an experimenter blinded to experiment conditions and applying identical inclusion/exclusion criteria across all groups; differences in object exploration times revealed reference memory impairment or retention. Consistent with our previous findings (Belmonte et al., 2022), mice in Groups 1 and 5 explored the novel object significantly longer ($p=0.02$ and 0.0001, respectively) than the familiar object, whereas Group 4 mice exhibited no difference in object exploration times ($p=0.43$). Mice in Group 2 spent significantly more time ($p=0.008$) exploring the novel object, indicating reference memory was retained as a result of paternal RHC treatment. In contrast, no significant difference in object exploration times ($p=0.34$) defined mice with maternal-only RHC treatment (Group 3). Collectively, these results indicate that epigenetically-induced, intergenerational resilience to VCID is paternally mediated. Future studies of the epigenetic modifications of male germ cells responsible for establishing intergenerational resilience to memory impairment, and the neurovascular and related phenotypes that ultimately defines such dementia resilience, are warranted.

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Poster

372. Cellular Mechanisms of Parkinson's Disease

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Program #/Poster #: 372.01

Topic: C.03. Parkinson’s Disease

Support: Augusta University Start-Up Fund

Title: Investigating the role of gut microbiome species Lactobacillus brevis in Parkinson's disease pathogenesis and healthy aging

Authors: *N. J. JOHNSON, D. E. MOR;
Neurosci. and Regenerative Med., Augusta Univ., Augusta, GA

Abstract: Aging is the greatest risk factor for age-related neurodegenerative diseases including Parkinson’s disease (PD), and the gut microbiome is emerging as a potential key modulator in early disease pathogenesis. PD is characterized by progressive degeneration of dopaminergic

neurons and the aggregation of the protein alpha-synuclein (α-syn) in the brain. Growing evidence suggests that the aggregation of α-syn may originate in the gut, and dysbiosis of the gut microbiome has been observed in PD patients. *Lactobacillus brevis* (*L. brevis*), a common constituent of the human gut microbiota, has been found to be increased in the gut microbiome of PD patients compared to healthy individuals. However, its role in α-syn aggregation and neurodegeneration in PD remains unknown. The nematode worm *C. elegans* is an ideal model system for studying the gut microbiome in aging and PD because they feed primarily on bacteria allowing easy manipulation of the gut microbiome and the study of the effects of a single bacterial species on health. *C. elegans* also have a short life span of 2-4 weeks, relative ease of cultivation, and allow for genetic manipulation of relevant genes/pathways having orthologs for 60-80% of human genes. Using established behavioral assays, preliminary data suggests that *L. brevis*-fed worms expressing human α-syn in muscle experience a decline in motor function compared to *E.coli*-fed controls. Confocal fluorescence microscopy revealed that the aggregation of α-syn is altered in *L. brevis*-fed worms expressing human α-syn::YFP in muscle. Basal slowing response was measured to evaluate dopamine neuron functions in worms expressing pan-neuronal human α-syn, and we found dopaminergic neuron functions to be reduced in worms fed *L. brevis*. Given that aging and neurodegenerative diseases are tightly interconnected and the gut microbiome plays central roles in both, we made the intriguing discovery that adult non-transgenic worms fed *L. brevis* also displayed striking health span phenotypes compared to *E. coli*-fed controls. These results included decreased body length, decreased motor functions, and increased egg retention indicative of a reproductive defect. Taken all together, our preliminary results show that the gut microbiome, and particularly *L. brevis*, has vast implications not only for neurodegenerative diseases such as PD, but also in the process of normal healthy aging.

Disclosures: **N.J. Johnson:** None. **D.E. Mor:** None.

Poster

372. Cellular Mechanisms of Parkinson's Disease

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Program #/Poster #: 372.02

Topic: C.03. Parkinson’s Disease

Support: NIH Grant R01 NS101628
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NIH Grant R01 AG065594
Helen Mendel Fund

Title: Pink1 regulates dendritic spine structure and function

Authors: P. OTERO[1], G. FRICKLAS[1], A. NIGAM[2], B. N. LIZAMA[1], Z. P. WILLS[3], J. W. JOHNSON[2], ***C. T. CHU**[1];
[1]Pathology, [2]Neurosci., [3]Neurobio., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Objectives and rationale. Recessive mutations in the gene for PTEN-induced kinase 1 (PINK1) are linked to Parkinson's disease (PD) and PD with dementia (PDD). We previously discovered that overexpression of PINK1 promotes dendritic complexity through activation of protein kinase A and phosphorylation of the NSFL1 cofactor p47. To further investigate the role of endogenous PINK1 in regulating dendritic architecture, we studied primary cortical neurons from *Pink1* knockout mice. **Methods.** Fluorescence microscopy was used to study dendritic branching and spine density in cortical neurons derived from *Pink1* knockout and wildtype E16 mouse embryos of unknown sex. In some preliminary experiments, cortical neurons differentiated from a familial patient iPSC line and its isogenic control were also studied. Images were analyzed using Sholl analysis and spine counting by individuals blinded to genotype and transfection status. Whole cell patch-clamp electrophysiology was used to evaluate synaptic function. **Results.** We found that loss of PINK1 expression results in diminished branching index, reduced spine density and maturation state, and diminished miniature excitatory postsynaptic current (mEPSC) frequency. Transfection of *Pink1* knockout mouse neurons with human PINK1, reversed changes in dendritic morphology. **Conclusions.** PINK1 plays a previously underappreciated role in regulating neuronal structure and function. Future studies are directed at understanding the signaling pathways involved, and the potential role of mitochondrial and non-mitochondrial mechanisms.
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Poster

372. Cellular Mechanisms of Parkinson's Disease

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 372.03

Topic: C.03. Parkinson's Disease

Support: H2020 MSCA Grant 888692
MJFF
Weston Brain Institute
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PSI Foundation

Title: Synaptic density in Parkinson's disease and atypical parkinsonism patients

Authors: *C. URIBE[1,2,3], K. L. DESMOND[2,4], R. RAYMOND[2], K. SMART[2], A. REILHAC[2], A. MENA[2,3,5], A. E. LANG[6], G. KOVACS[6], N. VASDEV[2,4], A. P. STRAFELLA[6,2,3,5]; [1]Departament de Medicina, Univ. de Barcelona, Barcelona, Spain; [2]Brain Hlth. Imaging Ctr., Ctr. for Addiction and Mental Hlth., Toronto, ON, Canada; [3]Krembil Brain Inst., Univ. Hlth. Network, Univ. of Toronto, Toronto, ON, Canada; [4]Dept. of Psychiatry, [5]Inst. of Med. Sci., Univ. of Toronto, Toronto, ON, Canada; [6]Dept. of Med., Toronto Western Hospital, Univ. Hlth. Network, Toronto, ON, Canada

Abstract: Rationale. The [^{11C}]UCB-J tracer has been used to investigate the synaptic density loss in Parkinson's disease (PD) patients. Recently, the synaptic vesicle protein 2A (SV2A) radioligand [^{18F}]SynVesT-1, that offers a longer half-life than the carbon labelled tracer, has been tested in healthy controls. To date, there are no studies in parkinsonian patients. **Objectives.** We investigated the *in vivo* binding properties of the [^{18F}]SynVesT-1 tracer for the quantification of synaptic density loss in PD and Multiple System Atrophy (MSA) patients, as well as the binding of a MSA post-mortem brain. **Methods.** *In vivo imaging:* Three healthy controls (46-67y), 2 PD patients (55y, 4y disease duration; and 66y, 15y disease duration) and one MSA patient (66y, 2y disease duration) were scanned. PET scans were acquired on a GE Discovery MI PET-CT. The radiotracer was injected via bolus injection (range 169-195MBq), and emission data acquired for 120 min with arterial blood collection. Low dose CT images were used for attenuation correction, and dynamic image reconstruction was performed using filtered back-projection. PET images were matched to the subject's T1-weighted MRI and processed using Pmod 4.2. A Simplified Reference Tissue Model 2 (SRTM2) to compute BP_{ND} values with the Centrum Semiovale as reference region was applied. *Postmortem imaging:* The [^{18}F](R)-SDM8 binding was assessed in the striatum and occipital lobe of a MSA patient. Sections were sliced at 10 µm and stored at -80°C. Slides were thawed and dried at room temperature for at least 30 minutes. Tissue was incubated for 1h with [^{18}F](R)-SDM8 (2nM and 12,400 mCi/µmole) or with 200µM levetiracetam. **Results.** Whole-brain BP_{ND} maps for PD patients displayed a similar distribution of synaptic density in cortical areas in comparison with age-matched healthy controls but lower density can be observed in subcortical areas, in particular in the striatum. Regarding the MSA patient BP_{ND} map, a lateralized rightwards density loss is found with respect to the left hemisphere in cortical areas as well as a significant striatal loss with respect to both PD patients and healthy controls. The post-mortem binding in sections of the striatum and occipital lobe of the MSA patient with [^{18F}](R)-SDM8 displays good specific binding distribution with respect to the levetiracetam blocking study. **Conclusion.** Preliminary data with [^{18F}]SynVesT-1 displays good binding distribution properties in parkinsonism patients. The use of this tracer is a promising tool for the investigation of synaptic loss in the striatum and to describe patterns of cortical synaptic depletion.

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Poster

372. Cellular Mechanisms of Parkinson's Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 372.04

Topic: C.03. Parkinson's Disease

Support: Canadian Institute of Health Research (CIHR)
Fonds de Recherche du Québec (FRQ-S)

Title: Post-mortem analysis of Parkinson's disease brains after long-term deep brain stimulation of the subthalamic nucleus

Authors: *J. D. MUNRO[1], F. DESMEULES[1], S. C. COTTIN[2], A. NOECKER[3], M.-È. TREMBLAY[4], P. V. GOULD[2], S. SAIKALI[2], M. LANGLOIS[2], C. MCINTYRE[3], M. PRUD'HOMME[2], L. CANTIN[2], M. PARENT[1];
[1]CERVO Brain Res. Ctr., Quebec, QC, Canada; [2]Hôpital de l'Enfant-Jésus, Quebec, QC, Canada; [3]Duke Univ., Durham, NC; [4]Ctr. de Recherche du CHU, Quebec, QC, Canada

Abstract: Deep brain stimulation (DBS) of the subthalamic nucleus (STN) is an effective surgical treatment for Parkinson's disease (PD), alleviating motor symptoms and restoring patients' quality of life. However, research into the long term effects of DBS on the human brain is lacking. This study aims to investigate the neuroanatomical and neurochemical alterations induced by chronic stimulations of the STN, and to correlate these changes with clinical outcomes and estimated electrical current delivered in the brain parenchyma. Brains of PD patients who had received more than 9 years of DBS treatment in the STN were used. For each brain, 3D graphical representations of the basal ganglia (BG) and DBS electrode were produced to determine the electrical current propagation using a patient-specific computational model (StimVision2). Immunofluorescence and confocal microscopy were used to determine the immunoreactivity of various proteins within the basal ganglia. Stereological quantification and morphological analyses of different cell types and blood vessels were performed. Along the electrode path, there was GFAP positive fibrillary gliosis with elevated expression of the growth factor GDNF. Near active contacts, astrocytes showed higher numbers of varicose processes that were in close apposition to GLUT1+ blood vessels. Additionally, elevated levels of GLUT1, VEGF and Claudin5 were observed, indicating increased blood vessel growth and blood brain barrier integrity. IBA1+ microglia count and CD68 expression was reduced while detailed morphological analysis of microglia indicates a more active state near the stimulated area of the STN. Overall, our post-mortem analysis indicates changes induced by long-term DBS of the STN involving glial cells, blood vessels and blood-brain barrier which do not appear to be detrimental to the patient.

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Poster

372. Cellular Mechanisms of Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: Department of Defense Grant W81XWH2010710, "Integrating Environmental,Genomic and Functional Data to Characterize Individual Risk for Parkinson's disease"

Title: Modeling gene-environment interactions in Parkinson's disease using iPSC-derived neurons

Authors: *E. MOCANU[1,2], K. L. HARRIS[1,2], Z. FAGHIHMONZAVI[1,3], J. KAYE[1,3], S. FINKBEINER[1,3], R. A. SWANSON[1,2];
[1]Dept. of Neurol., Univ. of California, San Francisco, San Francisco, CA; [2]Neurol. Service, San Francisco Veterans Affairs Hlth. Ctr., San Francisco, CA; [3]Ctr. for Systems and Therapeutics, Gladstone Inst., San Francisco, CA

Abstract: The cause of Parkinson's disease remains unknown, but epidemiological evidence points to both genetic and environmental risk factors. Purely environmental and purely genetic causes of PD are rare, and epidemiological studies suggest that most cases result from interactions between these factors. As a prime example, the FAME study of pesticide use and Parkinson's disease in agricultural workers (https://pubmed.ncbi.nlm.nih.gov/21269927/) found a strong positive interaction between genetic deficiency in glutathione-S-transferase theta 1 (GSTT1) and paraquat exposure. However, a limitation to epidemiological studies is that only correlative data can be gleaned. Here we describe an iPSC system we have developed for testing putative gene-environment interactions. This system employs the WTC11 iPSC line developed from a healthy volunteer engineered to constitutively express dCas9 from a "safe harbor" locus. Lentiviral infection with constructs expressing CRISPR guide mRNA and antibiotic resistance genes permit isolation of clones with desired gene expression knockdown. The cells are then differentiated toward a dopaminergic neuronal phenotype, yielding tyrosine hydroxylase expression in 20 - 50% of the differentiated neurons. The cultures are treated with pesticides or other chemicals of interest to identify potential synergistic relationships between the exposures and downregulation of specific genes. Our initial outcome measures have been markers of oxidative stress, DNA damage, and cell death. Using this system we have found that rotenone, paraquat, and (surprisingly) permethrin all produce oxidative stress (as measured by dihydroethidium oxidation) and variable degrees of DNA damage (as measured by gammaH2AX signal) in the dopaminergic neurons at concentrations below 1 μM. Higher concentrations of rotenone and paraquat to progressive cell death over 3-4 days. Ongoing studies are evaluating the influence of GSSTT1 knockdown on these chemical effects, as well as the effects of other candidate genes identified in the FAME study sample. This approach may be more widely used to assess putative gene-environment interactions, and even to build personalized toxicology profiles.

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Poster

372. Cellular Mechanisms of Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: This work was supported in part by the CRC1080 (German Research Council).

Title: Dnajc13 affects extracellular vesicle secretion from neurons

Authors: I. PFALZGRAF[1], M. W. BAEKEN[1], D. GOMEZ-ZEPEDA[2], S. TENZER[2], E.-M. KRÄMER-ALBERS[3], C. BEHL[1], ***A. M. CLEMENT**[1];
[1]Inst. of Pathobiochemistry, The Autophagy Lab., [2]Inst. of Immunol., Univ. Med. Ctr. Mainz of the Johannes Gutenberg Univ. Mainz, Mainz, Germany; [3]Inst. of Developmental Biol. and Neurobio., Johannes Gutenberg Univ. Mainz, Mainz, Germany

Abstract: To maintain neuronal function, the regulation of protein levels and their subcellular localization is essential. The endosomal system is a central switchboard for protein sorting in general but also in specialized compartments as the pre- and post-synapse. It consists of the early, the recycling, and the late endosome which is relevant for the lysosomal-autophagic way of protein degradation. The endosomal system directs proteins towards a recycling or the degradative pathway or gives rise to multivesicular bodies that allow a release of cellular components. The retromer and one of its associated proteins, DNAJC13, are essential for endosome function. Their importance for neuronal function and survival is documented since mutations of the retromer component VPS35 and DNAJC13 cause familial variants of Parkinson disease. We recently identified DNAJC13 as positive modulator of autophagy. We demonstrated that reduced levels of DNAJC13 or its C. elegans ortholog, RME-8, resulted in a decreased autophagic flux, whereas the overexpression of DNAJC13(WT), but not of the Parkinson associated DNAJC13(N855S) mutant, induced autophagic degradation. To further characterize DNAJC13 function in more detail, we employed human dopaminergic neurons derived from LUHMES cells (for Lund human mesencephalic). LUHMES cells lacking DNAJC13 show a decreased autophagic flux under basal conditions. As autophagic degradation and the release of extracellular vesicles (EVs) is cross-regulated, we quantitatively analyzed the release of EVs from differentiated LUHMES cells. Interestingly, DNAJC13 knockout cells released more EVs analyzed by nanoparticle tracking (NTA) and Western blotting. A comparative proteome analysis of EV preparations of wildtype and DNAJC13 knockout LUHMES cells by liquid chromatography-mass spectrometry (LCMS)-based label-free quantification revealed that the composition of these vesicles is different. STRING database analysis based on the cellular component (Gene Ontology) of the proteins revealed that a subset of in DNAJC13 knockout EVs enriched proteins is part of the ribonucleoprotein complex. Furthermore, proteins related to vesicles and their transport machinery were increased in EVs from DNAJC13 knockout cells, indicating that the sorting of proteins as well as the trafficking of vesicles might be affected in cells lacking DNAJC13. Interestingly, flotillin 1 and flotillin 2, two marker proteins for exosomes are increased in EVs from KO cells, indicating that DNAJC13 is tightly associated with the biogenesis of EVs.

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Poster

372. Cellular Mechanisms of Parkinson's Disease

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SNU-550-20210113

Title: Dysregulated phosphorylation of Rab GTPases induces axon degeneration

Authors: *E.-H. JANG[1,2], G. JEONG[5], Y. YAMAMOTO[6], K. TANAKA-YAMAMOTO[6], B. LEE[5], E.-M. HUR[1,3,4,2];
[1]Res. Inst. for Vet. Science, Col. of Vet. Med., [2]Lab. of Neuroscience, Col. of Vet. Med., [3]BK21 Four Future Vet. Med. Leading E&R Center, Col. of Vet. Med., [4]Interdisciplinary Program in Neuroscience, Col. of Natural Sci., Seoul Natl. Univ., Seoul, Korea, Republic of; [5]Kyung Hee Univ., Seoul, Korea, Republic of; [6]Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of

Abstract: Mutations in the *LRRK2* (leucine-rich repeat kinase 2) gene are the most common genetic cause of Parkinson's disease (PD), and LRRK2 has been associated with both familial and sporadic PD. A subset of Rab GTPases has been identified as authentic substrates of LRRK2, providing a link between intracellular trafficking and LRRK2 kinase activity. Previously, we have shown that dysregulation of Rab phosphorylation in the LRRK2 site induces neurodegeneration, and this study aims at investigating the mechanism by which dysregulation of Rab GTPases induces neurotoxicity. Here we show that dysregulated phosphorylation of Rab GTPases in the LRRK2 site causes defects in organelle trafficking and further support the notion that defects in intracellular trafficking can cause neurodegeneration.

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Poster

372. Cellular Mechanisms of Parkinson's Disease

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Program #/Poster #: 372.08

Topic: C.03. Parkinson’s Disease

Title: Study of effect induced by TiO2 vs SiO2 nano-matrices in differentiated SH-SY5Y cells

Authors: *E. A. RODRIGUEZ PEREZ[1], P. VERGARA-ARAGON[1], G. REYNOSO GALVEZ[1], A. ESPADAS ALVAREZ[1], B. HERNANDEZ TELLEZ[1], R. REYES RUIZ[1], R. BUSTAMANTE GARCIA[2], A. GOMEZ MARTINEZ[1];
[1]Physiology, Fac. of Med., [2]Histology, Fac. of Med., Natl. Autonomous Univ. of Mexico, Mexico City, Mexico

Abstract: We are interested in developing new and better biotechnological strategies for the treatment of diseases of the central nervous system. Silicon dioxide (SiO2) and Titanium dioxide (TiO2) has been used to build nanomatrices (NMs) capable of releasing and transporting dopamine (DA) to treat Parkinson's disease in animal model. We build, characterize SiO2 NMs and TiO2 NMs empty or DA-containing and evaluate the physiological effect of these on the differentiated or undifferentiated SH-SY5Y human cells. Observed that the SiO2 NMs empty increased the characteristics of the dopaminergic phenotype in both differentiated and undifferentiated SH-SY5Y cells and TiO2 did no increased the characteristics of the dopaminergic phenotype in both differentiated and undifferentiated SH-SY5Y cells. In addition, the TiO2 NMs empty did produced nitrosative stress in cultured cells SiO2 NMs empty did not produced nitrosative stress in cultured cells.

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Poster

372. Cellular Mechanisms of Parkinson's Disease

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Topic: C.03. Parkinson’s Disease

Support: NIH/NIEHS ES027245
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NIH/NINDS NS100090

Title: N6-methyladenosine RNA Modification Regulates Pro-inflammatory Cytokines Expression in a Neurotoxic Stress-Induced Neuroinflammatory Model of Parkinson’s Disease

Authors: *A. EALY[1], E. MALOVIC[2], H. JIN[1], V. ANANTHARAM[1], A. KANTHASAMY[1], A. KANTHASAMY[1];
[1]Physiol. and Pharmacol., Univ. of Georgia, Athens, GA; [2]Iowa State Univ., Ames, IA

Abstract: The etiopathogenesis of Parkinson's disease (PD) is mainly orchestrated by a complex interplay between genetic defects, environmental insults, and age-related neurotoxic stress exerted on the nigral dopaminergic system. Persistent neuroinflammation is a convergent pathophysiological process underlying gene-environment-aging-induced neurotoxic stress in PD. Although chronic neuroinflammatory responses can be self-perpetuated through a vicious cycle of pro-inflammatory cytokine release by astrocytes and microglia, the molecular mechanisms underpinning the dynamic regulation of cytokine expression during neurotoxic stress are yet to be defined. Therefore, understanding the mechanism governing the environmental stress-induced inflammatory actions of astrocytes will provide mechanistic insight into the neuroinflammatory pathogenesis of PD. N6-methyladenosine (m6A) RNA modification is the most prevalent epitranscriptomic modification in eukaryotic cells and is implicated in many cellular processes including the regulation of inflammation. Since environmental exposure to manganese (Mn) has been shown to induce proinflammatory responses in the basal ganglia by activating astrocytes, herein, we characterized the role of m6A in the regulation of neuroinflammation as it relates to PD. Our results demonstrate that Mn preferentially decreases the expression of the m6A reader protein YTHDF2 in both human U373 astrocytic cells and primary mouse astrocytes. Knockdown of YTHDF2 significantly increased Mn-induced pro-inflammatory cytokine mRNA expression while overexpression of YTHDF2 suppressed neuroinflammation. Our mechanistic studies further identified SEK1 mRNA as a direct target of YTHDF2 and discovered the ability of YTHDF2 to suppress the MAP2K4-SEK1-JNK-cJun pro-inflammatory signaling pathway in reactive astrocytes. Furthermore, astrocyte-specific YTHDF2 conditional knockout transgenic mice show increased astrogliosis with Mn treatment. Interestingly, LCMS/MS analysis of the total m6A/A ratio shows a global decrease of m6A modifications in Mn-treated astrocytic cells. To determine whether this decrease in global m6A expression is due to upregulation of m6A erasers, we profiled the m6A eraser proteins ALKBH5 and FTO. Both erasers were upregulated in Mn-treated astrocytes. Collectively, our data indicate that Mn-induced neurotoxic stress increases the expression of pro-inflammatory cytokines by decreasing global m6A modification through upregulation of m6A erasers and by increasing the stability of pro-inflammatory transcripts through downregulation of the mRNA decay-targeting reader protein YTHDF2.

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Poster

372. Cellular Mechanisms of Parkinson's Disease

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Program #/Poster #: 372.1

Topic: C.03. Parkinson's Disease

Support: NSF EHR 1700199
NSF DMS 1813819

Title: Mathematical model of subthalamic nucleus neuron - characteristic activity patterns and bifurcation analysis

Authors: *C. PARK[1], L. L. RUBCHINSKY[2], S. AHN[3];
[1]Mathematics and Statistics, North Carolina Agr. and Tech. State Univ., Greensboro, NC; [2]IUPUI and Indiana Univ. Sch. of Med., Indianapolis, IN; [3]Mathematics, East Carolina Univ., Greenville, NC

Abstract: The subthalamic nucleus (STN) has an important role in the pathophysiology of the basal ganglia in Parkinson's disease. The ability of STN cells to generate bursting rhythms under either transient or sustained hyperpolarization may underlie the excessively synchronous beta rhythms observed in Parkinson's disease. In this study, we developed a conductance-based single compartment model of an STN neuron, which is able to generate characteristic activity patterns observed in experiments including hyperpolarization-induced bursts and post-inhibitory rebound bursts. This study focused on the role of three currents in rhythm generation: T-type calcium (CaT) current, L-type calcium (CaL) current, and hyperpolarization-activated cyclic nucleotide-gated (HCN) current. To investigate the effects of these currents in rhythm generation, we performed a bifurcation analysis using slow variables in these currents. Bifurcation analysis showed that the HCN current promotes single-spike activity patterns rather than bursting in agreement with experimental results. It also showed that the CaT current is necessary for characteristic bursting activity patterns. In particular, the CaT current enables STN neurons to generate these activity patterns under hyperpolarizing stimuli. The CaL current enriches and reinforces these characteristic activity patterns. In hyperpolarization-induced bursts or post-inhibitory rebound bursts, the CaL current allows STN neurons to generate long bursting patterns. Thus, bifurcation analysis explained the synergistic interaction of the CaT and CaL currents, which enables STN neurons to respond to hyperpolarizing stimuli in a salient way. The results of this study implicate the importance of CaT and CaL currents in the pathophysiology of the basal ganglia in Parkinson's disease.

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Poster

372. Cellular Mechanisms of Parkinson's Disease

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Program #/Poster #: 372.11

Topic: C.03. Parkinson's Disease

Title: Analysis of insulin resistance as a risk factor for Parkinson's disease in human midbrain organoids

Authors: *A. ZAGARE[1], J. KURLOVICS[2], E. STALIDZANS[2], G. GOMMEZ GIRO[1], P. ANTONY[1], C. JÄGER[1], E. GLAAB[1], R. KRÜGER[1], J. SCHWAMBORN[1];

[1]Luxembourg Ctr. for Systems Biomedicine, Univ. du Luxembourg, Esch-sur-Alzette, Luxembourg; [2]Univ. of Latvia, Riga, Latvia

Abstract: Recent evidence indicates shared disease mechanisms between Type 2 Diabetes (T2D) and Parkinson's disease (PD), suggesting that T2D may contribute to the development and progression of PD. Insulin resistance, which is the main hallmark of T2D, has also been shown to play an important role in neurodegeneration by regulating neuronal metabolism, functionality and survival. To understand the importance of insulin signalling in the human midbrain we expose human midbrain organoids from healthy individuals and GBA-N409S mutation-carrying PD patients to either high insulin concentrations, leading to insulin resistance, or to low insulin concentrations to restore normal insulin function. We characterise midbrain organoid transcriptional and metabolic profiles in order to identify the most insulin signalling-dependent dysregulated cellular processes. Furthermore, we show that insulin resistance compromises dopaminergic neuron maturity and increases cellular death. Our study suggests that defective insulin signalling contributes to the vulnerability of dopaminergic neurons that may lead to the development of PD and aggravates existing PD phenotypes. These results highlight insulin resistance as an important target in PD prevention and therapy.

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Poster

372. Cellular Mechanisms of Parkinson's Disease

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Program #/Poster #: 372.12

Topic: C.03. Parkinson's Disease

Support: Association France Parkinson

Title: Phosphodiesterase 2A : functional role in the striatum and potentially new therapeutic target in Parkinson's disease

Authors: *S. BOMPIERRE, L. CASTRO, V. PIERRE;
Sorbonne Univ., Paris, France

Abstract: Aims: In Parkinson's disease (PD), the degeneration of dopaminergic neurons results in a deficit of dopamine. This situation is commonly treated pharmacologically by the administration of L-DOPA, a precursor of dopamine. However, after about 10 years of treatment, 80% of patients develop L-DOPA-induced dyskinesia (LID). In the dopamine depleted striatum, D1 type medium-sized spiny neurons (D1 MSN) become hyper-responsive to the stimulation of type 1 dopamine receptors. This hypersensitivity leads to an over-activation of the cAMP/PKA signaling pathway, resulting in the progressive development of LID. Our aim is to evaluate the potential of phosphodiesterase 2A (PDE2A), which degrades cAMP, to reduce D1 MSN

hypersensitivity associated with LID. Because of its low affinity for cAMP, the stimulation of PDE2A activity through the nitric oxide (NO)/cGMP pathway could reduce excessive cAMP levels while preserving proper responses.
Methods: Biosensor imaging reports the dynamics of cAMP/PKA signaling in MSNs in striatal brain slices from young mice, or adult mice in PD and dyskinetic situation.
Results: In PD and dyskinetic mouse model, D1 MSN display a larger cAMP response to transient dopamine compared to normal mice. The larger cAMP response is similar to the response measured in immature brain. Interestingly, PDE2A activation by the NO/cGMP pathway efficiently reduces the amplitude of the dopamine response in PD and dyskinetic mouse model.
Conclusion: the stimulation of PDE2A activity moderates excessive cAMP levels in the response to dopamine in dyskinetic mice. These results highlight the therapeutic potential of PDE2A stimulation in the treatment of LID.

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Poster

372. Cellular Mechanisms of Parkinson's Disease

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Program #/Poster #: 372.13

Topic: C.03. Parkinson’s Disease

Support: NIH Grant RO1ES021656

Title: Inhibition of canonical IKK2/NF-kappaB signaling in microglia does not protect against neurodegeneration in a dual-hit environmental exposure model of Parkinson's Disease

Authors: *S. M. ROCHA[1], M. J. EDMONDS[2], D. CHATTERJEE[4], R. J. SMEYNE[4], R. B. TJALKENS[3];
[1]Dept. of Microbiology, Immunology, and Pathology, [2]Dept. of Molecular, Cell. and Integrative Neurosciences, [3]Dept. of Envrn. and Radiological Hlth. Sci., Colorado State Univ., Fort Collins, CO; [4]Thomas Jefferson Univ., Farber Inst. of Neurosci. Thomas Jefferson Univ., Philadelphia, PA

Abstract: Parkinson’s Disease (PD) is the most common motor movement disorder of aging. Although the precise etiology of PD remains elusive, recent scientific advances have demonstrated that genetics, aging and environmental exposures function in concert to promote disease progression and pathology. Late-stage PD is characterized clinically by bradykinesia, short shuffling gate, resting tremor, postural instability, and difficulty balancing. Pathologically, PD is characterized by a loss of dopaminergic neurons (DAn) in the substantia nigra pars compacta (SNpc), accumulation of misfolded alpha-synuclein (a-syn) in surviving DAn, known as Lewy bodies, and neuroinflammatory activation of glia. Microglia contribute to neuronal injury in PD through chronic activation of innate immune inflammatory pathways that are linked

in part to environmental exposures. However, how multiple environmental exposures prime innate immunity in microglia to induce a neurotoxic phenotype is not well understood. We postulated that inhibition of NF-kappaB signaling in microglia would impair immunological priming and reduce overall inflammatory injury in lesioned animals. To test this hypothesis, we generated mice deficient in NF-kappaB activation in microglia (CX3CR1-Cre::IKK2$^{fl/fl}$). These mice were then exposed to 50mg/kg/day of manganese (Mn) in drinking water during juvenile development (PN21-PN51), followed by later exposure to rotenone (2.5mg/kg/day for 14 days) as adults (4 months old). Subsequent analysis revealed knockout (KO) mice had an accumulation of misfolded a-syn in DAn and microglial cells in animals exposed to either rotenone or Mn and rotenone. Although KO animals showed less DAn neurodegeneration after exposure to rotenone, animals exposed to Mn and rotenone had comparable DAn loss to wildtype (WT) mice. These data suggest that inhibition of primary immune recognition and signaling through canonical NF-kappaB in microglia is protective. However, dual exposure modeling, which more closely recapitulates patient lifetime exposures, facilitated resident immunological 'training' through microglial priming. The resulting pathology is independent of microglial canonical NF-kappaB signaling but instead is due to astrocytic, microglial, and neuronal responses to misfolded a-syn accumulation, mitochondrial dysfunction, alternate inflammatory pathway activation, and microenvironment changes, which are highly exposure dependent.

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Poster

372. Cellular Mechanisms of Parkinson's Disease

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Program #/Poster #: 372.14

Topic: C.03. Parkinson's Disease

Support: Novo Nordisk: ESC-derived human microglia as a platform to study neurodegenerative disease

Title: Investigating the role of microglia in Parkinson's disease using human-mouse microglial chimeras

Authors: *M. A. KRZISCH[1], W. CHEN[2], D. FU[2], C. M. GARRETT-ENGELE[2], K. A. ANDRYKOVICH[2], R. JAENISCH[2];
[1]Whitehead Inst. for Biomed. Res., Cambridge, MA; [2]Whitehead Inst. for Biomed., Cambridge, MA

Abstract: Parkinson's disease is the second most common neurodegenerative disorder, and is characterized by the aggregation of intracellular inclusions of alpha-synuclein named Lewy bodies, Lewy neurites, and the loss of dopaminergic neurons in the substantia nigra. Microglia-driven neuroinflammation is thought to contribute to neuronal death in Parkinson's disease,

however the exact role of microglia in Parkinson's disease remains unclear. Mutations in alpha-synuclein found in familial PD increase the potential of microglia for neuroinflammation and increase the Lewy body pathology and death of dopaminergic neurons. In particular, the A53T mutation in alpha-synuclein increases its aggregation, promotes pro-inflammatory cascades in microglia and increases the reactivity of microglia. Primary microglia isolated from the human brain shows significant changes in gene expression after only 6 hours in culture, with downregulation of key microglial genes and upregulation of genes related to microglial activation. Human stem cell-derived myeloid precursors transplanted in the brain of human CSF1 knock-in immune-deprived mouse neonates colonize the mouse brain and yield microglia that retains its human identity and more closely ressembles *ex vivo* human microglia than *in vitro* 2D cultures. Additionally, a recent study found that out of 20 Parkinson's disease related genes, 18 genes had higher expression in transplanted human microglia than in mouse microglia. These results indicate that transplantation of human microglia carrying Parkinson's disease-related mutations in the mouse brain may yield new insights on the dysfunction of microglia in Parkinson's disease. Here, we generated human-mouse microglial chimeras carrying A53T-mutant human microglia and isogenic control microglia by transplanting human myeloid precursors in the brain of human CSF1 knock-in Rag2 IL2rg mouse neonates, in order to study their morphological and functional phenotype in an *in vivo* context, and assessed alterations in their morphology, reactivity marker expression and gene expression profile at 2 months post-injection.

Disclosures: M.A. Krzisch: None. **W. Chen:** None. **D. Fu:** None. **C.M. Garrett-Engele:** None. **K.A. Andrykovich:** None. **R. Jaenisch:** None.

Poster

372. Cellular Mechanisms of Parkinson's Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 372.15

Topic: C.03. Parkinson's Disease

Support: CIHR

Title: Characterizing the Bcl-2 Associated Athanogene 5 Interactome in the Context of Parkinson's Disease

Authors: M. KAPADIA[1], E. L. FRIESEN[2], A. KRIZUS[1], X. WANG[2], D. WILLIAMS[2], G. SCHMITT-ULMS[2], L. KALIA[1], S. KALIA[1];
[1]Krembil Res. Inst., Univ. Hlth. Network, Toronto, ON, Canada; [2]Univ. of Toronto, Univ. of Toronto, Toronto, ON, Canada

Abstract: Parkinson's disease (PD) is a common neurodegenerative disorder deranging the nigro-striatal circuits of the brain and particularly dopaminergic neurons in the substantia nigra pars compacta. PD pathogenesis is complex and includes alterations in multiple proteostasis

pathways that promote the progressive accumulation of macromolecular damage. Molecular chaperones are a class of proteins that assist the conformational folding or unfolding and the assembly or disassembly of other macromolecular structures, making them an enticing therapeutic target in PD. BAG5 is a co-chaperone protein that directly inhibits the chaperone activity of Hsp70 and promotes dopaminergic neuronal death *in vivo* in models of PD. The mechanisms of how BAG5 impairs proteostasis and promotes cell death remain unclear. The purpose of this project was to characterize the BAG5 interactome to guide further studies of its role in physiological and disease states. Dopaminergic SH-SY5Y tetracycline-inducible cell lines with stable overexpression of GFP-BAG5 or GFP were harvested and labelled with *i*sobaric *t*ags for *r*elative and *a*bsolute *q*uantification (iTRAQ) by mass spectrometry (MS). Consistent with the previous knowledge of BAG5 function, Gene Ontology and Pathway analysis of our iTRAQ-MS-BAG5 interactome showed an enrichment of terms relating to protein folding, autophagy, modulation of ubiquitin-proteasome system function, and regulation of apoptosis. A novel theme that surfaced in this analysis pointed to a role of BAG5 in nuclear functions such as mRNA splicing and DNA damage response. Mechanistic studies to elucidate the molecular consequences of these interactions under steady-state and genotoxic stress conditions are currently ongoing. The findings may offer a novel therapeutic target for PD and other neurodegenerative conditions.

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Poster

372. Cellular Mechanisms of Parkinson's Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 372.16

Topic: C.03. Parkinson’s Disease

Title: Cell-specific changes in ion channel expression are associated with differential neuronal vulnerability in Parkinson’s disease and its A53T α-synuclein mouse model.

Authors: *N. BURKERT[1], T. ARZBERGER[2,3], D. SPAICH[1], C. PÖTSCHKE[1], S. MÜLLER[1], J. ALY[1], N. WATTAD[4], T. SNUTCH[5], J. ROEPER[6], J. GOLDBERG[4], J. HERMS[3], D. WUTTKE[7], M. MÜNCHMEYER[7,8], J. DUDA[1], B. LISS[1,9];
[1]Inst. of Applied Physiol., Ulm Univ., Ulm, Germany; [2]Dept. of Psychiatry and Psychotherapy, [3]Ctr. for Neuropathology and Prion Res., Ludwig-Maximilians-University Munich, Munich, Germany; [4]Dept. of Med. Neurobio., The Hebrew Univ. of Jerusalem, Jerusalem, Israel; [5]Dept. of Psychiatry, Univ. of British Columbia, Vancouver, BC, Canada; [6]Inst. of Neurophysiol., Goethe Univ. Frankfurt, Frankfurt, Germany; [7]Wolution GmbH & Co. KG, Munich, Germany; [8]Dept. of Physics, Univ. of Wisconsin-Madison, Madison, WI; [9]Linacre and New Col., Univ. of Oxford, Oxford, United Kingdom

Abstract: The two main neuropathological hallmarks of Parkinson's disease (PD) are differential neuronal vulnerability and formation of toxic α-synuclein aggregates, so-called Lewy-bodies (LB). While dopaminergic (DA) neurons within the Substantia nigra (SN) exhibit the highest cell loss during PD, cholinergic neurons within the dorsal motor nucleus of the vagus (DMV) show early LB-formation and a similar pacemaker-activity, but are less affected by degeneration. The cause for this differential neuronal vulnerability is still unclear, but cell type specific ion channel activity, activity-related metabolic stress, and calcium (Ca^{2+}) homeostasis are important factors. To identify mechanisms defining differential neuronal vulnerability in PD, we analyzed mice overexpressing human mutant α-synuclein (A53T), causing familial forms of PD (PARK4/1), as well as *post mortem* human brains from PD patients. We previously reported that SN DA neurons of A53T mice display elevated metabolic stress, accompanied by redox-impaired A-type K^+ channels (particularly Kv4.3), while in DMV neurons metabolic stress levels were even lower, compared to wildtype (WT). Here, we addressed differential expression of voltage gated Ca^{2+} channels (Cav) and Ca^{2+} and voltage gated A-type channels as underlying mechanism. We quantified mRNA of Cav and Kv4 channel α and β subunits in mouse and human neurons via RNAscope *in situ* hybridization, followed by automated cell-recognition and signal-quantification (Wolution). In WT mice, selectively Cav3.1 mRNA levels were ~70% higher in SN DA compared to DMV neurons. In A53T mice, DMV Cav3.1 levels were further reduced by ~30%. A-type channel α (Kv4.3/4.2) and β (KChip3/4) subunits were also differentially expressed in mouse DA and DMV neurons. Moreover, mRNA-levels for Cav3.1 as well as Kv4.3 and KChip3 were ~2-fold higher in remaining human SN DA neurons from PD patients compared to age-matched unaffected controls, while Cav3.1 mRNA levels in remaining human DMV neurons were ~50% lower in PD samples. Increased Cav3.1 T-type channel activity in SN DA neurons could constitute a compensatory response to their progressive loss in PD, to stimulate Ca^{2+} dependent ATP-production and dopamine-release, but it also could elevate metabolic stress. To address this hypothesis, we studied mitochondrial respiration on tissue-punches from vital mouse brain slices (Seahorse). In the SN from A53T mice, we detected ~50% higher metabolic stress levels that were reduced to WT levels in response to pharmacological Cav3 T-type channel inhibition. In contrast, for DMV tissue-punches, we detected no differences between A53T and WT mice.

Disclosures: **N. Burkert:** None. **T. Arzberger:** None. **D. Spaich:** None. **C. Pötschke:** None. **S. Müller:** None. **J. Aly:** None. **N. Wattad:** None. **T. Snutch:** None. **J. Roeper:** None. **J. Goldberg:** None. **J. Herms:** None. **D. Wuttke:** None. **M. Münchmeyer:** None. **J. Duda:** None. **B. Liss:** None.

Poster

372. Cellular Mechanisms of Parkinson's Disease

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Program #/Poster #: 372.17

Topic: C.03. Parkinson's Disease

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Stanford Maternal and Child Health Research Institute Uytengsu-Hamilton 22q11 Neuropsychiatry Research Program

Title: Investigating genes within the 22q11.2 deletion region as potential risk factors for Parkinson's disease in human iPSC models.

Authors: *L. HEINRICH, M. Y. CHEN, B. SCHUELE;
Dept. of Pathology, Stanford Univ. Sch. of Med., Stanford, CA

Abstract: Adults with genomic deletions on chromosome 22q11.2, which include a 3 Megabase (Mb) genomic region with approximately 41 protein-coding genes expressed in the human brain, have a higher risk of developing typical Parkinson's disease (PD), however, it is unclear which of these genes contribute to the neurodegenerative process.
The goal is to identify genes in the 22q11 deletion region that contribute to causal molecular mechanisms of neuronal dysfunction and predispose to a neurodegenerative process reminiscent of PD due to diminished gene dosage. (i) Using human induced pluripotent stem cells (iPSCs), we investigate whether iPSC-derived dopaminergic neurons from 22q11DS patients replicate pathophysiological phenotypes associated with PD. (ii) Implementing CRISPR perturbation that will allow us to identify biological targets and pathways.
We show successful differentiation of human iPSCs from 22q11DS patients and controls to neurons that express genetic markers for midbrain dopaminergic identity and neuronal maturation. In iPSC-derived neuronal progenitors, we found no differences in reactive oxygen species (ROS), mitochondrial dysfunction, or endolysosomal activity in cultures from 22q11DS compared to controls. By contrast, we detected increased levels of ROS in neurons (30 days *in vitro*) derived from 22q11DS patients compared to controls. Both mitochondrial activity and density were altered in 22q11DS neurons. We further detected enhanced endolysosomal activity with Lysotracker in 22q11DS neurons. We currently optimize Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) perturbation in 22q11DS and control iPSCs to reversibly regulate gene expression and systematically increase protein levels of deleted genes in the 22q11.2 deletion region in human iPSC-derived neurons to identify causative genes.
Mitochondrial phenotypes could only be detected in iPSC-derived post-mitotic dopaminergic neurons from patients with 22q11DS and not in corresponding neuronal progenitor cells. Our findings are consistent with data from 22q11DS mouse models indicating increased ROS levels in cortical projection neurons and replicate mitochondrial phenotypes found in human iPSC-derived dopaminergic neurons and animal models of PD.

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Poster

372. Cellular Mechanisms of Parkinson's Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 372.18

Topic: C.03. Parkinson's Disease

Title: Intracranial basal ganglia LFP show increases in event related beta synchrony during beat entrainment of finger movements in Parkinson's disease

Authors: *M. THAUT[1], V. SHARMA[2], U. SAHA[3], J. SAHA[4], R. CHEN[5];
[1]Fac. of Music and Fac. of Medicine, Univ. iof Toronto, Toronto, ON, Canada; [2]SICK KIDS HOSPITAL, UHN UNIVERSITY OF TORONTO, Toronto, ON, Canada; [3]UHN, Univ. of Toronto, Toronto, ON, Canada; [4]Electrical Engineering, Univ. of Waterloo, Waterloo, ON, Canada; [5]Krembil Res. Institute, Fac. of Medicine, Univ. of Toronto, Toronto, ON, Canada

Abstract: Parkinson's disease, a neurodegenerative condition characterized by the death of neurons in the basal ganglia, results in severe gait and motor impairments, which are alleviated by rhythmic auditory stimulation (RAS). The mechanism for this alleviation is not known. Understanding this mechanism may lead to RAS-based therapeutics, diagnostics, prognostics, or give insights that improve current stimulation methods. We recorded local field potentials (LFPs) in the globus pallidus with Medtronic model 3387 electrodes at a sample rate of 5000 Hz in a single Parkinson's patient case study. Neural data was acquired with a Neuroscan SynAmps RT EEG system (Compumedics Neuroscan USA, Ltd. Charlotte, NC, USA). The patient finger tapped synchronized to RAS at two tempos (0.8 Hz and 2 Hz), and finger tapped self-paced after being prompted by four beats at these two tempos. Neural data were bandpassed between 1 to 60 Hz and epoched ± 20 ms around tapping response. Montage was bipolar. Results show that RAS significantly reduced variation between finger taps. We found significantly increased post-tap beta event-related synchonry during the RAS conditions compared to the self-pace tapping. Results demonstrate RAS produces increased neuroelectric response in basal ganglia via the ascending auditory pathway, which facilitated rhythmic coupling between basal ganglia and motor cortex during motor output in Parkinson's disease.

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Poster

372. Cellular Mechanisms of Parkinson's Disease

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Program #/Poster #: 372.19

Topic: C.03. Parkinson's Disease

Support: NSF 1944247
McCamish Parkinson's Disease Innovation Program at Georgia Institute of Technology and Emory University

Title: Cross-domain literature-based discovery to better elucidate Parkinson's Disease

Authors: *M. WANG, R. MATHEW, G. TANDRA, A. YOONE, C. S. MITCHELL;
Georgia Inst. of Technol., Georgia Inst. of Technol., Atlanta, GA

Abstract: Simultaneous examination of the multifactorial nature of Parkinson's disease is difficult using traditional experimental methods. Yet, ability to assess risk factors or multiple etiological factors simultaneously is pivotal for better understanding of and treatment discovery for Parkinson's Disease (PD). Literature-based discovery (LBD) is a new branch of natural language processing (NLP) that simultaneously integrates text relationships across multiple domains to better examine complex multifactorial pathology. The objective of this study was to use cross-domain LBD of 30+ million PubMed articles to elucidate lesser known associative or causal etiologies of PD. SemNet 2.0, an optimized text mining tool used to query the National Library of Medicine's SemMedDB repository and identify relationships among biomedical concepts, was used to assess PD. Unsupervised learning rank aggregation in SemNet 2.0 ranked the HeteSim relevance score of metapaths that connect PD to more distant domains, like cardiovascular or endocrine diseases. Identified source nodes (or concepts) originated from the following Unified Medical Language System (UMLS) ontology: genes or genomes; amino acids, peptides, proteins; and pharmacological substances. Besides having a strong HeteSim relevance ranking, resultant node inclusion criteria required physiological evidence for relatedness. Node exclusion criteria consisted of rejecting generic nodes or nodes with clear, prominent relationships to PD, like movement disorders. Seven diseases and twenty-two genes or proteins were determined to have significantly increased cross-domain relatedness to PD. Subsequently, a novel "hub" bioinformatics network analysis was adapted for NLP to identify more distant or indirect connections that could identify even lesser-known PD risks, new etiological ties to PD, or new therapeutic targets. Seven diseases or symptoms with high cross-domain HeteSim scores to PD were selected for further simulation. Subsequently, the top 2% non-generic nodes with the highest connectivity (n = 30) were chosen as the hubs for hub network analysis. A unifying theme among the resultant cross-domain nodes was various forms of multi-scalar inflammation, which tie back to PD. Combined, fully ranked results prioritizing specific nodes (genes, proteins, drugs, diseases, etc.) for future research will be provided in full in the presentation.

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Poster

372. Cellular Mechanisms of Parkinson's Disease

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Title: Tet family proteins are dispensable for the structure and function of dopamine neurons in health and Parkinson's disease

Authors: *H.-E. LEE[1], H. KIM[1], B. LEE[1], J. AN[2], M. KO[1], J.-I. KIM[1];
[1]Dept. of Biol. Sci., Ulsan Natl. Inst. of Sci. and Technol., Ulsan, Korea, Republic of; [2]Dept. of Life Sci., Jeonbuk Natl. Univ., Jeonju, Korea, Republic of

Abstract: DNA undergoes demethylation via the oxidation of 5-methylcytosine (5mC), which is mediated by the Ten Eleven Translocation (TET) family of proteins. Notably, 5hmC is highly enriched in the brain than in other tissues, the level of which is dynamically regulated during development, aging, and in brain disorders. In addition, accumulating evidence has recently revealed that 5-hmC and TETs play a significant role in synaptic functions, anxiety, addiction, and cognition in several brain regions. Furthermore, TET enzymes have turned out to be essential for diverse types of neurons in health and brain disorders. In this study, by generating triple knockout (TKO) mice of TET family proteins (TET1, 2, and 3) selectively in dopamine (DA) neurons, we investigated the functional roles of TET proteins in the structure and the function of DA neurons, which are pivotal for voluntary movement, reward-related behaviors, and motivation. Immunohistochemistry analysis of dopaminergic neuronal markers revealed that DA neuron-specific TET1, 2, 3 TKO does not alter cellular structure and survival of DA neurons. Furthermore, whole-cell patch clamp recordings from substantia nigra pars compacta (SNc) DA neurons show that intrinsic properties and synaptic transmission of DA neurons are unchanged by disruption of TET family proteins. Thus, unexpectedly, cell type-specific KO of all three TET proteins did not lead to critical alterations of neuronal structure and function in DA neurons. Moreover, we revisited the pathophysiological importance of TET enzymes in Parkinson’s disease (PD) by utilizing both pharmacological and genetic mouse models of PD. Against our expectation, however, we found that PD pathology induced by two types of PD models is largely unaffected by disruption of TET family proteins, which suggests that the role of TET family proteins in the pathophysiology of PD can be weak. Thus, contrary to the previous reports, TET family enzymes may be dispensable for the structure and function of specific neurons in health and disease.

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Poster

372. Cellular Mechanisms of Parkinson's Disease

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Program #/Poster #: 372.21

Topic: C.03. Parkinson’s Disease

Support: NINDS R01 grant R01NS112390

Title: Synj1 deficiency leads to lysosomal changes in cultured midbrain neurons

Authors: *X. ZHU, S. S. PRAKASH, A. RIZVI, A. CAPUANO, P. PAN;
RWJMS, Rutgers Univ., Rutgers Univ. Behavioral and Systems Neurosci., Piscataway, NJ

Abstract: One of the pathological hallmarks of Parkinson's Disease (PD) is the manifestation of Lewy Body in the brain, which comprises of alpha-synuclein (a-syn) and related non-digestible materials. The accumulation of a-syn enriched protein aggregates is thought to be contributed by dysfunction of the degradation system within the brain. Recently, missense mutations of *SYNJ1* encoding two lipid phosphatase domains are found in families with hereditary early-onset Parkinsonism. Our previous study showed that *Synj1* haploinsufficiency (*Synj1+/-*) leads to PD-like behavioral and pathological changes in the male mice, including the accumulation of the autophagy substrate p62 and an aggregation-prone form of a-syn, pS129 a-syn in the midbrain (MB) and striatum. In this study, we further investigated the underlying mechanisms related to the neuronal degradation pathway using the *Synj1+/-* MB culture as a model. We found that the autophagosome formation and maturation is not affected in the *Synj1+/-* MB neurons. But, the lysosome number is reduced in the soma of *Synj1+/-* neurons, with a similar reduction in lysosomal proteins, such as LAMP1, LAMP2 and LAMP2A, suggesting that the lysosomal capacity may be impaired in *Synj1+/-* neurons. However, we were unable to detect defects in the clearance of either the wild-type or the A53T a-syn by expressing a photo-switchable fluorescent protein-tagged a-syn. The lack of difference is likely associated with the increase of lysosomal acidity and enzymatic activity detected in *Synj1+/-* MB neurons. In conclusion, our study suggests that Synj1 deficiency impacts lysosomal function in a complex manner but does not contribute to a-syn accumulation in MB neuron.

Disclosures: X. Zhu: None. **S.S. Prakash:** None. **A. Rizvi:** None. **A. Capuano:** None. **P. Pan:** None.

Poster

372. Cellular Mechanisms of Parkinson's Disease

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Topic: C.03. Parkinson's Disease

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Title: Increased expression of IL-13 receptor in the substantia nigra of Parkinson's disease patients and A53T alpha-synuclein rat model

Authors: H. PARK, Y. CHUNG, *W.-H. SHIN;
Korea Inst. of Toxicology, Korea Inst. of Toxicology, Daejeon, Korea, Republic of

Abstract: Accumulating evidences have shown that an increased expression of IL-4 and IL-13 is detectable in the brain under neuropathological conditions. Since IL-13 receptor (IL-13R) alpha

1 mRNA is present in the dopamine (DA) neurons of the substantia nigra (SN) and down-regulation of IL-4 and IL-13 leads to neuroprotection or neurotoxicity *in vivo*, we aimed to examine the expression and possible function of IL-13R in the SN of animal models and Parkinson's disease (PD) patients. Using Western blot analysis and double fluorescent immunohistochemistry, we found a significant increase in IL-13R expression on tyrosine hydroxylase (TH)-positive DA neurons in the SN of postmortem PD brains compared to age-matched controls. In parallel, IL-13R expression on DA neurons and IL-13 on microglia are upregulated in the A53T alpha-synuclein rat model. These data suggest that IL-13R may be associated with degeneration or survival of DA neurons in PD pathological conditions. Therefore, further studies will be conducted to determine the death or protection of DA neurons by regulating IL-13R expression and to identify the signaling mechanisms involved.

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Poster

372. Cellular Mechanisms of Parkinson's Disease

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Program #/Poster #: 372.23

Topic: C.03. Parkinson's Disease

Title: Phosphorylation of carboxyl terminus of hsp70-interacting protein (CHIP) modulates its molecular co-chaperone function

Authors: *S. HUI[1], S. ZHANG[1], R. EARNSHAW[1], M. KAPADIA[3], E. FREISEN[2], H. CHAU[3], D. WILLIAMS[2], G. SCHMITT-ULMS[2], L. V. KALIA[3], S. K. KALIA[3];
[1]Lab. Med. and Pathobiology, [2]Univ. of Toronto, Toronto, ON, Canada; [3]Krembil Res. Inst., Univ. Hlth. Network, Toronto, ON, Canada

Abstract: Parkinson's Disease (PD) is the most common neurodegenerative movement disorder and is characterized by the loss of dopaminergic neurons within the substantia nigra pars compacta (SNpc) leading to motor and non-motor impairments. The presence of intraneuronal protein aggregates, known as Lewy bodies, is a key pathological hallmark of PD, and these aggregates are largely comprised of the protein alpha-synuclein (asyn). Asyn is associated with both genetic and sporadic forms of PD, and it is the misfolding and accumulation of asyn as well as the ability of asyn to self-associate and form insoluble, toxic oligomers that are critical factors implicated in the pathogenesis of PD.
C-terminus of HSP70 interacting protein (CHIP) is a molecular co-chaperone and E3 ubiquitin ligase that plays a role in regulating degradation of multiple proteins implicated in PD. We have previously shown that asyn is directly ubiquitinated by CHIP, demonstrating that CHIP is an important regulator of asyn levels within the cell. However, while CHIP may have an important role in the pathogenesis of PD, how posttranslational modifications (PTMs) regulate CHIP function remains poorly understood. Through mass spectrometry, we identified that CHIP is phosphorylated in response to stress, and *in silico* analysis identified potential kinases that

phosphorylate CHIP at this residue. We have identified a novel kinase that can phosphorylate CHIP, leading to alterations of its cochaperone activity, and potential influencing CHIP as a modulator of asyn pathology. Thus, phosphorylation is a potential modulator of CHIP function with implications for CHIP mediated regulation of asyn.

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Poster

372. Cellular Mechanisms of Parkinson's Disease

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Topic: C.03. Parkinson's Disease

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Michael J. Fox Foundation, Invited Priority Biology Program

Title: Investigation of NLRP3-dependent, microglia-derived extracellular vesicle cargo in synucleinopathies using TMT-based mass spectrometry

Authors: *K. E. BIGGS[1], A. N. KETTENBACH[2], M. C. HAVRDA[1];
[1]Mol. and Systems Biol., [2]Biochem. and Cell Biol., Geisel Sch. of Med. at Dartmouth, Lebanon, NH

Abstract: The activation of the NLRP3 inflammasome is widely implicated in the pathogenesis of many neurodegenerative diseases, including Parkinson's disease (PD). The NLRP3 inflammasome is activated in microglia in response to danger signals which include pathologic forms of the PD associated protein, alpha synuclein. Activation of the inflammasome results in the release of proinflammatory cytokines and a distinct subtype of NLRP3 activation-dependent extracellular vesicles (EVs). Inflammasome-dependent EVs are poorly characterized but contain inflammasome-related cargo and aberrant protein aggregates. A growing body of evidence indicates that inflammasome-dependent EVs participate in cell-to-cell signaling and likely facilitate the spread of protein aggregates. We have initiated an effort to define the NLRP3 secretome and identify intracellular mechanisms governing inflammasome-related EV release. We used tandem-mass-tag (TMT) based, quantitative proteomics to deeply identify and quantify the proteome of EVs released from reactive primary wild type and *Nlrp3*$^{-/-}$ mouse microglia . We compare the microglial EV proteome in response to different stressors, including the canonical inflammasome activators LPS and nigericin, as well as PD-associated triggers such as alpha synuclein preformed fibrils (PFFs). We discover a diverse array of differentially packaged EV proteins and validate proteins of interest using western blot. Using functional studies, we determine the role of inflammasome-associated EVs on downstream immune signaling events

and the spread of protein aggregation. Our data include a deep, quantitative coverage of the NLRP3-dependent EV proteome, identify putative intracellular modifiers of inflammasome function, and provide early evidence of a functional role for inflammasome-related EVs in cell-to-cell signaling and the spread of protein aggregation in the context of Parkinson's disease.

Disclosures: K.E. Biggs: None. **A.N. Kettenbach:** None. **M.C. Havrda:** None.

Poster

372. Cellular Mechanisms of Parkinson's Disease

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Program #/Poster #: 372.25

Topic: C.03. Parkinson's Disease

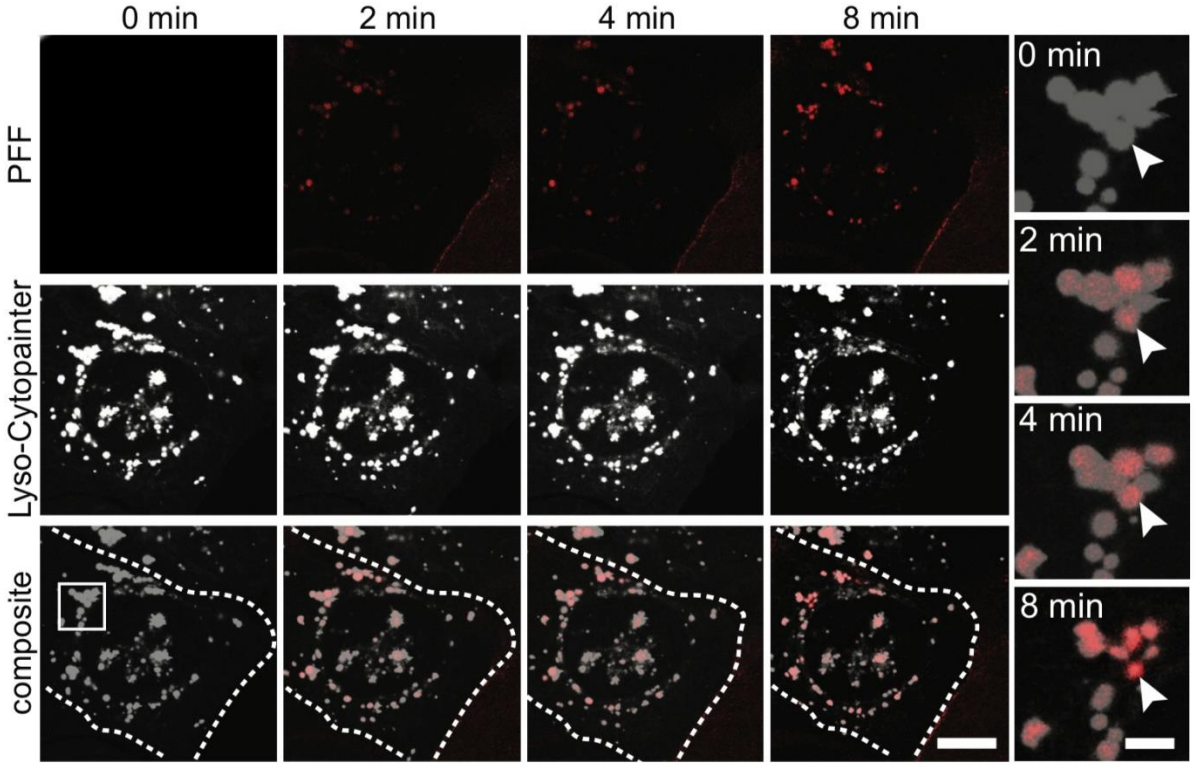
Support: Canada First Research Excellence Fund
Healthy Brain, Healthy Lives
FRQS
Parkinson Canada

Title: Rapid macropinocytic transfer of α-synuclein to lysosomes

Authors: *A. BAYATI[1], E. BANKS[1], C. HAN[1], H. M. MCBRIDE[1], I. SHLAIFER[1], E. D. PELLITERO[1], B. VANDERPERRE[2], W. REINTSCH[1], W. LUO[1], C. E. ZORCA[1], E. A. FON[3], T. DURCAN[1], P. S. MCPHERSON[3,1];
[1]Neurol. and Neurosurg., McGill, Montreal, QC, Canada; [2]UQAM, Montreal, QC, Canada; [3]Montreal Neurolog. Inst., Montreal Neurolog. Inst., Montreal, QC, Canada

Abstract: Background and rationale: Recent evidence suggests that the neurodegeneration characteristic of disorders such as Parkinson's disease (PD) is caused by the cell-to-cell spread of misfolded pathological proteins (1). In the case of PD, the most prominent misfolded protein is alpha-synuclein (α-syn), a component of Lewy Bodies which are aberrant aggregates found to be a hallmark of PD. Injection of α-syn protofibrils into the brains of mice leads to spread of misfolded protein to anatomically connected areas (2). However, the mechanisms by which α-syn fibrils are taken up by cell and form aggregates remain enigmatic (3). Specifically, the form of uptake remains unclear, as do the mechanisms by which fibrils are propagated to other cells. We found that misfolded α-syn is rapidly taken up by lysosomes, through macropinocytosis and utilizes the exosomal system to be transported to neighbouring cells. Methodology: Further verification and the mechanisms underlying uptake of α-syn preformed fibrils (PFF) by lysosomes was tested in several human cell lines using confocal and super-resolution microscopy (STED). Electron microscopy will be used to investigate the contents of lysosomes showing PFF colocalization. Contents of PFF containing lysosomes were observed using electron microscopy, giving us further insight regarding the structure of PFF within lysosomes. Concurrently, we knockdown selective proteins vital to conventional membrane trafficking pathways, including clathrin-mediated endocytosis. We ascertained the role of these proteins in PFF internalization.

Once we addressed these questions in immortalized cell lines, our work extended to human dopaminergic neurons generated from induced pluripotent stem cells (iPSCs).
1) Jucker, M. & Walker, L. C. (2013) Nature 501, 45-51; 2) Luk, K. C. et al. (2012) Science 338, 949-953; 3) Karpowicz, R. J. et al. (2017) Journal of Biological Chemistry 292, 13482-13497.



Disclosures: **A. Bayati:** None. **E. Banks:** None. **C. Han:** None. **H.M. McBride:** None. **I. Shlaifer:** None. **E.D. Pellitero:** None. **B. vanderperre:** None. **W. Reintsch:** None. **W. Luo:** None. **C.E. Zorca:** None. **E.A. Fon:** None. **T. Durcan:** None. **P.S. McPherson:** None.

Poster

372. Cellular Mechanisms of Parkinson's Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 372.26

Topic: C.03. Parkinson’s Disease

Support: NIH/NINDS Grant NS123153-01
TEDCO Grant MSCRFD-5037

Title: Deregulation of mTOR-TFEB axis in GBA1-associated Parkinson’s disease

Authors: F. MUBARIZ[1], A. SAADIN[1], N. LINGENFELTER[1], C. SARKAR[2], M. M. LIPINSKI[2], ***O. AWAD**[1];
[1]Microbiology and Immunol., [2]Anesthesiol., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Mutations in the *GBA1* gene, which encodes the lysosomal enzyme Glucocerebrosidase (GCase), are the single most frequent genetic risk factor for Parkinson's disease (PD). *GBA1* mutations result in reduced GCase enzyme activity and subsequent accumulation of its lipid substrates. Biallelic *GBA1* mutations cause Gaucher's Disease (GD), the most common lysosomal storage disorder. Neurodegenerative changes in *GBA1*-associated PD have been linked to the defective lysosomal clearance of autophagic substrates and aggregate-prone proteins. We previously demonstrated that lysosomal alterations in GD neurons are mediated by dysfunction of the transcription factor EB (TFEB), the master regulator of the autophagy-lysosomal pathway (ALP). In this study, we investigated whether TFEB dysfunction is involved in *GBA1*-associated PD pathogenesis. Using PD patients' induced-pluripotent stem cells (iPSCs), We examined TFEB activity and regulation of the ALP in dopaminergic neuronal cultures generated from PD iPSC lines harboring heterozygous *GBA1* mutations and the CRISPR/Cas9-corrected isogenic controls. Our data showed a significant decrease in TFEB transcriptional activity and downregulation of the CLEAR network gene expression in *GBA1* mutant neurons but not in the isogenic corrected control cells. Further analysis demonstrated that *GBA1*-mediated lipid accumulation results in increased activity of mTORC1, the main upstream negative regulator of TFEB. Increased mTORC1 activity resulted in excess TFEB phosphorylation and decreased nuclear translocation in PD neurons. Treatment with the mTOR inhibitor, Torin1 restored TFEB activity, improved autophagic clearance, and reduced Alpha-synuclein accumulation. Our study demonstrates that *GBA1* mediated deregulation of mTOR-TFEB axis plays a role in PD-associated proteinopathy. It also indicates that pharmacological restoration of TFEB activity could be a promising therapeutic approach in *GBA1*-associated neurodegeneration.

Disclosures: F. Mubariz: None. **A. Saadin:** None. **N. Lingenfelter:** None. **C. Sarkar:** None. **M.M. Lipinski:** None. **O. Awad:** None.

Poster

372. Cellular Mechanisms of Parkinson's Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 372.27

Topic: C.03. Parkinson's Disease

Title: Modelling MSA disease through the generation of brain organoids

Authors: *M. MAGNI, E. FRATTINI;
Ospedale Maggiore, MILANO, Italy

Abstract: Multiple System Atrophy (MSA) is a progressive neurodegenerative disorder due to the loss of various neuronal populations, including the GABAergic and the dopaminergic systems in the striatum and in the midbrain. The resulting degeneration of the nigro-striatal circuit is responsible for a severe neurological involvement characterized by parkinsonism and cerebellar phenotypes. The neuropathological hallmarks of MSA consist of alpha-synuclein aggregations in oligodendrocytes and neurons. No disease-modifying therapy is available for this disorder, resulting in a reduced life-span of affected patients with a median survival of 6-9 years. This is largely due to the lack of reliable human models capable of reproducing key features of the disease and that could be tested for drug-development applications. Here, we aim to generate advanced 3D models of MSA derived from patients by developing brain region-specific organoids with midbrain and striatum identities. These innovative cultures have proven particularly useful to mimick the embryonic organogenesis and for disease-modeling purposes. However, their application to the study of MSA has yet to be explored. We generated long-term culture of midbrain and striatum organoids from one MSA subject and one healthy donor and have extensively characterized these cultures demonstrating their appropriate differentiation. Specifically, organoids showed a correct spatial and temporal progression in the expression of genes involved in the development of both midbrain and striatum, mimicking the embryogenesis of the human brain. These models display several cell populations, particularly enriched in neurons (GABAergic, dopaminergic, glutamatergic) and glia (astrocytes, oligodendrocytes). We are currently exploring the fusion of these two brain region-specific organoids into a nigro-striatal assembloid which may be able to recapitulate the fundamental pathology observed in MSA brains.

Disclosures: **M. Magni:** None. **E. Frattini:** None.

Poster

372. Cellular Mechanisms of Parkinson's Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 372.28

Topic: C.03. Parkinson’s Disease

Support: NIH Grant R01NS115809
American Parkinson's Disease Association (APDA) Grant

Title: Effects of extracellular S100B on voltage-gated channel activity in dopaminergic neurons

Authors: *E. A. BANCROFT, M. DE LA MORA, G. PANDEY, S. M. ZARATE, R. SRINIVASAN;
Dept. of Neurosci. and Exptl. Therapeut., Texas A&M Univ. Sch. of Med., Bryan, TX

Abstract: Abnormal increases in secreted S100B within the midbrain and cerebrospinal fluid is associated with Parkinson's disease (PD) and correlate with symptom severity. Furthermore, overexpression of S100B in mice accelerates the loss of dopaminergic (DA) neurons in the

substantia nigra pars compacta (SNc), suggesting a function for this protein in PD etiology. We found that S100B labeled astrocytic processes completely encompass the somata of tyrosine hydroxylase (TH) expressing DA neurons in the mouse SNc. These findings demonstrate that S100B containing astrocytic processes within the SNc are uniquely positioned to contribute to DA dysfunction in early PD. Consequently, we used primary mouse midbrain cultures to determine if acute exposure to extracellular S100B modulates the activity of TH+ DA neurons. Acute exposure to 50 pM S100B specifically inhibited A-type voltage-gated potassium currents in TH+ , but not TH- neurons. This was accompanied by an approximate 2-fold increase in the frequency of both intrinsic firing, and L-type voltage-gated calcium channel-mediated calcium fluxes only in TH+ neurons. Furthermore, the S100B-mediated increase in calcium fluxes in TH+ neurons was mimicked by exposure to 100 µM 4-aminopyridine (4-AP), an A-type voltage-gated potassium channel inhibitor. Our discovery that extracellular S100B modulates the activity of native DA neurons via inhibiting A-type voltage-gated potassium channels has crucial implications for understanding the pathogenesis of early PD. Additionally, these results reveal novel molecular targets for mitigating DA dysfunction, which will aid in developing neuroprotective therapies for the treatment of PD.

Disclosures: E.A. Bancroft: None. **M. De La Mora:** None. **G. Pandey:** None. **S.M. Zarate:** None. **R. Srinivasan:** None.

Poster

372. Cellular Mechanisms of Parkinson's Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 372.29

Topic: C.03. Parkinson’s Disease

Support: Aufzien Family Center for the Prevention and Treatment of Parkinson’s Disease

Title: Influence of membrane phospholipids scramblase on α-synuclein pathological behavior in Parkinson’s disease

Authors: *S. COHEN ADIV[1], A. ASHKENAZI[2];
[1]Tel Aviv Univ., Ramat Gan, Israel; [2]Tel Aviv Univ., tel aviv, Israel

Abstract: There are two main hallmark of Parkinson’s disease (PD) pathology: aggregation and spreading of the protein α-synuclein (α-syn) known to form a primary structural component of dense protein-rich and lipid-rich cellular neuronal inclusions called Lewy bodies. Emerging research suggests that lipid membranes have a role in α-syn misfolding and aggregation. Thus-as it may tip the balance between physiological and pathological α-syn, we hypothesized that the cellular membrane composition may hold a central role in PD pathology. Here, we established a transgenic mouse line lacking a phospholipid-scrambling protein in order to investigate the effect of lipid scrambling in cell membranes on α-syn aggregation and spreading. We generated P2A skipping system modeling PD pathology in primary cortical neurons knockout (KO) and wild-

type (WT) littermates for the scramblase. The system is based on adeno-associated virus encodes one mRNA: eGFP-P2A-α-syn A53T-HA. Since ribosomes skip on glycine-proline peptide bonds in P2A translation, two proteins are created to enable discrimination between neurons that were transduced and neurons that received mutant α-synA53T protein through transmission. We found a significant elevation in α-synA53Ttransmission rate together with a decrease in phosphorylated S129 α-syn in KO neurons compared to WT, indicating enhanced membrane association. In addition, we observed downregulation in authophagy and a reduction in the insoluble α-syn fraction in KO neurons. Further investigation is required to understand the role of lipid scrambling in cellular mechanisms influencing α-syn pathological behavior. This may also lay the groundwork for future therapeutic approaches for α-synucleinopathies, of a particular relevance are “bottom-up” models where α-syn is propagated from the periphery to the brain.

Disclosures: S. cohen adiv: None. **A. Ashkenazi:** None.

Poster

372. Cellular Mechanisms of Parkinson's Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 372.30

Topic: C.03. Parkinson’s Disease
Michael J. Fox Foundation, Invited Priority Biology Program (MCH)
R01ES033462-0, NIH/NIEHS ViCTER, (MCH)
R01ES024745-06, NIH/NIEHS, (MCH)
F31ES030982, NIH/NIEHS (FA)

Title: Identification and characterization of peripheral immune modulator genes in Parkinson's disease

Authors: *K. C. PAUL[1], O. M. WILKINS[2], K. E. BIGGS[1], F. ANDERSON[1], S. L. LEE[3], F. W. KOLLING[4], D. SHOKEEN[1], M. C. HAVRDA[1];
[1]Dept. of Mol. and Systems Biology, Geisel Sch. of Med., Dartmouth Col., Hanover, NH; [2]Norris Cotton Cancer Ctr., Dartmouth Col., Lebanon, NH; [3]Dept. of Neurology, Geisel Sch. of Med., [4]Genomics Shared Resource, Geisel Sch. of Med., Dartmouth Col., Hanover, NH

Abstract: Neuroinflammation is a hallmark of the pathogenesis of Parkinson’s disease (PD), but peripheral immune involvement during the onset and progression of PD remains poorly characterized. To address this, we systematically evaluated 17,000 peripheral immune cells obtained from three male PD patients and two demographically matched controls using single cell RNA-sequencing to identify a PD-specific immune transcriptional profile. We identified hundreds of differentially expressed genes in PD patients compared to healthy controls indicating PD-specific immune transcriptional profiles in multiple cell lineages. Deeper analysis of monocytes allowed categorization into two clusters: a CD14 classical monocyte cluster and a CD16 non-classical monocyte cluster. In CD14 and CD16 monocytes, 45 genes and 54 genes, respectively, were significantly different between PD patients and controls. Most notably, *BRI3*,

ATP5E, *TPGS1*, *FAM89B*, and *CAPG* are highly upregulated in PD CD16 monocytes. We validated *BRI3*, a gene consistently upregulated in PD CD16 monocytes, as a candidate for further evaluation. In current studies, CRISPR/Cas9 mediated inactivation of *BRI3* modifies the inflammatory and metabolic phenotypes of THP-1 cells, a human monocyte-like cell line. Preliminary studies indicate a role for *BRI3* in peripheral immune modulation in PD. BRI3 function is likely related to non-classical CD16 monocytes, an immune subset involved in patrolling vascular endothelium and responding to distressed tissues.

Disclosures: **K.C. Paul:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); U.S. Prov. Appl. No. 63/318,612. **O.M. Wilkins:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); PCT/US21/52964. **K.E. Biggs:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); U.S. Prov. Appl. No. 63/318,612. **F. Anderson:** A. Employment/Salary (full or part-time):; Merck. **S.L. Lee:** None. **F.W. Kolling:** None. **D. Shokeen:** None. **M.C. Havrda:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Takeda Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); PCT/US21/52964, U.S. Prov. Appl. No. 63/318,612.

Poster

373. Parkinson Disease: Animal Models and Therapeutics

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 373.01

Topic: C.03. Parkinson's Disease

Support: Lundbeckfonden
Danmarks Frie Forskningsfond
Swedish Research Council
Knut och Alice Wallenberg Foundation

Title: Exploring direct glia-to-neuron reprogramming in vivo using a pre-clinical rat model of Parkinson's Disease

Authors: *M. HABEKOST[1], J. GIACOMONI[1], D. HOBAN[2], S. A. GOLDMAN[3], M. PARMAR[4];
[1]Lund Univ., Lund, Sweden; [2]Lund Univ., Lund, Denmark; [3]Dept of Neurol., Univ. Rochester Med. Ctr., Rochester, NY; [4]Wallenberg Neurosci Ctr., Lund 22184, Sweden

Abstract: Parkinson's Disease (PD) is a neurodegenerative disease, which is associated with focal loss of dopaminergic neurons in the substantia nigra. The therapeutic potential of cell

replacement therapy for treating PD has been established through clinical trials transplanting human fetal tissue and stem cell-based therapies are under development. Direct reprogramming of resident glia cells *in situ* into therapeutic neurons represents a strategy based on cell replacement but circumvents the need for cell transplantation. Proof-of-concept experiments has been performed in rodent models using genetic overexpression of lineage-specifying transcription factors or downregulation of key barriers such as *Ptbp1*. *In vitro* studies have demonstrated the ability to convert human glia into dopaminergic neurons, but the question whether human glia have the same capacity to reprogram *in vivo* is unclear. To study this, we have established a human stem cell-based *in vitro* conversion model (Nolbrant et al., 2020) as well as a transplantation-based *in vivo* conversion model. In the rodent model, human glia progenitor cells (hGPCs) derived from embryonic stem cells are transplanted into a pre-clinical rat model of PD. The hGPCs engraft and proliferate, and over time the rodent brain contains large numbers of hGPCs and astrocytes. We have transduced the hGPCs to express CRE recombinase, which allows us to specifically target the human cells *in vivo* upon stereotactic injection of CRE-dependent neural conversion factors. Using the *in vitro* and *in vivo* conversion models, we are currently exploring both transcription factor-mediated conversion and *PTBP1* knockdown-mediated conversion, with the goal to select neural conversion factors that specify the reprogramming of dopaminergic neurons from human glia directly in the brain.

Disclosures: M. Habekost: None. **J. Giacomoni:** None. **D. Hoban:** None. **S.A. Goldman:** None. **M. Parmar:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Owner of Parmar Cells AB - U.S. patent 15/093,927; EP17181588; PCT/EP2018/062261. F. Consulting Fees (e.g., advisory boards); Paid Consultancy, steering group member and commissioned research for Novo Nordisk AS Cell Therapy Research and Development unit SAB/ Paid Consultancy of Arbor Bio. Other; Academic research collaborations with Novo Nordisk AS.

Poster

373. Parkinson Disease: Animal Models and Therapeutics

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 373.02

Topic: C.03. Parkinson's Disease

Support: NIH/NINDS Grant R56 NS109608
NIH/NINDS Grant R01 NS122805-01
ABRC Grant ADHS18-198846

Title: Phase-locking of motor cortex neurons to ketamine-generated slow gamma oscillations and 80-Hz gamma oscillations in parkinsonian rats with L-DOPA-induced dyskinesias

Authors: *A. VISHWANATH[1], M. J. BARTLETT[3], J. L. KWIDZINSKI[1], M. KAMINSKI[2], T. FALK[3], S. L. COWEN[1];

[1]Dept. of Psychology, [2]Dept. of Neurosci., Univ. of Arizona, Tucson, AZ; [3]Dept. of Neurol., Col. of Med., Tucson, AZ

Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disease, and it is accompanied by debilitating motor and cognitive deficits. L-DOPA is the gold-standard treatment for PD, but long-term administration leads to side effects such as L-DOPA-induced dyskinesias (LID) which causes uncontrollable involuntary movements. During LID, a common neurophysiological feature is a narrow-band 80Hz oscillation (fine-tuned gamma) observed in the motor cortex (M1). Our goal is to understand the mechanisms involved in the generation of this oscillation by identifying the neuronal subtypes in M1 and the basal ganglia that are most synchronized to LID-associated 80Hz gamma. We also aim to understand the mechanism by which low-dose ketamine reduces LID. Ketamine, a N-methyl-D-aspartate (NMDA) receptor antagonist, has recently been shown to alleviate LID symptoms in human case studies and preclinical models. Ketamine induces a slower gamma oscillation (~50Hz) in M1 in healthy animals and in an animal model of LID. In LID, ketamine disrupts the 80Hz gamma. It is conceivable that the slow-gamma oscillation generated by ketamine competes with LID-induced 80Hz oscillations and this underlies the alleviation of behavioral LID symptoms. We predict that the 50Hz oscillations and the LID-associated 80Hz oscillations are generated by distinct neural circuits, with interconnected groups of interneurons driving fast 80Hz oscillations and interacting groups of principal cells and interneurons driving slower 50Hz ketamine-induced oscillations. We implanted dual-bundle 16-tetrode hyperdrives bilaterally (AP: 1.5, ML: +/-2.2mm) in 6-hydroxydopamine (6-OHDA) hemi-lesioned PD rats treated daily with L-DOPA (12 mg/kg for ≥10 days) to induce LID. The LID-expressing (n=4) and sham-lesioned (n=2) rats were administered L-DOPA (12 mg/kg, *i.p.*) or saline followed by ketamine (20 mg/kg, *i.p.*). Preliminary data shows that in the lesioned hemisphere of LID rats, ~20% of M1 neurons are phase locked (effect size of magnitude of phase locking: Cohen's D=.84) to the 80Hz oscillations during peak LID and ~27% (D=.54) of M1 neurons are phase locked to the 50Hz oscillations during peak ketamine. Contrary to our hypothesis, there was no difference in the proportion of interneurons and principal cells phase-locking to either oscillation. The lack of differential recruitment of distinct neuronal subtypes might indicate that more complex interactions between principal cells and interneurons are involved in these two oscillations.

Disclosures: A. Vishwanath: None. **M.J. Bartlett:** None. **J.L. Kwidzinski:** None. **M. Kaminski:** None. **T. Falk:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); TF have a pending patent application for the use of ketamine as a novel treatment for levodopa-induced dyskinesia associated with Parkinson's disease, that has been licensed to PharmaTher Inc.. **S.L. Cowen:** None.

Poster

373. Parkinson Disease: Animal Models and Therapeutics

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 373.03

Topic: C.03. Parkinson's Disease

Support: CAPES
CNPq

Title: Effects of pramipexole on depressive-like behaviors and IDO expression in the 6-OHDA animal model of Parkinson's disease

Authors: *D. C. RAMOS[1], D. G. C. SILVA[1], L. C. SOUZA[1], R. K. SCHWARTING[2], R. ANDREATINI[1], M. A. B. F. VITAL[1];
[1]Pharmacol., Federal Univ. of Parana, Curitiba, Brazil; [2]Exptl. and Biol. Psychology, Philipps-University of Marburg, Dept. of Psychology, Marburg, Germany

Abstract: Depression is a common psychiatric disorder present in patients with Parkinson's disease (PD), which etiology is not fully understood. It is hypothesized that the natural chronic neurodegeneration of PD leads to the reduction of dopamine, serotonin and noradrenaline concentrations. Furthermore, other hypothesis suggests that chronic inflammation may be a causative pathological process of depression. This inflammatory process is characterized by the increased levels of pro-inflammatory cytokines, which can be able to reduce serotonin levels through the activation of the indoleamine 2,3-dioxygenase (IDO), enzyme that converts tryptophan into kynurenine metabolites. Pramipexole is a dopamine agonist, approved both as a single therapy and as an adjunct to levodopa for the treatment of PD patients. The aim of this study was to evaluate the antidepressant and IDO expression effects of the pramipexole in the animal model of PD induced by 6-OHDA. Male Wistar rats were subjected to the bilateral infusion of 6-OHDA, or sterile saline, into the substantia nigra compacta. One hour later, lesioned and SHAM animals received pramipexol (PPX) at 1 mg/kg, i.p, or its vehicle, for 28 days. The results demonstrated that the 6-OHDA lesioned rats presented a reduction in sucrose consumption, indicating anhedonia. However, the lesioned group treated with PPX showed a partial reversion of this condition on day 21. Moreover, in the forced swim test, 6-OHDA animals treated with PPX showed a reduction in the immobility time compared to SHAM + vehicle and 6-OHDA + vehicle groups. When submitted to the ultrasonic vocalization test, 6-OHDA + PPX rats emitted a higher number of appetitive 50-kHz calls, in comparison to 6-OHDA + vehicle group. The neurochemical analysis indicated an increase of striatal levels of dopamine and serotonin in 6-OHDA + PPX group, when compared to 6-OHDA + vehicle animals. Moreover, western blot data revealed a reduced IDO expression in the hippocampus in the 6-OHDA + PPX animals in comparison with the 6-OHDA + vehicle group. The prolonged administration of PPX partially reversed the depressive-like behavior induced by 6-OHDA. In addition, PPX presented an anti-inflammatory like-effect, by reducing the IDO expression in the hippocampus.

Disclosures: D.C. Ramos: None. **D.G.C. Silva:** None. **L.C. Souza:** None. **R.K. Schwarting:** None. **R. Andreatini:** None. **M.A.B.F. Vital:** None.

Poster

373. Parkinson Disease: Animal Models and Therapeutics

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 373.04

Topic: C.03. Parkinson's Disease

Support: NIH Grant R21

Title: Deletion of USP30 enchances mitophagy, suppresses synucleinopathy and prevents dopaminergic neuronal loss in a Parkinson's disease mouse model

Authors: *T.-S. FANG[1], S. ELEUTERI[2], D. K. SIMON[1];
[2]Neurol., [1]Beth Israel Deaconess Med. Ctr., Boston, MA

Abstract: Alpha-synuleinopathy is the pathological hallmark of Parkinson's disease in the postmortem brains. Mutations or overexpression of SNCA, which is the gene encoding alpha-synuclein, is associated with early onset familial Parkinson's disease. Recently, accumulating evidence also shown the importance of mitochondria dysfunction in the progression of dopaminergic neurodegeneration both in Parkinson's disease patients and in animal models. USP30 counteracts with parkin's function by deubiquitination of mitochondrial outer membrane proteins and plays a role in maintaining the mitochondrial dynamics through regulating mitochondrial clearance through mitophagy. In this study, we investigated the effects of USP30 knockout on mitophagy, alpha-synucleinopathy and dopaminergic neurodegeneration in an AAV-based alpha-synuclein mouse model. We found the increase of mitophagy puncta in dopaminergic neurons in the USP30 knockout mice comparing with USP30 wildtype mice. The USP30 knockout rescues the behavioral deficits in the AAV-based alpha-synuclein mouse model at 28 weeks post-AAV-SNCA stereotaxic intranigral injection. We also found significantly decreased pathological s129 phospho-aSyn in the dopaminergic neurons of SNpc in USP30 knockout mice comparing with robust development of s129 phospho-aSyn in wildtype mice with AAV-SNCA injection. More interestingly, the decrease of alpha-synucleinopathy is consistent with the findings of preserved TH densitometry, increased levels of dopamine and its metabolites in striatum, and decreased loss of TH-positive neurons in SNpc in the USP30 knockout mice comparing with WT mice after AAV-SNCA injection. Taken together, our study suggests that manipulating USP30 is a potential strategy for treating Parkinson's disease in the future.

Disclosures: T. Fang: None. **S. Eleuteri:** None. **D.K. Simon:** None.

Poster

373. Parkinson Disease: Animal Models and Therapeutics

Location: SDCC Halls B-H

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Program #/Poster #: 373.05

Topic: C.03. Parkinson's Disease

Support: ERC starting grant 805426-FutureTrophicFactors

Title: Effects of a novel neuroprotective compound in a toxin model of Parkinson's disease

Authors: *T. VILJAKAINEN[1], M. H. VOUTILAINEN[2];
[1]Fac. of Pharm., Univ. of Helsinki, Helsinki, Finland; [2]Fac. of Pharm., Helsinki Univ., Helsinki, Finland

Abstract: Background. Parkinson's disease (PD) is a progressive neurodegenerative disease characterized by the degeneration of dopaminergic neurons of the nigrostriatal pathway. Currently, all treatments for PD are symptomatic. Mesencephalic astrocyte-derived neurotrophic factor (MANF) has shown neuroprotective and neurorestorative effects in animal models of Parkinson's disease. MANF does not penetrate the blood-brain barrier (BBB), for this reason, it needs to be delivered directly into the brain. We have developed a MANF variant (vMANF) which can cross the BBB enabling peripheral administration. The aim of this study was to investigate the neuroprotective effect of vMANF after intracranial and peripheral delivery in the 6-hydroxydopamine (6-OHDA) rat model.
Methods. 6-OHDA was unilaterally injected into the striatum of male Wistar rats. Two weeks post-lesioning, rotational behavior was assessed after a subcutaneous amphetamine injection by using automatic rotometry. Rats were divided into treatment groups based on their ipsilateral rotations. Rats received either vMANF or vehicle as intrastriatal injections or chronic subcutaneous infusion (n=8-10). The amphetamine-induced rotation test was repeated every second week. Rats were sacrificed 8 weeks after lesioning by transcardial perfusion. Free-floating coronal striatum and substantia nigra sections were cut and tyrosine hydroxylase (TH) staining was performed.
Results. We saw a reduction in amphetamine-induced ipsilateral turns in the group that received vMANF, which indicates improvement in motor imbalance seen in the unilateral 6-OHDA model. However, this reduction was not statistically significant. vMANF, when injected intrastriatally, protected TH-positive neurons, which was seen in significantly higher number of remaining TH-positive neurons in the substantia nigra compared to the vehicle group ($p=0.0034$, one-way ANOVA with Tukey's post-hoc test). When vMANF was given subcutaneously, a promising tendency in motor behavior, but no protection of TH-positive neurons was observed.
Conclusions. We show significant neuroprotection against 6-OHDA-induced toxicity, and a positive trend in the reduction of amphetamine-induced rotations. With further compound optimization, we hope to achieve neuroprotection after subcutaneous delivery of vMANF, which would make it a potential disease modifying treatment for PD where currently only symptomatic treatments exist.

Disclosures: T. Viljakainen: None. **M.H. Voutilainen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent holder.

Poster

373. Parkinson Disease: Animal Models and Therapeutics

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 373.06

Topic: C.03. Parkinson's Disease

Support: Seelos Therap. Inc

Title: The role of alpha-synuclein dysregulation in synucleinopathies: establishing new therapoetic target for for next-generation drug discovery

Authors: *B. KANTOR[1], J. RITTINER[2], O. CHIBA-FALEK[3];
[1]Duke Univ., Duke Univ., Durham, NC; [2]Neurobio., Duke, Durham, NC; [3]Duke Med., Duke Med., Durham, NC

Abstract: Elevated levels of *SNCA* are causative in the pathogenesis of Parkinson's Disease (PD) and other synucleinopathies, while, normal physiological levels of *SNCA* are crucial to maintain neuronal function. A so-far unmet need is the development of new therapeutic strategies targeting the regulatory mechanisms of *SNCA* expression to fine-tune *SNCA* levels, versus previous approaches that targeted directly the mRNA or the protein product resulting in robust reduction of *SNCA* levels associated with neurotoxicity. We developed a novel strategy targeting the transcription regulation of *SNCA*, based on targeted epigenome editing. Specifically, we established a system for targeted DNA-methylation at *SNCA*-intron 1 that comprises of an all-in-one lentiviral vector, for the delivery of CRISPR/dCas9 fused with the catalytic domain of DNA-methyltransferase3A (DNMT3A). To facilitate the drug discovery pipeline, we first applied the gRNA-dCas9-DMNT3A system into human induced pluripotent stem cells (hiPSC)-derived 'aged' dopaminergic neurons from a PD-patient with the *SNCA* triplication. The experiment resulted in fine-tuned downregulation of *SNCA*-mRNA and protein levels mediated by targeted DNA-methylation at intron 1. Furthermore, the reduction in *SNCA* levels by the gRNA-dCas9-DMNT3A system rescued disease-related cellular-phenotypes characteristics of the *SNCA*-triplication/hiPSC-derived dopaminergic neurons, *e.g.* mitochondrial ROS-production and cellular viability, and nuclear aging signatures. This novel CRISPR/dCas9 technology offers the unprecedented tool to modify a particular epigenetic mark resulting in effective fine-tuned reduction of *SNCA* expression levels sufficient for reversing PD-associated phenotypic perturbations. Collectively these proof-of-concept experiments provide a foundation for advancing the novel epigenetic editing-based system further towards PD therapeutic strategy for application in a clinical setting.

Disclosures: B. Kantor: None. **J. Rittiner:** None. **O. Chiba-Falek:** None.

Poster

373. Parkinson Disease: Animal Models and Therapeutics

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 373.07

Topic: C.03. Parkinson's Disease

Support: NINDS/NIH grant -R01 NS108968-01

Title: Minimally Invasive Nasal Depot (MIND) Approach for CNS Delivery of Oligonucleotides in Parkinson's Disease

Authors: *A. DSOUZA[1,2], P. KULKARNI[3], C. F. FERRIS[3], B. BLEIER[1], M. AMIJI[2]; [1]Massachusetts Eye and Ear Infirmary, Harvard Med. Sch., Boston, MA; [2]Dept. of Pharmaceut. Sci., Sch. of Pharmacy, Northeastern University, Boston, Boston, MA; [3]Ctr. for Translational NeuroImaging, Northeastern Univ., Boston, MA

Abstract: Delivery of disease-modifying biological therapeutics to the brain for chronic age-related neurodegenerative diseases is very challenging primarily due to the presence of the blood-brain barrier (BBB). Our group has developed the Minimally Invasive Nasal Depot (MIND) approach for CNS delivery that overcomes the limitations of BBB penetration and traditional nasal administration, such as poor delivery efficiency and variable dose uniformity. The MIND approach enables reproducible therapeutic exposure directly to the olfactory epithelium and has been evaluated in both naïve Long Evans and a combination traumatic brain injury (TBI) and PINK-1 knock-out Parkinson's disease rat models.
In the current study, we have utilized the MIND approach for sustained delivery of brain-derived neurotrophic factor (BDNF) upregulating oligonucleotide (antagoNAT) using an osmotic core-shell implant. Initially, the development of BDNF deficiency and PD markers were screened in Long Evans rats subjected to TBI of different hit velocities (7.4,9.3 and 11.2m/s) using magnetic resonance imaging (MRI) and biochemicals markers by ELISA and histology, including BDNF, alpha-synuclein, tyrosine hydroxylase using ELISA. Further, the model development was validated in the TBI+PINK-1 rat model of PD again using ELISA, MRI, and behavioral analysis. Long Evans rats subjected to three mild TBI hits showed a significant decrease in BDNF levels from an average of 207 pg/mg protein to 110 pg/mg protein in the hippocampus on day 12 after the first hit compared to sham. Moreover, a ~1.9-fold decrease in tyrosine hydroxylase in the frontal cortex and a ~2.3-fold increase in alpha-synuclein in the striatum were also observed. In the TBI+PINK-1 KO animals, MRI analyses showed a reduced apparent diffusion coefficient (ADC), which is an indicator for vascular edema and damage to white fibers, at 12^{th} and 40^{th} days post TBI in the BDNF-antagoNAT treated group as compared to the control. BDNF antagoNAT treated TBI+PINK KO rats also showed a higher rotarod performance compared to the sham-injured group.

Disclosures: **A. DSouza:** A. Employment/Salary (full or part-time):; Massachusetts Eye and Ear, Harvard Medical School, Boston, Department of Pharmaceutical Sciences, School of Pharmacy, Northeastern University, Boston. **B. Bleier:** A. Employment/Salary (full or part-time):; Massachusetts Eye and Ear, Harvard Medical School, Boston, MA. **M. Amiji:** A. Employment/Salary (full or part-time):; School of Pharmacy, Northeastern University, Boston.

Poster

373. Parkinson Disease: Animal Models and Therapeutics

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 373.08

Topic: C.03. Parkinson's Disease

Support: Pfizer Scientific Institute (México) grant under project number WI246311

Title: Crispr/sgrna directed synergistic activation mediator (SAM) as a therapeutic tool for Parkinson´s disease.

Authors: *L. NARVÁEZ PÉREZ[1], F. PAZ BERMÚDEZ[1], J. AVALOS FUENTES[1], A. CAMPOS-ROMO[2], G. B. FLORAN[1], J. V. SEGOVIA-VILA[1];
[1]DEPARTMENT OF PHYSIOLOGY, BIOPHYSICS AND NEUROSCIENCES, CINVESTAV, MEXICO CITY, Mexico; [2]Unidad Periférica de Neurociencias, Facultad de Medicina., UNAM, MEXICO CITY, Mexico

Abstract: Introduction: Parkinson`s disease (PD) is the second most prevalent neurodegenerative disease. The death of dopaminergic neurons of the substantia nigra, causes a dysfunction of the basal ganglia circuits, which affects motor control. Different gene therapy strategies have been used as experimental treatments for PD. CRISPR-Cas is a successful gene-editing tool that can modify gene transcription and can be used to activate gene expression in specific brain regions or cells. As a proof of concept for the treatment of PD, we used SAM, a CRISPR gene activation system, to produce Dopamine (DA) on the striatum of 6-OHDA-lesioned rats. Astrocytes were modified to synthesize tyrosine hydroxylase (TH), the only enzyme these cells lack to produce DA, and tested in a murine PD model. **Methodology:** Different candidate sgRNAs within the rat *th* promotor region were tested, and the expression of the endogenous *th* gene and the presence of the TH protein expression were determined in the C6 glial cell line by RT-PCR, western blot, and immunofluorescence assays. Employing pseudo-lentivirus, the SAM complex, and the sgRNA were transferred into primary cultures of rat astrocytes, gene expression, and TH protein synthesis were determined; furthermore, DA release into the culture medium was determined. Then, the modified astrocytes (producing DA) were injected into the striatum of 6-OHDA hemiparkinsonian rats, Motor behavior was evaluated using a cylinder test, the amphetamine-induced rotation test, and the inclined beam test. **Results:** The expression of TH in glial cells produce a release of dopamine to the culture medium (11.78±6.358 nmol/µg on TH-astrocytes). Moreover, we also observed motor behavior improvement in the 6-OHDA lesioned rats that received TH-astrocytes compared to lesioned rats receiving astrocytes that did not produce DA. On the other hand, there were no differences between the group implanted with TH-producing astrocytes compared to the Sham (non-lesioned group) on the motor behavior tests. **Discussion:** The SAM co-transcriptional complex induced the expression of the endogenous *th* gene of astrocytes, and these cells were then capable of expressing the TH protein and synthesizing DA. Moreover, when TH-producing astrocytes were implanted in a PD rat model, they were able to produce DA and elicit motor recovery. Thus, the endogenous activation of the *th gene* using the SAM system can make motor improvement in the 6-OHDA rat model, demonstrating the benefits of the therapeutic use of CRISPR on a neurogenerative disease.

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Poster

373. Parkinson Disease: Animal Models and Therapeutics

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 373.09

Topic: C.03. Parkinson's Disease

Support: 1ZIAES103310

Title: Silencing Dopamine Neurons During Sleep Slows the Progression of Parkinson's Disease in Mice

Authors: *C. MENG[1], J. B. COOK[2], L. HUO[3], S. SAHA[3], B. HOU[3], A. YEH[3], N. P. KOBZAR[4], A. PAPANERI[3], G. CUI[5];
[1]Natl. Inst. of Envrn. Hlth. Sci., Natl., Rtp, NC; [2]Dignify Therapeut., Research Triangle Park, NC; [3]Natl. Inst. of Envrn. Hlth. Sci., RTP, NC; [4]Natl. Inst. of Envrn. Hlth. Sci., North Port, FL; [5]Neurobio. Lab., NIH/NIEHS, Rtp, NC

Abstract: Parkinson's disease (PD) is a highly debilitating neurodegenerative disorder featuring progressive loss of dopamine (DA) neurons in the substantia nigra pars compacta (SNc). Currently, there are no treatments that can stop or slow the progression of PD. Although the etiology of PD remains elusive, it has been suggested that SNc DA neurons are more vulnerable than other neurons during aging because of their high bioenergetic demand. Here we test whether reducing the energy expenditure in DA neurons by inhibiting their activity during sleep, when DA is not needed for movement control, can protect DA neurons and slow the progression of PD. We show that silencing DA neurons for 6-8 hours per day, either chemogenetically or pharmacologically, improves motor and cognitive functions and reduces the loss of SNc DA neurons in MitoPark mice, a genetic PD model with age-dependent loss of DA neurons. We further confirm the protective effect of daily DA neuron silencing in 6-OHDA lesioned parkinsonian mice. Furthermore, silencing DA neurons during sleep does not cause motor or cognitive side effects, nor does it impair memory consolidation. These findings suggest that sleep-time DA neuron silencing is a promising therapeutic strategy that may slow the progression of PD in humans.

Disclosures: C. Meng: None. **J.B. Cook:** None. **L. Huo:** None. **S. Saha:** None. **B. Hou:** None. **A. Yeh:** None. **N.P. Kobzar:** None. **A. Papaneri:** None. **G. Cui:** None.

Poster

373. Parkinson Disease: Animal Models and Therapeutics

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 373.10

Topic: C.03. Parkinson's Disease

Support: Mitacs IT23474

Title: Intracerebral administration of Botulinum toxin A (BoNT-A) ad an alternative for the improvement of gait disturbances in a neurotoxic model of Parkinson's disease

Authors: *A. PARRA[1], R. RAJAKUMAR[2], M. JOG[1];
[1]Clin. Neurolog. Sci., Western Univ., London, ON, Canada; [2]Anat. & Cell Biol., Univ. Western Ontario, London, ON, Canada

Abstract: Parkinson's disease (PD) is a neurodegenerative disorder characterized by the loss ofdopaminergic neurons in the nigrostriatal pathway and the consequent changes in theneuronal activity of subcortical areas, such as overactivation of the subthalamic nucleus(STN) that is associated with the appearance of various motor symptoms (Kuhn et al., 2006).For this reason, the modulation of the electrical activity of the STN has been proposed as apossible therapeutic target. Previously collected evidence suggests that the administration ofbotulinum toxin type A (BoNT-A) in the central nervous system could modulateglutamatergic activity, inhibiting glutamate release (McMahon et al. 1992). In this study, weevaluated whether administration of BoNT-A to the entopeduncular nucleus (EPN), which issuggested to inhibit glutamate release from afferents such as the STN, leads to relief of motorsymptoms in a neurotoxic model of PD. For this, male Sprague Dawley rats (n= 12, 250-300g) were unilaterally injected into the medial forebrain bundle with 6-OHDA or saline(control), and 1 month later a subgroup of 6-OHDA and control rats were injected with 1 ngof BoNT-A in the EPN. Using behavioral tests, we have demonstrated that the administrationof 1 ng of BoNT-A completely abolishes apomorphine-induced rotational behavior(apomorphine test) and improves speed, body speed variation, cadence, and gait pattern(Catwalk test) for up to 1 month after treatment. These results suggest that BoNT-A couldexert neuromodulatory effects on the activity of the basal ganglia, which results in animprovement in motor parameters such as gait.**References.** McMahon et al., 1992. “Tetanus Toxin and Botulinum Toxins Type A and BInhibit Glutamate, γ- Aminobutyric Acid, Aspartate, and Met-Enkephalin Release fromSynaptosomes: Clues to the Locus of Action.” Journal of Biological Chemistry. Kühn et al.,2006. “Reduction in Subthalamic 8-35 Hz Oscillatory Activity Correlates with ClinicalImprovement in Parkinson’s Disease.” European Journal of Neuroscience.

Disclosures: A. Parra: None. **R. Rajakumar:** None. **M. Jog:** None.

Poster

373. Parkinson Disease: Animal Models and Therapeutics

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 373.11

Topic: C.03. Parkinson’s Disease

Title: Investigating the pharmacological contributions of the multimodal serotonergic drug Vortioxetine on L-DOPA induced dyskinesia in a hemiparkinsonian rat model

Authors: *C. BUDROW[1], K. ELDER[2], A. CENTNER[3], N. LIPARI[6], E. WHEELIS[7], S. NEZARIA[2], M. COYLE[4], F. MANFREDSSON[8], C. R. BISHOP[5];
[1]Binghamton Univ., SUNY - Binghamton Univ. Behavioral Neurosci., Apalachin, NY; [2]Binghamton Univ., Binghamton, NY; [3]Binghamton Univ., Binghamton Univ., Pennellville, NY; [4]Michael Coyle, [5]Binghamton Univ., Binghamton Univ., Binghamton, NY; [6]State Univ. of New York, Binghamton, State Univ. of New York, Binghamton, Binghamton, NY; [7]State Univ. of New York, Binghamton Integrative Neurosci., Binghamton, NY; [8]561789949, Barrow Neurolog. Inst., Phoenix, AZ

Abstract: Parkinson's Disease (PD) is a progressive, neurodegenerative disorder characterized by both non-motor and motor symptoms, which arise in part as a result of dopamine (DA) neuronal loss within the substantia nigra pars compacta (SNc). While L-DOPA is widely accepted as a gold-standard DA replacement therapy, chronic use often results in the development of levodopa-induced dyskinesia (LID), characterized by choreaic and dystonic movements. Underlying mechanisms of LID and multifaceted evidence supports neuroplasticity within the serotonin (5-HT) system may drive LID onset, persistence and severity. This mechanism has been supported through prior studies of 5-HT compounds that target the 5-HT_{1A} receptor and the 5-HT transporter (SERT). In prior work, Vortioxetine, a multimodal 5-HT compound, demonstrated clear anti-dyskinetic effects. However, the underlying pharmacology of Vortioxetine's action have yet to be delineated. Thus, the current experiment employed 5-HT_{1A} or 5-HT_{1B} antagonists in conjunction with L-DOPA and Vortioxetine, after which LID and motor performance were assayed. To do so, Sprague-Dawley rats (N = 10, 6 female, 4 male) were rendered hemiparkinsonian via 6-OHDA left medial forebrain bundle lesioning. Following post-operative recovery, rats underwent baseline forepaw adjusting steps (FAS) testing to verify lesion severity, followed by 14 days of chronic L-DOPA administration in order to induce LID, during which rats were assessed for abnormal involuntary movements (AIMs) on days 1, 7 and 14. Thereafter, rats underwent a within-subjects, counterbalanced treatment paradigm, where all rats received both L-DOPA (6 mg/kg) and Vortioxetine (0, 3 and 10 mg/kg), as well as 5-HT_{1A} antagonist WAY-100635 (0, 0.5 mg/kg) or 5-HT_{1B} antagonist SB-224289 (0, 2.5 mg/kg). On test days, rats underwent AIMs and FAS assessments. Results revealed that Vortioxetine reduced LID in a dose-dependent manner. It was also found that blocking the 5-HT_{1A} and 5-HT_{1B} receptors differentially reversed Vortioxetine's effects, suggesting its high 5-HT_{1A} and moderate 5-HT_{1B} agonistic affinity, supporting this pharmacological profile in concert with SERT inhibition as promising for treating LID while preserving L-DOPA efficacy.

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Poster

373. Parkinson Disease: Animal Models and Therapeutics

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Program #/Poster #: 373.12

Topic: C.03. Parkinson's Disease

Support: NIH RO1-EB003268
Canadian Institutes of Health Research (FDN 154272)
Temerty Chair in Focused Ultrasound Research at Sunnybrook Health Sciences Centre

Title: Delivery of Mesenchymal Stem Cells using MR-Guided Focused Ultrasound Exerts Therapeutic Effects on Rodent Parkinsonian Model.

Authors: *S.-K. WU[1], C.-L. TSAI[3], A. MIR[4], K. HYNYNEN[2];
[2]Med. Biophysics / Physical Sci., [1]Sunnybrook Hlth. Sci. Ctr., Toronto, ON, Canada; [3]Dept. of Neurology, Tri-Service Gen. Hospital, Natl. Def. Med. Ctr., Taipei, Taiwan; [4]Sunnybrook Res. Inst., Toronto, ON, Canada

Abstract: Delivery of Mesenchymal Stem Cells using MR-Guided Focused Ultrasound Exerts Therapeutic Effects on Rodent Parkinsonian Model. Sheng-Kai Wu [a, b, *], Chia-Lin Tsai [a, c], Aisha Mir [a], and Kullervo Hynynen [a, b, d]
[a] Physical Sciences Platform, Sunnybrook Research Institute, Toronto, ON, Canada [b] Department of Medical Biophysics, University of Toronto, Toronto, ON, Canada [c] Department of Neurology, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan[d] Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, ON, Canada

AbstractTitle: Delivery of Mesenchymal Stem Cells using MR-Guided Focused Ultrasound Exerts Therapeutic Effects on Rodent Parkinsonian Model

Objectives: To investigate the effects of MR-guided focused ultrasound (MRgFUS) delivery of human mesenchymal stem cells (MSCs) on the 6-HODA induced Parkinson's disease rat model.Methods:6-OHDA was stereotaxically injected into the right substantia nigra (SN) two weeks before the treatment. Baseline and endpoint behaviour tests were performed, including cylinder test and apomorphine-induced rotations. MRgFUS-induced BBB permeability modulation was conducted using an acoustic controller with the targets at the striatum (ST) and SN. Human MSCs were injected immediately before sonication. Immunohistochemical stains were used to identify the stem cells. Tyrosine hydroxylase (TH) expression was quantified in the ST and SN.Results: Here we show that we can deliver human mesenchymal stem cells into Parkinsonian rats by MRgFUS-induced BBB modulation using the acoustic controller. The CD90+ and CD105+ labelled cells were identified in the sonicated brain regions, indicating the feasibility of stem cell delivery via MRgFUS. MSCs+FUS treatment significantly improves the behaviour outcomes compared with control, FUS alone, and MSCs alone groups. In the quantification analysis of the TH stain, a significant reservation of dopamine neurons can be seen in the MSCs+FUS group (ST: 33.22±6.44%, p=0.034; SN: 40.5±3.33%, p=0.0005 to the contralateral side) as compared with MSCs group (ST: 28.24±2.36%; SN: 32.24±5.2%).Conclusions: Mesenchymal stem cell therapy may be a viable option in treating neurodegenerative diseases such as Parkinson's disease. Transcranial MRgFUS serves as a better method for targeted and minimally-invasive stem cell homing. Further investigations on the optimal conditions, such as an alternate injection route, are necessary.
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Poster

373. Parkinson Disease: Animal Models and Therapeutics

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Topic: C.03. Parkinson's Disease

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MOST-108-2314-B-016-058-MY2
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TSGH-E-110213
TSGH-D109-100

Title: Tetrabenazine Mitigates Aberrant Release and Clearance of Dopamine in the Nigrostriatal System, and Alleviates L-DOPA-Induced Dyskinesia in a Mouse Model of Parkinson's Disease

Authors: *K.-Y. TSENG[1], Y.-H. CHEN[2], B. J. HOFFER[3], L. OLSON[4];
[1]Inst. of Biotechnology, Helsinki Univ., Taipei, Taiwan; [2]Neurolog. Surgery, Natl. Def. Med. Ctr., Taipei, Taiwan; [3]Scientist Emeritus, NIDA/NIH, Lyndhurst, OH; [4]Dept. of Neurosci., Karolinska Inst., Solna, Sweden

Abstract: BACKGROUND: L-DOPA-induced dyskinesia (LID), occurring with aberrant processing of exogenous L-DOPA in the dopamine-denervated striatum, is a main complication of levodopa treatment in Parkinson's disease. **OBJECTIVE:** To characterize the effects of the vesicular antagonist tetrabenazine (TBZ) on L-DOPA-induced behavior, neurochemical signals, and underlying protein expressions in an animal model of Parkinson's disease. **METHODS:** 20-week-old MitoPark mice were co-treated or separately administered TBZ and L-DOPA for 14 days. Abnormal involuntary movements (AIMs) and locomotor activity were analyzed. To explore dopamine (DA) transmission, fast scan cyclic voltammetry was used to assess presynaptic DA dynamics in striatal slices following treatments. PET imaging with 4-[18F]-PE2I, ADAM and immunoblotting assays were used to detect receptor protein changes in the DA-denervated striatum. Finally, nigrostriatal tissues were collected for HPLC measures of DA, serotonin and their metabolites. **RESULTS:** A single injection of TBZ given in the interval between the two L-DOPA/Carbidopa treatments significantly attenuated L-DOPA-induced AIMs expression and locomotor hyperactivity. TBZ was shown to reduce tonic and phasic release of DA following L-DOPA treatment in DA-denervated striatal tissue. In the DA-depleted striatum, TBZ decreased the expression of L-DOPA-enhanced D1 receptors and the serotonin reuptake transporter. Neurochemical analysis indicated that TBZ attenuated L-DOPA-induced surges of DA levels by promoting DA turnover in the nigrostriatal system. **CONCLUSIONS:** Our findings demonstrate that TBZ diminishes abnormal striatal DA transmission, which involves the

ability of TBZ to modulate the presymptomatic dynamics of DA, and then mitigate aberrant release of exogenous L-DOPA from nerve terminals. The results support the potential of repositioning TBZ to counteract LID development.

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Poster

373. Parkinson Disease: Animal Models and Therapeutics

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Topic: C.03. Parkinson's Disease

Support: DOD Award W81XWH-19-1-0776

Title: Contralateral Non-degenerative Dopaminergic Phenotype Changes Associated with Exercise in the Unilateral 6-Hydroxydopamine Model of Parkinson's Disease

Authors: *H. SKELTON[1,2], A. KOTLURE[1], A. FERNANDEZ[3], K. BERGLUND[3], C.-A. N. GUTEKUNST[5], R. E. GROSS[4];
[1]Emory Univ., Atlanta, GA; [2]Biomed. Engin., Georgia Inst. of Technol., Atlanta, GA; [3]Neurosurg., [4]Dept Neurosurg., Emory Univ. Sch. of Med., Atlanta, GA; [5]Neurosurg., Sch. of Med., Atlanta, GA

Abstract: Exercise is associated with reduced Parkinson's Disease (PD) risk and slower progression, suggesting a neuroprotective effect. This has prompted studies of exercise in animal models of PD with mixed results- sometimes attributed to the time course of intervention relative to the PD model or the details of the exercise paradigm. We developed a forced running wheel platform that allowed for rapid initiation of exercise after 6-hydroxydopamine lesioning. Mice (C57Bl/6J, male, 8 weeks) were started on daily forced exercise one day after unilateral, intra-striatal lesioning, with sedentary animals in a stationary wheel. Exercise was continued for 4-weeks until tissue collection. Neuroprotection was quantified using stereologic counts of tyrosine hydroxlase immunoreactive (TH+) cells in the substantia nigra pars compacta (SNC). The sample size (n=5) determined *a priori* to provide sufficient power was not reached, due to mortality amongst sedentary controls. When the resulting cell counts were quantified according to the proportion of cells on the lesioned side relative to the unlesioned of each animal, exercise was associated with a small, inconsistent improvement compared to sedentary controls (mean +10%, Cohen's D 0.858). There was a much stronger trend towards increased cell number on the lesioned side of exercised animals (mean +286 TH+ cells, Cohen's D 1.66). In subsequent experiments using alternative exercise paradigms, we have observed significant dissociation between the number of neurons and the number of TH+ neurons on the unlesioned side of TH+ animals, without any evidence of a bilateral lesion. These preliminary results suggest that exercise can induce changes in dopaminergic phenotype prevalence among a stable reserve pool of potentially dopaminergic neurons, which complicates the assessment of neuroprotection.

Notably, the unlesioned side may not be an appropriate control in a unilateral model of PD where both sides are affected by exercise, as any neuroprotection on the lesioned side may be obfuscated by changes on either side not directly related to suppression of neurodegeneration. Future work will attempt to more rigorously distinguish phenotype switching from neurodegeneration, in order to determine the contribution of each to the effects of exercise.

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Poster

373. Parkinson Disease: Animal Models and Therapeutics

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Topic: C.03. Parkinson's Disease

Support: Micheal J Fox Foundation Grant
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Fonds de recherche du Québec - Santé

Title: Virus-mediated single-chain antibody treatment against alpha-synuclein to block Parkinson's disease progression

Authors: *A.-M. CASTONGUAY[1], B. MORIN[1], T. DURCAN[2], C. GRAVEL[1], M. LÉVESQUE[1];
[1]CERVO Brain Res. Ctr., Univ. Laval, Québec, QC, Canada; [2]McGill Univ., Montréal, QC, Canada

Abstract: Parkinson's disease (PD) in mainly characterized by the degeneration of dopaminergic neurons from the substantia nigra. Degenerating neurons present intracytoplasmic inclusions called Lewy bodies. These insoluble inclusions are mainly composed of hyperphosphorylated and misfolded alpha-synuclein (aSyn). Evidence in various animal and cell models suggest that pathological aSyn can be transmitted from cell-to-cell in a prion-like manner. Blocking either the aggregation process or the transmission of the pathological protein across the brain could therefore be effective therapeutic strategies. We aim to use single-chain antibodies against aSyn to stop the propagation of the pathology in a mouse model of PD. We generated mini-antibodies in the form of secreted single-chain variable fragments (scFv) or intracellular antigen-binding fragments (Fab) that bind to aSyn. We also generated a control antibody that targets GFP. We encoded the single-chain antibodies in an adeno-associated viral vector (AAV) of a serotype that can cross the blood-brain barrier when injected intravenously. After intravenous injection, we verified the expression of the antibodies in the brain with immunohistochemistry for the tag expressed by the antibodies. We validated the specificity of our antibodies using dot blots and immunoprecipitation and revealed that one of our antibodies is specific for phosphorylated aSyn, and one is specific for total aSyn. We also verified the efficiency of our intracellular Fabs to bind

and degrade aSyn by co-transfecting them with aSyn in HEK cells and by measuring the aSyn protein level after 72h. Our Fabs significantly reduced aSyn protein level compared to the control antibody. We are currently testing our viral encoded mini antibodies *in vivo*. We injected pre-formed fibrils (PFF) of aSyn in the dorsal striatum of transgenic mice overexpressing A53T-aSyn (M83) and 7 days later, we administered intravenously an AAV encoding the scFvs against aSyn. The experimental groups were composed of an equal number of males and females aged 2 to 3 months old at the time of injection. Control groups were injected with PBS instead of PFF. We closely monitored the motor impairments arising after the injection of PFF. Preliminary observations suggest a protective effect of our scFvs against motor impairments. We will sacrifice the mice 4.5 months post-PFF-injection for histopathological analysis. In conclusion, we generated single-chain antibodies targeting phosphorylated or total aSyn intra- and extracellularly and found that they could engage their target. These antibodies show great potential in reducing the pathology *in vitro* but also *in vivo*.

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Poster

373. Parkinson Disease: Animal Models and Therapeutics

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Topic: C.03. Parkinson's Disease

Support: W81XWH-19-0771
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Title: Treadmill exercise and the alpha-synuclein preformed fibril rat model of Parkinson's disease

Authors: E. HAMAD[1], J. PATTERSON[2], C. J. KEMP[2], J. LEPP[1], *S. SCOTT[1], J. HOLDEN[3], A. DAVIS[1], C. SZAROWICZ[2], J. W. LIPTON[2], M. KUBIK[2], N. KUHN[2], A. STOLL[2], K. C. LUK[4], C. E. SORTWELL[2], S. M. FLEMING[1];
[1]Dept. of Pharmaceut. Sci., Northeast Ohio Med. Univ., Rootstown, OH; [2]Dept. of Translational Neurosci., Michigan State Univ., Grand Rapids, MI; [3]Dept. of Psychology, Univ. of Cincinnati, Cincinnati, OH; [4]Dept of Pathology and Lab. Med., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Exercise is recommended for Parkinson's disease (PD) patients to promote and preserve mobility. Clinical studies indicate benefits of exercise on gait and balance, however, whether it is protective against PD pathology and neurodegeneration remains unclear. Animal toxin models of PD show mixed results regarding exercise's influence on nigrostriatal degeneration and there are limited studies on the effect of exercise on alpha-synuclein (a-Syn) pathology. Therefore, in present study, treadmill exercise was examined in the a-Syn preformed fibril (PFF) rat model of PD. Fischer 344 rats received unilateral intrastriatal injections of saline

or mouse a-syn PFFs. One week after injection, treadmill training was initiated. Animals were brought up to a maximum speed of 10 meters/minute for 20 minutes, five days/week for seven weeks. Non-exercising rats remained in the homecage. Sensorimotor function, emotional reactivity, olfaction, and cognitive function were measured during the last 10 days of treadmill training. Brains were examined for phosphorylated a-Syn accumulation (p-Syn), tyrosine hydroxylase immunoreactivity, reactive microglia, striatal dopamine, and hippocampal brain-derived neurotrophic factor (BDNF). The results revealed that both saline and a-Syn PFF exercise rats showed decreased bodyweight, increased activity in the cylinder, and increased object investigation compared to homecage non-exercise rats. In movement initiation, a significant deficit in time to initiate a step was detected in a-Syn PFF homecage rats; however, there was no deficit in a-Syn PFF exercise rats. At two months post injection in a-Syn PFF rats, exercise did not affect p-Syn accumulation, nigral tyrosine hydroxylase immunoreactivity, reactive microglia, or striatal dopamine levels. Hippocampal BDNF was significantly decreased in both exercised and non-exercised a-Syn PFF rats. The data suggests moderate short-term treadmill exercise impacts aspects of behavioral function but not early PD-related pathology.

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Poster

373. Parkinson Disease: Animal Models and Therapeutics

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 373.17

Topic: C.03. Parkinson’s Disease

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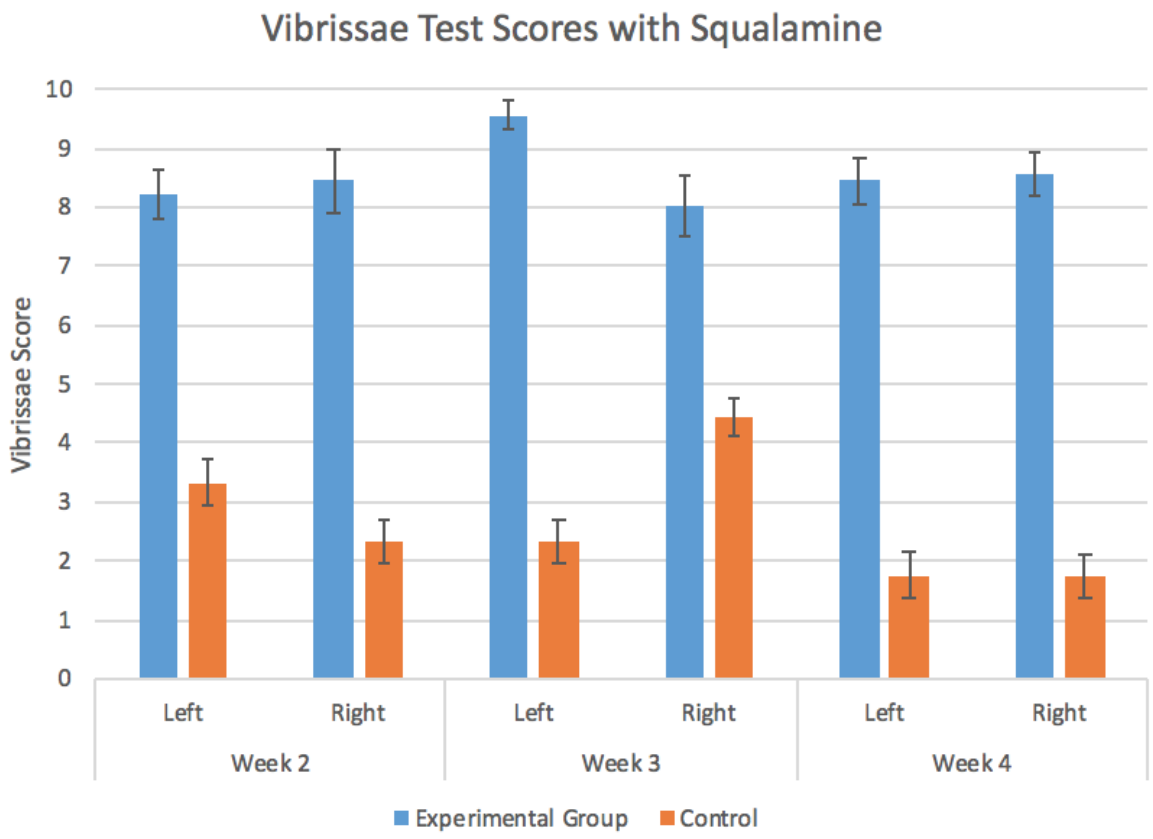
Title: Squalamine is protective against paraquat and lectin model of Parkinson's disease

Authors: *C. SWAIN[1], K. LE[1], J. ARNOLD[1], K. SAUTER[4], T. SUBRAMANIAN[2], K. VENKITESWARAN[3];
[1]Dept. of Neurol., [2]Neurology, Neurosci. and Bioengineering, [3]Dept. of Neurol. and Neurosci., Univ. of Toledo, Toledo, OH; [4]Col. of Med., Penn State Univ., Red Lion, PA

Abstract: Exposure to pesticides has been linked to the development of Parkinson’s Disease (PD). A model of PD using oral administration of paraquat and lectin has shown that this pathology is mediated via the monosynaptic dopaminergic nigrovagal pathway, which connects

the gut to the substantia nigra (SN) via the vagus nerve. In the disease state, α-synuclein that is phosphorylated at the Ser129 site (pSyn) travels retrogradely via the vagus nerve to reach the SN via the nigrovagal pathway. Gut dysmotility and constipation is a characteristic of early stages of PD, which is captured later in this model. Our aim was to utilize the prokinetic squalamine lactate (SL) to target the early pathology and symptoms in the gut, to prevent the progression of PD through the nigrovagal pathway. Squalamine acts on neuronal membranes in the gut and displaces pSyn aggregates. We administered subthreshold doses of paraquat and lectin (P+L) to Sprague Dawley rats (n=7, 250-400g) via oral gavage for 7 days. An experimental group (n=3) were given squalamine in their drinking water for 30 days post-P+L treatment. A control group (n=4) received no squalamine post P+L treatment. A vibrissae-evoked forelimb placement test (VEFPT) was used to detect motor deficits at 2, 3, and 4 weeks post-P+L treatment. The animals were then euthanized, perfused with 4% PFA, and their brains were processed for histology. Animals that received P+L showed significantly lower VEFPT scores (*$p<0.5$) than animals treated with P+L+SL. P+L animals showed significantly more neuronal loss in the SN than P+L+SL animals depicted by TH+ immunohistochemistry staining. Additional studies to quantify pSyn in the SN of P+L and P+L+SL animals using primary antibody against pSyn are ongoing. Our study indicates that squalamine has a protective role in preventing α-synuclein misfolding, and therefore may represent a translational opportunity for pharmacotherapy of early PD.



Disclosures: **C. Swain:** None. **K. Le:** None. **J. Arnold:** None. **K. Sauter:** None. **T. Subramanian:** B. Contracted Research/Research Grant (principal investigator for a drug study,

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Poster

373. Parkinson Disease: Animal Models and Therapeutics

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 373.18

Topic: C.03. Parkinson's Disease

Title: Ipsc-derived dopamine neurons show engraftment and rescue of behavioral deficits in a 6-ohda lesion model of parkinson's

Authors: C. J. ANDERSON, **L. HARVEY**, *J. XU, R. BENDRIEM, F. LAFAILLE, C. A. PALADINI, T. HUDZIK;
BlueRock Therapeut., New York, NY

Abstract: BlueRock's dopamine program seeks to generate a best in-class dopamine cell therapy for the treatment of Parkinson's disease (PD). Here, we describe a series of experiments aimed at demonstrating functionality of our cell product in vivo using the unilateral 6-OHDA lesion rat model of PD. Functional integration of transplanted cells was measured using a series of motor behavioral assays. These assays include the amphetamine-induced rotation test, a well-established test of unilateral motor deficit characteristic of this rodent model, as well as the stepping task, which measures forelimb use and dexterity. Amphetamine-induced rotational behavior revealed an improvement in lesioned animals transplanted with iPSC-derived dopamine cells compared to vehicle controls, beginning at 2 months post-graft with nearly complete rescue at 4-months post-graft. In addition, the stepping task demonstrated behavioral rescue of cell-transplanted animals compared to vehicle at 4-months post-graft. Graft composition, maturity, and speed of engraftment were assessed with immunohistochemical methods. Taken together, this work demonstrates the ability of BlueRock's iPSC-derived cells to make functional projections in vivo and provides a potential biological basis for observed behavioral benefits in a rat model of PD.

Disclosures: **C.J. Anderson:** A. Employment/Salary (full or part-time):; BlueRock Therapeutics. **L. Harvey:** A. Employment/Salary (full or part-time):; BlueRock Therapeutics. **J. Xu:** A. Employment/Salary (full or part-time):; BlueRock Therapeutics. **R. Bendriem:** A. Employment/Salary (full or part-time):; BlueRock Therapeutics. **F. Lafaille:** A. Employment/Salary (full or part-time):; BlueRock Therapeutics. **C.A. Paladini:** A.

Employment/Salary (full or part-time):; BlueRock Therapeutics. **T. Hudzik:** A. Employment/Salary (full or part-time):; BlueRock Therapeutics.

Poster

373. Parkinson Disease: Animal Models and Therapeutics

Location: SDCC Halls B-H

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Program #/Poster #: 373.19

Topic: C.03. Parkinson's Disease

Support: National Research Foundation of Korea (2019R1A2C1085130)
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Korea Health Technology R&D Project through the Korea Health Industry Development Instiute (HF21C0053)

Title: Anti-inflammatory effect of Korean red ginseng in a mouse model of Parkinson's disease

Authors: H. LEE[1], J. SEO[1], C.-H. BAE[1], H.-Y. KIM[2], K.-T. HA[1], *S. KIM[1];
[1]Korean Med. Sci., [2]Korean Med. Res. Ctr. for Healthy Aging, Pusan Natl. Univ., Mulgeum-eup, Yangsan-si, Korea, Republic of

Abstract: Parkinson's disease (PD) is a neurodegenerative disease involving dopaminergic neuronal death in the substantia nigra (SN); recent studies have shown that interactions between gut and brain play a critical role in the pathogenesis of PD and gut microbiota influences the interactions. In this study, the anti-inflammatory effect of Korean red ginseng (KRG) and the changes in gut microbiota were evaluated in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD mouse model. Male nine-week-old C57BL/6 mice were injected intraperitoneally with 30 mg/kg of MPTP at 24-h intervals for 5 days. Two hours after the daily MPTP injection, the mice were orally administered 100 mg/kg of KRG, which continued for 7 days beyond the MPTP injections, for a total of 12 consecutive days. Eight days after the final KRG administration, the pole and rotarod tests were performed and brain and colon samples of the mice were collected. Dopaminergic neuronal death, activation of microglia and astrocytes, α-synuclein and inflammatory cytokine expression were evaluated. In addition, 16S ribosomal RNA gene sequencing of mouse fecal samples was performed to investigate microbiome changes. KRG treatment prevented MPTP-induced behavioral impairment, dopaminergic neuronal death, activation of microglia and astrocytes in the nigrostriatal pathway, disruption of occludin in the colon, and the increase in α-synuclein, interleukin-1β and tumor necrosis factor-α expression in the SN and colon. The 16S rRNA sequencing revealed that MPTP altered the number of bacterial species and their relative abundances, which were partially suppressed by KRG treatment. Especially, KRG suppressed the abundance of the inflammation-related phylum Verrucomicrobia and genera Ruminococcus and Akkermansia (especially Akkermansia muciniphila), and elevated the abundance of Eubacterium, which produces the anti-inflammatory substances. These findings suggest that KRG prevents MPTP-induced inflammation and

dopaminergic neuronal death in the SN and colon, and changes in gut microbiota influences the effect.

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Poster

373. Parkinson Disease: Animal Models and Therapeutics

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Program #/Poster #: 373.20

Topic: C.03. Parkinson's Disease

Title: Subthalamic nucleus deep brain stimulation restores motor and sensorimotor cortical neuronal oscillatory activity in the free-moving 6-OHDA lesion rat Parkinson model

Authors: A. ABDULBAKI, M. ALAM, J. K. KRAUSS, ***K. SCHWABE**;
Neurosurg., Hanover, Germany

Abstract: Background: Altered oscillatory activity in cortical-basal ganglia thalamic circuitries, especially enhanced activity in the beta band, have been linked to motor symptoms in Parkinson`s disease (PD). The subthalamic nucleus nucleus (STN) is targeted for deep brain stimulation (DBS) in PD and chronic stimulation has been shown to reduce beta band activity. We here assessed the effect of STN DBS on the spectral power of oscillatory activity in the classic frequency bands in the motor cortex (MCtx) and sensorimotor cortex (SMCtx) in free-moving 6-hydroxydopamine (6-OHDA) lesioned hemiparkinsonian (HP) rats and sham-lesioned controls.
Methods: Thirteen male Sprague Dawley rats (250-350g) were either rendered HP by unilateral injection of 6-OHDA (n=6), or by injection of saline (sham-lesioned; n=7) in the right medial forebrain bundle. After three weeks of surgical recovery, a DBS electrode was implanted in the STN, and an electrocortigram (ECoG) recording array was placed under the dura above the MCtx and SMCtx areas of the right hemisphere. All surgeries were performed under chloral hydrate (360 mg/kg; i.p.) anesthesia. Seven days after surgery free-moving rats were individually recorded in three conditions: (1) basal activity, (2) during STN DBS, and (3) after STN DBS. Spectral power of oscillatory activity of theta (4-8 Hz), alpha (8-12 Hz), beta (12-30 Hz) and gamma (30-100 Hz), and average number of bursts in the oscillatory activity were analyzed in the MCtx and SMCtx areas and compared between HP and sham-lesioned rats.
Results: In HP rats, the relative power of theta band activity was lower, and beta and gamma activity was higher in MCtx and SMCtx, which was reverted towards control level by STN DBS both during and after stimulation ($p<0.001$). Further analysis showed a higher count of bursts in beta and gamma oscillatory activity in HP rats in the MCtx and SMCtx, which was suppressed by STN DBS. No differences were found between MCtx and SMCtx.
Conclusion: Our results provide evidence that loss of nigrostriatal dopamine leads to increased

beta and gamma oscillatory activity in motor and sensorimotor cortical areas, which is compensated by STN stimulation.

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Poster

373. Parkinson Disease: Animal Models and Therapeutics

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Program #/Poster #: 373.21

Topic: C.03. Parkinson's Disease
Ron and Pratima Gatehouse Fund
Anne M. and Phillip H. Glatfelter, III Family
Department of Defense NETP GRANT13204752, NIDDK R01 DK124098, NINDS R01 NS104565

Title: Chemogenetic unilateral nigrovagal inhibition protects the ipsilateral nigra from alpha synucleinopathy in the P+L rat model of parkinsonism

Authors: *J. ARNOLD[1], C. C. SWAIN[1], K. LE[1], T. SUBRAMANIAN[3], K. VENKITESWARAN[2];
[1]Neurol., [2]Neurol. and Neurosci., Univ. of Toledo Col. of Med. and Life Sci., Toledo, OH;
[3]Neurology, Neurosci. and Bioengineering, Univ. of Toledo, Toledo, OH

Abstract: Chemogenetic unilateral nigrovagal inhibition protects the ipsilateral nigra from alpha synucleinopathy in the P+L rat model of parkinsonismJulia Arnold, Caroline Swain, Khoi Le, Thyagarajan Subramanian, Kala Venkiteswaran
We demonstrated that there is a direct monosynaptic nigrovagal dopaminergic pathway in the rat and that oral exposure of Paraquat and dietary lectin (P+L) causes bilateral parkinsonism accompanied by substantia nigra pars compacta (SNpc) neuronal degeneration (Anselmi, et. al., npj Parkinson's disease 4:30, 2018). In this model misfolded alpha-synuclein ascends the vagus nerve to reach the dorsal motor nucleus of the vagus (DMV) and then into the SNpc causing neuronal degeneration in the nigrovagal pathway. We tested the hypothesis that unilateral chemogenetic inhibition of the nigrovagal pathway will provide contralateral protection and cause ipsilateral hemiparkinsonism. Adult Sprague Dawley (SD) rats were stereotactically injected with AAV2- Ef1a-eYFP-IRES-WGA-Cre into the DMV and AAV8-hSyn-DIO-hM4Di-mCherry into the SNpc on the left side. This allowed chemogenetic inhibition of the left nigrovagal pathway when P+L was given with chemogenetic activators Clozapine nitric oxide (CNO) or JHU compound in the water supply. Control animals received inactive vectors. In a second experiment DAT-Cre or TH-cre transgenic rats received AAV8-hSyn-DIO-hM4Di-mCherry into the SNpc on the left side followed by P+L oral gavage for a week while on CNO/JHU. Behavioral assessments for parkinsonism was performed at 2, 4, 8, 12, 16 and 20 weeks followed by histology as we have described in Lieu. et al. Parkinsonism Relat. Disord. (2010)16, 458-465. All test animals developed stable left hemiparkinsonism and control animals

developed bilateral parkinsonism as per Vibrissae and stepping tests. Histology showed unilateral expression of hM4Di and mCherry exclusively in the left nigrovagal pathway in the wild type SD rats and ipsilateral SNpc neuronal preservation with all its projections expressing hM4Di and mCherry in the transgenic rats. Contralateral SNpc demonstrated >50% neuronal degeneration with Lewy body-like inclusions. Preliminary findings show excess phosphorylated S129 misfolded alpha-synuclein in the unprotected SNpc. We show that maladaptive nigrovagal neuronal hyperactivity plays a critical role in accelerating synucleinopathy and SNpc neurodegeneration providing proof of concept that the nigrovagal pathway is a novel therapeutic target to prevent disease progression in Parkinson's disease
Key words: Parkinson's disease, gene therapy, basal ganglia, neuroprotection, neurodegeneration

Disclosures: J. Arnold: None. **C.C. Swain:** None. **K. Le:** None. **T. Subramanian:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Research Grant from Enterin, Research Grants from Eli Lily, Intec, Pharma2B, NIH and PA Tobacco Settlement funds., Department of Defense NETP GRANT13204752, NIDDK R01 DK124098, NINDS R01 NS104565. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Squalamine is supplied by Enterin. D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); Honoraria from Adamas, Teva, Kyowa Krin, Supernus, Neurocrine, Acorda and Acadia.. F. Consulting Fees (e.g., advisory boards); Consulting fees from Enterin, Adamas, Teva, Kyowa Krin, Supernus, Neurocrine, Acorda and Acadia.. **K. Venkiteswaran:** None.

Poster

373. Parkinson Disease: Animal Models and Therapeutics

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Program #/Poster #: 373.22

Topic: C.03. Parkinson's Disease

Title: Therapeutic Effects of Cortical Stimulation by Graphene Electrode Compared to Subthalamic Nucleus Deep Brain Stimulation

Authors: *H. SHIN[1], K. KIM[1], S. YANG[1], C. YOU[1], S. LEE[2], M. KIM[2];
[1]Incheon Natl. Univ., Incheon Natl. Univ., Incheon, Korea, Republic of; [2]gbrain, Incheon, Korea, Republic of

Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disease which largely arises from the degeneration of the dopaminergic system. Various biomedical devices have been developed as an alternative and effective treatment for PD. Deep brain stimulation (DBS) installed into deep brain structures such as subthalamic nucleus (STN) has shown some therapeutic effects on PD. However, DBS-induced adverse complications such as headache, local pain, depression, anxiety, cognitive impairments, and suicide have been issued

due to the non-specific stimulation of the STN having multi-functional potency. To avoid these unwanted effects, our group has fabricated a graphene electrode array that holds superior biocompatibility and flexibility. It could specifically activate only the motor cortex for promoting therapeutic effects without adverse effects. When the therapeutic effect of STN-DBS was compared with that of graphene electrode-induced cortical surface stimulation, Graphene electrode stimulation showed better therapeutic effects than DBS in a 6OHDA-intoxicated PD model with 18% improvement in gait test. Furthermore, cortical surface stimulation maintained greater synaptic plasticity even in the PD animal model. This data demonstrate surface stimulation over the motor cortex could be an electrotherapeutic target for PD.

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Poster

373. Parkinson Disease: Animal Models and Therapeutics

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Program #/Poster #: 373.23

Topic: C.03. Parkinson's Disease

Support: National Institute of Neurological Disorders and Stroke (NINDS) of the National Institutes of Health (NIH) R01 NS091238.

Title: Glycosylated PACAP Peptides as Novel Agents to Address Parkinson's Disease

Authors: *K. BERNARD[1], A. LUJAN[2], M. J. CORRENBLUM[3], J. L. SAEZ[4], M. J. BARTLETT[3], P. TANGUTURI[5], C. R. APOSTOL[2], L. SZABÒ[2], M. L. HEIEN[2], J. M. STREICHER[5], R. POLT[2], T. FALK[6], L. MADHAVAN[3];
[1]Physiological Sci. GIDP, [2]Chem. and Biochem., [3]Neurol., [4]Physiol., [5]Pharmacol., Univ. of Arizona, Tucson, AZ; [6]Dept. Of Neurol., Col. of Med., Tucson, AZ

Abstract: Parkinson's Disease (PD) is a chronic age-related neurodegenerative disorder characterized by debilitating motor deficits and neurocognitive decline. Currently, there are no treatments that can lessen the progression of this disease. Pituitary adenylate cyclase-activating peptide (PACAP) is an endogenous neuropeptide hormone with neuroprotective abilities especially given its trophic and anti-inflammatory properties. However, its poor stability and low blood-brain barrier (BBB) permeability limit its therapeutic potential. To address this problem, we have designed and synthesized a glycosylated PACAP analog (2ls98lac) that shows increased **peptide stability (and therefore half-life) and enhanced CNS penetration.** We have also determined through in vitro analysis that 2ls98lac can target specific PACAP receptors to activate downstream pathways and showed a protective effect of systemically administered 2ls98lac against a mild 6-hydroxydopamine (6-OHDA) lesion in rats. To further examine the in vivo effects of 2ls98lac in relation to PD, mice overexpressing human wild-type alpha-synuclein (Thy1-Syn, line 61) were treated with 1 mg/kg 2ls98lac (SC, alternate days) or saline (n = 7-12

/group) starting at 2 months of age. After one month, mice were assessed behaviorally through specific motor and cognitive tasks, and subsequently sacrificed for immunohistochemical studies to assess Syn pathology, inflammation, and trophic sequelae. We did not observe significant behavioral improvements in the Thy1-Syn mice compared to wild-type controls. However, a 50% reduction in the degree of Syn expression within the striatum and substantia nigra (SN) of the parkinsonian Thy1-Syn mice was seen. We also found that 2ls98lac exerted an immunomodulatory influence on the IBA1-positive microglia in the SN. The number of branches per cell in the Thy1-Syn mouse is reduced (121.2, compared to 154.7, $p = 0.09$) and is increased in those treated with 2ls98lac (145.1), suggesting a reduction in activation status. Although not significant, a similar pattern was observed for other measures of morphology. We are also evaluating the overall trophic effect of 2ls98lac within these regions, by assessing the production of growth factors such as Brain-Derived Neurotrophic Factor (BDNF), as well as determining if there are size or morphologic changes to the cells within the SN region. In essence, these data suggest that 2ls98lac may have the ability to dampen PD pathology by reducing the Syn load and modulating the immune response. These encouraging results make a strong case for the evaluation of longer treatment duration of PACAP glycopeptides in the future.

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Poster

373. Parkinson Disease: Animal Models and Therapeutics

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Topic: C.03. Parkinson's Disease

Support: NIH R61NS112441

Title: Sustained Levodopa Delivery by Genetically Engineered Microbiome Technology for Parkinson's Disease

Authors: *P. PADHI[1], A. ABDALLA[4], B. K. SCHEIDER[5], N. BACKES[6], G. D. ZENITSKY[2], H. JIN[2], V. ANANTHARAM[3], A. KANTHASAMY[3], K. ALLENSPACH-JORN[7], J. P. MOCHEL[4], G. PHILLIPS[8], A. G. KANTHASAMY[3];
[1]Physiol. and Pharmacol., [3]Physiol. and Pharmacology, Ctr. of Brain Sci. and Neurodegenerative Dis., [2]Univ. of Georgia, Athens, GA; [4]Biomed. Sci., [5]Dept. of Biomed. Sci., [7]Vet. Clin. Sci., [8]Vet. Microbiology, [6]Iowa State Univ., Ames, IA

Abstract: After six decades, the combination of Levodopa and an L-DOPA decarboxylase inhibitor, either carbidopa or benserazide, remains the gold-standard dopamine replacement therapy for Parkinson's disease (PD). Although oral administration of the conventional L-DOPA formulation 3-4 times a day is initially effective in managing PD symptoms, its long-term treatment is often linked to L-DOPA-induced dyskinesia (LID) and motor fluctuations due to non-continuous, pulsatile delivery of L-DOPA to the brain. To address this issue, we developed an innovative strategy to augment the host's microbiota with a human probiotic, *E.coli* Nissle 1917 (EcN), genetically engineered to continuously produce L-DOPA (EcN_{LDOPA}). Thus far, we have developed three generations of the $EcN_{LDOPA}^{(1-3)}$ utilizing modern synthetic biology and genome engineering techniques and characterized their ability to endogenously synthesize L-DOPA from tyrosine using the recombinant *hpaB/C* genes. The initial two generations of EcN_{LDOPA} were plasmid-based, and we further improved it by integrating the *hpaB/C* genes into the chromosome with a rhamnose (Rha) regulatable promoter system using advanced Sce-ROPE genetic engineering. Pharmacokinetics, pharmacodynamics, microbial kinetics, and safety and tolerability measures were assessed over time. Our results demonstrate that the two chromosome-integrated, rhamnose-inducible EcN_{LDOPA} systems are more effective than plasmid-based $EcN_{LDOPA}^{1,2}$ at producing L-DOPA in a Rha concentration- and time-dependent manner *in vitro*, implying the biotherapeutic dosage can be personalized to each patient's needs. Our pharmacokinetic studies in both healthy wild-type and diseased mouse models of PD revealed steady-state levels of plasma L-DOPA and heightened brain dopamine concentrations in both acute and chronic oral dosing paradigms. More importantly, our efficacy studies revealed that EcN_{LDOPA} improved performance in motor and mood-related tasks in the progressive neurodegenerative MitoPark mouse model of PD. Furthermore, in healthy canines, EcN_{LDOPA} achieved steady-state levels of L-DOPA, all while being safe and well-tolerated. Microbial kinetic, colonization and pharmacokinetic modeling studies reveal once to twice-daily administration of the EcN_{LDOPA} biotherapeutic to be sufficient. In summary, our novel, engineered, self-replicating Levodopa bacterial live-therapeutic (LD-LB) offers a paradigm-altering treatment capable of ensuring long-term efficacy, while minimizing side effects compared to traditional forms of therapy in all our preclinical models.

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Poster

373. Parkinson Disease: Animal Models and Therapeutics

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 373.25

Topic: C.03. Parkinson's Disease

Support: NIH RO1 NS107336
The Grainger Foundation

Title: Investigating the relationship between high frequency stimulation of the subthalamic nucleus and risk avoidant behaviors

Authors: *S. G. HILLAN[1], S. B. DE SOUZA[2], C. MULDER[4], J. SILVERNAIL[3], W. LUJAN[3], J. LUJAN[3];
[1]Mayo Clin. Grad. Sch. of Biomed. Sci., Mayo Clin. Grad. Sch. of Biomed. Sci., Rochester, MN; [2]Neurologic surgery, Mayo Clin., Jacksonville, FL; [3]Neurologic Surgery, Mayo Clin., Rochester, MN; [4]Minnesota State Univ., Mankato, MN

Abstract: Deep brain stimulation (DBS) of the subthalamic nucleus (STN) is a well-established treatment for the motor symptoms of Parkinson's disease (PD). While DBS has been an effective treatment for the motor symptoms of PD, patients occasionally experience side effects from stimulation. These undesirable effects include psychiatric symptoms, with one example being anxiety. Anxiety evoked by stimulation usually presents in patients at least six months following the onset of stimulation, however anxiety has also been seen as a transient side effect immediately following surgery. One reason for these effects is believed to be the result of stimulation spreading from the motor component of the STN to the limbic region, however the mechanisms responsible for these psychiatric side-effects are not well understood. Additionally, severe psychiatric disorders are an exclusion criterion for receiving DBS as a treatment for PD due to the possibility of worsening psychiatric symptoms. In order to expand the utility of DBS for the treatment of PD, it is necessary to better understand how DBS of the STN may influence these psychiatric side effects.
To improve understanding of the effects of stimulation on anxiety, this study investigates changes in exploratory behaviors and associated electrophysiological activity in a pre-motor animal model of PD. The pre-motor 6 hydroxydopamine lesioned model of PD preserves motor function, which limits confounding factors such as movement deficits that can confound behavioral analysis, while still exhibiting psychiatric symptoms associated with PD. Behavior is evaluated under chronic and acute high frequency stimulation, where chronic stimulation

investigates long-term behavioral changes associated with stimulation, and acute stimulation investigates transient effects. Preliminary results indicate that there is no difference in risk avoidant behaviors in groups that received chronic, acute, or no stimulation, suggesting that stimulation alone may not be able to induce risk avoidant behaviors in an animal model. Continuing work focuses on characterizing changes in neural activity in brain regions related to anxiety-like behaviors, such as the medial prefrontal cortex and medial amygdala, with the goal of revealing the mechanism related to changes in anxiety in relation to stimulation of the STN.

Disclosures: S.G. Hillan: None. **S.B. De Souza:** None. **C. Mulder:** None. **J. Silvernail:** None. **W. Lujan:** None. **J. Lujan:** None.

Poster

373. Parkinson Disease: Animal Models and Therapeutics

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 373.26

Topic: C.03. Parkinson's Disease

Support: Ambizione Fellowship

Title: Principles of gait encoding in the subthalamic nucleus of people with Parkinson's diseas

Authors: *Y. THENAISIE[1], K. LEE[2], C. MOERMAN[1], S. SCAFA[1], G. COURTINE[3,1], J. BLOCH[1], E. MARTIN MORAUD[1];
[1].Neurorestore, Lausanne, Switzerland; [2]Wyss Ctr. for Bio and Neuroengineering, Genève, Switzerland; [3]Swiss Federal Inst. of Technol., Lausanne, Switzerland

Abstract: Disruption of subthalamic nucleus (STN) dynamics in Parkinson's disease leads to impairments during walking. Commonly used therapies such as STN deep brain stimulation has limited efficacy on severe gait deficits. Here, we aimed to uncover the principles through which the STN encodes functional and dysfunctional walking in people with Parkinson's disease.
We first conceived a neurorobotic platform that allowed us to deconstruct the key components of walking under well-controlled conditions. We exploited this platform in 18 patients with Parkinson's disease, which allowed us to demonstrate that the subthalamic nucleus encodes the initiation, termination, and vigor of leg muscle activation during single-joint leg motor tasks. We identified low (~13-20Hz) and high beta (~20-35Hz) desynchronization time-locked to effort initiation, adaptation and termination, followed by a beta rebound. These modulations were present during passive and active movements. Surprisingly, these modulations were present in STN ipsi- and contralaterally to the active leg. Maintaining stronger efforts implied stronger desynchronizations.
We then mapped STN activity patterns to whole-body kinematics, and leg muscle activity during a variety of locomotor tasks (standing, walking with different step length, turning). We found that the same fundamental principles determine the encoding of walking. Despite patient-specific idiosyncrasies, we identified similar patient-specific band modulations during gait tasks. Gait

initiation, adaptation and termination were marked by beta desynchronizations and were efficiently decoded. STN activity also exhibited a phasic step length-dependent pattern during the gait cycle, which allowed decoding left versus right gait phases.
We finally translated this understanding into a machine-learning framework that decoded muscle activation, walking states, locomotor vigor, and freezing of gait. These results may guide the decoding of abnormal gait patterns in Parkinson's disease, such as asymmetry, shuffling steps or freezing of gait. Such biomarkers guide the design of adaptive stimulation protocols specifically addressing gait deficits.

Disclosures: Y. Thenaisie: None. **K. Lee:** None. **C. Moerman:** None. **S. Scafa:** None. **G. Courtine:** None. **J. Bloch:** None. **E. Martin Moraud:** None.

Poster

373. Parkinson Disease: Animal Models and Therapeutics

Location: SDCC Halls B-H

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Program #/Poster #: 373.27

Topic: C.03. Parkinson's Disease

Support: Defitech foundation
Parkinson Schweiz foundation
EU Joint Program for Neurodegenerative Disease Research (project NeuWalk)
ONWARD medical

Title: A spinal cord neuroprosthesis that alleviates locomotor deficits in Parkinson's disease

Authors: *E. MARTIN MORAUD[1,4], T. MILEKOVIC[1,4], R. DEMESMAEKER[1,4], F. RASCHELLÀ[1], M. G. PERICH[5], C. MOERMAN[1,4], N. MACELLARI[5,4], S. SUN[5], G. SCHIAVONE[5], C. VARESCON[1,4], L. BOLE FEYSOT[5,4], S. D. HERNANDEZ-CHARPAK[5,4], G. DUMONT[5,4], N. HANKOV[1,4], G. CARPARELLI[5,4], S. BORGOGNON[6], E. PRALONG[2], M. CASTRO JIMÉNEZ[3], J. BALLY[3], D. BORTON[7], Q. LI[8], M. CAPOGROSSO[9], L. ASBOTH[1], T. DENISSON[10], S. P. LACOUR[5], S. MICERA[5], C. QIN[11], E. BEZARD[12], J. BLOCH[2,4], G. COURTINE[5,4];
[2]Neurosurg., [3]Neurol., [1]CHUV, Lausanne, Switzerland; [4]Defitech Ctr. for Interventional Neurotherapies .NeuroRestore, Lausanne, Switzerland; [5]EPFL, Lausanne, Switzerland; [6]Univ. Fribourg, Fribourg, Switzerland; [7]Brown Univ., Providence, RI; [8]Motac Neurosci., Beijing, China; [9]Univ. Pittsburgh, Pitsburgh, PA; [10]Oxford Univ., Oxford, United Kingdom; [11]Chinese Acad. of sciences, Beijing, China; [12]Inst. of Neurodegenerative Dis., Bordeaux, France

Abstract: People with late-stage Parkinson's disease (PD) suffer from debilitating locomotor deficits. Here, we introduce a spinal cord neuroprosthesis that targets leg motor neurons in real-time to alleviate these deficits. We first optimized this neuroprosthetic in the gold-standard (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, MPTP) nonhuman primate model of PD. We established an analytical platform combining high-resolution kinematic and muscle activity

recordings to characterize locomotor deficits and found that nonhuman primates exhibited locomotor deficits comparable to those quantified in humans with PD. Projection of muscle activity onto the location of motor neuron pools in the spinal cord showed that walking involves the sequential activation of spatially-restricted regions, and that this activation is altered when PD symptoms appear. To restore the natural dynamics of motor neurons, we engineered a spinal cord implant incorporating precise configurations of electrodes that recruit the individual dorsal root entry zones to modulate specific ensembles of leg motor neurons. We controlled this spinal cord neuroprosthesis based on real-time decoding of motor cortex activity in order to replicate the natural spatiotemporal activation of leg motor neurons underlying walking. This neuroprosthesis alleviated gait and balance deficits in nonhuman primates, enabling both natural and skilled locomotion despite severe Parkinsonism. Spinal cord stimulation and deep brain stimulation of the subthalamic nucleus interacted synergistically to reduce motor signs of PD. Translation of the spinal cord neuroprosthesis in one patient with PD alleviated locomotor deficits and suppressed freezing of gait, including at home and in challenging community settings.

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Poster

373. Parkinson Disease: Animal Models and Therapeutics

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 373.28

Topic: C.03. Parkinson’s Disease

Support: Defitech foundation
Parkinson Schweiz foundation
EU Joint Program for Neurodegenerative Disease Research (project NeuWalk)
ONWARD, medical

Title: A TASK-ADAPTIVE SPINAL NEUROPROSTHESIS TO IMPROVE GAIT DEFICITS IN PEOPLE WITH PARKINSON DISEASE

Authors: *N. MACELLARI[1,2,3], C. MOERMAN[1,2,3], C. D. SASPORTES[1], C. HARTE[1,2,3], C. VARESCON[1,2,3], L. BOLE FEYSOT[1,3], G. CARPARELLI[1,3], S. HERNANDEZ-CHARPAK[1,2,3], G. DUMONT[1,3], N. HANKOV[1,2,3], M. VAT[1,3], A. WATRIN[5], A. PALEY[1,3], M. TSCHOPP[4], N. HERMANN[4], V. LUPI[4], F. BECCE[6], E. PRALONG[7], M. CASTRO JIMENEZ[9], J. F. BALLY[9], L. ASBOTH[1,3,2], R. DEMESMAEKER[1,2,3], E. MARTIN MORAUD[1,3], T. MILEKOVIC[1,2,3], J. BLOCH[1,2,3,8], G. COURTINE[1,2,3,8];
[1]Defitech Ctr. for Interventional Neurotherapies (.NeuroRestore), Lausanne, Switzerland, Lausanne, Switzerland; [2]Ctr. for Neuroprosthetics and Brain Mind Institute, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland, Lausanne, Switzerland; [3]Dept. of Clin. Neuroscience, Lausanne Univ. Hosp. (CHUV) and Univ. of Lausanne (UNIL), Lausanne, Switzerland, Lausanne, Switzerland; [4]Dept. of Clin. Neuroscience, Lausanne Univ. Hosp. (CHUV) and Univ. of Lausanne (UNIL), Lausanne, Switzerland, Department of Clinical Neuroscience, Lausanne Univ, Switzerland; [5]ONWARD Medical, Lausanne, Switzerland., Lausanne, Switzerland; [6]Dept. of Diagnos. and Interventional radiology, CHUV/UNIL, Lausanne, Switzerland, Department of Diagnostic and Interventional radiol, Switzerland; [7]Dept. of Neurosurgery, CHUV, Lausanne, Switzerland, Department of Neurosurgery, CHUV, Lausanne, Switze, Switzerland; [8]Dept. of Neurosurgery, CHUV, Lausanne, Switzerland, Lausanne, Switzerland; [9]Dept. of Neurology, CHUV, Lausanne, Switzerland, Department of Neurology, CHUV, Lausanne, Switzerla, Switzerland

Abstract: More than 90% of people with Parkinson's disease (PD) suffer from gait deficits that affect their quality of life, increase their comorbid condition and respond poorly to current available therapies. Here, we present a novel neuroprosthesis that targets leg motor neurons to alleviate gait deficits in PD patients. This neuroprosthesis controls the delivery of epidural electrical stimulation (EES) to the lumbar spinal cord in real-time by inferring different phases of gait from signals recorded by wearable movement sensors (IMUs). We use these feedback signals to adapt EES protocols to different activities of daily life: walking, standing, stair-climbing, turning and obstacle avoidance. We tested this neuroprosthesis in a 61-year-old participant (enrolled in the STIMO-PARK clinical trial) with long-standing PD (>20years) and severe gait deficits despite DBS therapy. A personalized computational framework guided the neurosurgical positioning of a Medtronic 5-6-5 paddle lead to target specific sensorimotor reflex pathways. The lead was connected to an Activa RC implantable pulse generator (Medtronic, USA) that is wirelessly controlled in real-time by the neuroprosthesis controller. The neuroprosthesis controller detected gait events with an accuracy of 93% ± 2% and inferred activities with an accuracy of 80% ± 5%. We projected IMU recordings from multiple days into a four-dimensional space using principal component analysis. We then used the manifolds representing the average daily gait to realign the data using iterative algorithms. The re-alignment method maintained the accuracy of the gait event detection above 85% independently of changes in IMU placement. The use of the task-adapted stimulation protocol was rewarded with a higher preference score by both the participant and the observing physiotherapist (+36% compared to neuroprosthesis without task adaptation and +56% compared to non-changing EES). Our results represent a step towards an EES-based neuroprosthetic therapy that has the potential to successfully alleviate gait deficits of people with PD.

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Poster

374. CMT and Other Degenerative Diseases

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 374.01

Topic: C.06. Neuromuscular Diseases

Support: NIH grant R37NS054154

Title: Preclinical studies inhibiting GCN2 and the integrated stress response in mouse models of CMT2D

Authors: A. L. D. TADENEV, T. MULLINS, B. PATTAVINA, R. SCHNEIDER, ***R. BURGESS**;
The Jackson Lab., Bar Harbor, ME

Abstract: Charcot-Marie-Tooth disease is a collection of inherited peripheral neuropathies associated with mutations in at least 100 different genes. The gene family with the most CMT-associations is the aminoacyl tRNA-synthetase family (aaRSs), with dominant mutations in at least five aaRS gene leading to CMT. There are currently no approved treatments for any form of CMT, but recent results have identified a promising pharmacological target. Dominant mutations in tRNA synthetase genes in mice activate the integrated stress response (ISR) through the sensor kinase GCN2. Inhibiting GCN2 at or before disease-onset prevented ISR activation and greatly mitigated the neuropathy phenotype. Here we provide additional preclinical data on GCN2 inhibition in mouse models of *Gars*/CMT2D. We performed two studies using an experimental GCN2 inhibitor (GCN2iB) in *Gars*-ΔETAQ mice modeling CMT2D. First, mice were treated with GCN2iB starting after disease onset, at 5-weeks-of-age. Second, mice were treated at disease-onset (two-weeks-of-age), but treatment stopped after 4 weeks to test necessity of life-long treatment. When treatment with GCN2iB was started post-onset (postnatal day 35, P35) and continued for 5 weeks, *Gars* mice showed improvement over the course of the study, gaining body weight, improving motor performance and showing better neurophysiological outcomes.

The basis for this post-onset improvement is under investigation, but the ability of the drug to improve function and not just prevent disease is important for eventual clinical relevance. In a second cohort of mice, treatment was started at disease onset (P14) and continued for 4 weeks, then treatment stopped, and mice were followed for an additional 4 weeks. These mice showed an improvement in motor performance during the treatment phase, but performance declined when treatment stopped. Neurophysiology measures showed some benefit persisted even four weeks after treatment was ended. However, this was insufficient to produce behavioral improvement and continued treatment is likely required to maintain benefit. In conclusion, inhibiting GCN2 is beneficial even when treatment is started after the onset of disease and when treatment was stopped, some of the improvements were lost.

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Poster

374. CMT and Other Degenerative Diseases

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 374.02

Topic: C.06. Neuromuscular Diseases

Title: Intermediate sterols are severely dysregulated in Charcot-Marie-Tooth 1A mouse models

Authors: *S. MANCA[1], C. GARRON[3], T. TRELEAVEN[2], J. MCINNIS[2], L. GUO[2], M. GONCALVES[2], B. ZHANG[2], J. DODGE[1], S. GIERA[1];
[1]Sanofi-Aventis Pharmaceuticals, Cambridge, MA; [2]Sanofi-Aventis Pharmaceuticals, cambridge, MA; [3]Sanofi, cambridge, MA

Abstract: Charcot-Marie-Tooth (CMT) disease, the most common inherited disorder of the peripheral nervous system (PNS) is caused by gene duplications or mutations in *PMP22* (CMT1A), *MPZ* (CMT1B), *GJB1* (CMTX) or *MFN2* (CMT2A) genes. CMT1A represents 60% of the overall cases and it is due to duplication of the PMP22 gene. Differentiation is impaired in Schwann cells overexpressing PMP22 protein and this leads to a wide range of neurological phenotypes in CMT patients including dysmyelination, axonal loss, and muscular weakness and atrophy in the hands and feet. It is known that cholesterol regulates peripheral myelination and impairment in its biosynthesis leads to hypomyelination and motor deficits. In our current experiments we found that the intermediate sterols that are necessary for cholesterol biosynthesis were significantly reduced in the sciatic nerves, but not in the brain or plasma of two different well-characterized CMT mouse models. Specifically, C3-PMP22 mice at 3 months of age displayed significant reductions in lanosterol, MAS-412, FF-MAS, zymostenol, zymosterol, lathosterol, 7-DHC and cholesterol in the sciatic nerve compared to wild-type mice. Interestingly, in 3-week-old $Pmp22^{TrJ}$ mice, which have a more severe and earlier motor disease phenotype, we found an even greater reduction in intermediate sterol and cholesterol levels in

sciatic nerve samples. Taken together, these results demonstrate for the first time a broad dysregulation of the cholesterol biosynthesis pathway in CMT mouse models. Additional studies are underway to elucidate the relationship between the lack of sterols and etiology of CMT.

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Poster

374. CMT and Other Degenerative Diseases

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 374.03

Topic: C.06. Neuromuscular Diseases

Support: DOD Grant W81XWH2010423
NIH Training Grant F30

Title: Toward a treatment for Duchenne muscular dystrophy by expanded capacity AAV gene-therapy

Authors: *R. H. HSU[1,2,3], L. C. BACHMANN[1], C. WILLIAMS[1], G. MAIER[1], K. HERMANN[1], S. L. PFAFF[1,4];
[1]The Salk Inst. for Biol. Studies, Salk Inst. for Biol. Studies, La Jolla, CA; [2]Bioengineering, [3]Med. Scientist Training Program, [4]Neurobiology, Biol. Sci., Univ. of California - San Diego, La Jolla, CA

Abstract: Duchenne muscular dystrophy (DMD) is a devastating genetic muscular disease that arises from a mutation in the dystrophin gene. Dystrophin plays a major structural role in muscles and defects in the gene lead to muscle degeneration in the limbs, heart, and diaphragm. About 1 in 3,500 male births are affected, leading to paralysis by age 12 and death by age 26 on average. To date, there is no cure and treatments merely achieve minor quality of life improvements.Gene therapy is an emerging field that aims to cure genetic diseases by restoring healthy DNA in patients. Specifically, gene-replacement therapy delivered by Adeno-associated virus (AAV) is a promising strategy, which aims to replace the defective gene. However, a major limitation of AAV gene delivery is its limited cargo-capacity of 4.7 kilobases (kb), posing a significant hurdle in delivering larger genes such as dystrophin (12 kb) and many CRISPR-Cas9 gene editing systems. To date, clinical testing has been restricted to using highly truncated micro-dystrophins, engineered to fit in AAV, but have resulting limited efficacy even at toxic doses.The Pfaff Laboratory recently developed a novel RNA trans-splicing technology that overcomes the cargo-capacity of AAV, allowing the delivery of full-length gene-replacement therapies. First, split-gene constructs are designed by dividing the full gene into DNA fragments, flanked by regions containing intronic and base-pairing binding sequences, where each fragment is delivered by a sub-population of an AAV cocktail. Within the cell, the expressed RNA fragments are locally stabilized by the base-pairing of RNA binding domains, then undergo a

spliceosome-mediated joining reaction, thus concatenating the fragments into full-length mRNA for translation the original protein. We leveraged this novel technology to deliver three candidate genetic therapeutics: a medium-length mini-dystrophin (7 kb), a full-length dystrophin (12 kb), and a CRISPR-Cas9 adenine base editor (6 kb). We demonstrate efficacious treatment, with minimal off-target activity, of DMD mouse models evaluated by transcriptomics, protein quantification, immunohistochemistry, and functional assays. Continued work will further validate efficacy by systemic administration. This project demonstrates the potential of a novel class of genetic therapies and lays the groundwork for future studies in higher mammals and for a potential cure of Duchenne muscular dystrophy in patients.

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Poster

374. CMT and Other Degenerative Diseases

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Program #/Poster #: 374.04

Topic: C.06. Neuromuscular Diseases

Support: NHLBI F30 HL151138
NIGMS T32 GM008244
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R01 AR078571
R56 AR05529

Title: In vitro modeling of Duchenne muscular dystrophy using patient-specific iPS cells

Authors: *B. I. GARAY[1,2,3], R. PERLINGEIRO[3,1];
[1]Univ. of Minnesota Grad. Program In Neurosci., Minneapolis, MN; [2]Med. Scientist Training Program, Univ. of Minnesota Med. Sch., Minneapolis, MN; [3]Medicine, Cardiovasc. Div., Univ. of Minnesota, Minneapolis, MN

Abstract: Duchenne muscular dystrophy (DMD) is a relentlessly degenerative neuromuscular disorder that affects both skeletal and cardiac muscles. Despite over 30 years of research, our current standard of care only delays its clinical progression. DMD pathophysiology is characterized by loss of the dystrophin protein that results in sarcolemmal damage, mislocalization of ion channels, efflux of creatine kinase (CK) and influx of calcium that culminates in cell death. Understanding why the clinical manifestations of DMD start in the skeletal muscle and then progress to the cardiac muscle remains a critical gap in our knowledge. Here, we leverage the use of a panel of patient-derived human induced pluripotent stem cells (hiPSCs) with a spectrum of mutations and disease severity to unravel the physiologic adaptations and transcriptional changes specific and/or common to both skeletal (SkM) and cardiac myocytes (CMs) upon stress via electrical pacing or adrenergic/cholinergic receptor

agonists. Using our optimized differentiation and maturation protocols, we generated highly pure skeletal and cardiac myocytes. Preliminary data in unaffected, isogenic corrected and DMD hiPSC lines show that at baseline, there is increased CK release in both muscle cell types: 1-4±1 U/L in control *vs*. 10±1 U/L CK activity in DMD lines differentiated into SkM (****p<0.0001), and 27±5 U/L in control *vs*. 234±49 U/L in DMD lines differentiated into CMs (**p<0.001). Additionally, under continuous electrical pacing conditions for 4hrs in SkM only DMD SkM showed an increase in CK from 10±1 U/L to 29±6 U/L (****p<0.0001). After 240hrs of continuous electrical pacing, SkM from all hiPSC lines showed an increase in CK release, but only DMD had a significantly greater fold-change increase compared to baseline and control lines (****p<0.0001). These results validate key pathophysiological manifestations of DMD and serve as the basis for further studies dissecting the transcriptional changes of DMD upon stress.

Disclosures: B.I. Garay: None. **R. Perlingeiro:** None.

Poster

374. CMT and Other Degenerative Diseases

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Program #/Poster #: 374.05

Topic: C.06. Neuromuscular Diseases

Support: NINDS R37 NS054154

Title: Towards in vivo studies of C12ORF65/MTRFR deficiency in mitochondrial translation in neurons

Authors: *S. L. PRATT[1,2], B. J. BATTERSBY[3], R. W. BURGESS[1,2];
[1]Jackson Lab., Bar Harbor, ME; [2]Neurosci. Program, Grad. Sch. of Biomed. Sci., Tufts Univ., Boston, MA; [3]Inst. of Biotech., Univ. of Helsinki, Helsinki, Finland

Abstract: The nuclear gene *C12ORF65* encodes mitochondrial translation release factor rescue-1 (MTRFR), a small protein vital for proper mitochondrial translation that acts as a mitoribosome release factor when translation is terminated. Recessive mutations in *C12ORF65* lead to failure of resolution of non-stop mitoribosome stalling in mitochondrial translation and subsequently neuropathy and neurodegeneration. Patients presenting with *C12ORF65* deficiencies often have optic and peripheral neuropathies characteristic of Behr's syndrome, Leigh syndrome, and Charcot-Marie-Tooth disease (CMT). Many of these patients have disease onset in early childhood with progressive neuronal degeneration. However, the underlying mechanisms and neuronal sensitivity have not yet been explained. To fully understand how *C12ORF65* deficiency leads to neurodegeneration, a mouse model mimicking patient phenotype is necessary. Currently we are developing four new mammalian models of *C12ORF65* deficiency that will clarify the genetic mechanism and cell-specificity of this peripheral neuropathy. The mouse models include a null mutation, resulting in a total loss of function, which dies embryonically as a homozygote, and a knock-in with a premature truncation in the C-

terminus of the gene on endogenous Chromosome 5, providing a partial loss of function model. The two other models are Cre-inducible transgenes expressing versions of the human *C12ORF65* sequence inserted in a safe harbor locus on Chromosome 6. The first transgene carries three lysine to alanine substitutions, disrupting the lysine-rich C-terminal region, also leading to a partial loss of function. The final model is a wild-type human transgene, allowing for the analysis of a fully functioning allele. We will generate allelic combinations to produce a viable mouse model, and use these mice to determine the genetic mechanism driving the pathology of these diseases and identify activation of downstream pathways that may provide therapeutic targets. The mice will also inform gene therapy approaches and provide a preclinical platform for future studies. Understanding *C12ORF65* deficiency will provide insight into how mitochondrial translation plays a role in neuropathy and neurodegeneration.

Disclosures: S.L. Pratt: None. **B.J. Battersby:** None. **R.W. Burgess:** None.

Poster

374. CMT and Other Degenerative Diseases

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 374.06

Topic: C.06. Neuromuscular Diseases

Support: NIGMS Grant GM103440
NIGMS Grant GM104944

Title: Investigating the Proteomic Heterogeneity of Peripheral Neuropathies Caused by Genetic Abnormalities of Peripheral Myelin Protein-22

Authors: *V. DEFILIPPI, J. PETEREIT, Y. WOO, L. NOTTERPEK;
Univ. of Nevada, Reno, Reno, NV

Abstract: Investigating the Proteomic Heterogeneity of Peripheral Neuropathies Caused by Genetic Abnormalities of Peripheral Myelin Protein 22
Victoria Defilippi, Juli Petereit, Yeon Hwa Woo, and Lucia Notterpek
Hereditary demyelinating neuropathies caused by abnormal expression of the peripheral myelin protein 22 (*PMP22*) gene comprise a heterogeneous group of disorders with differences in disease onset and severity. Specifically, Charcot-Marie-Tooth (CMT) type1A is caused by trisomy of *PMP22*, hereditary neuropathy with liability to pressure palsies (HNPP) is due to a heterozygous deletion of *PMP22*, and point mutations cause CMT type1E. To better understand the pathogenic mechanisms underlying the unique phenotypes of these disorders, we used data-independent acquisition (DIA) mass spectrometry to quantify proteins of neuropathic nerves. This approach provides higher sensitivity and protein coverage than typical data-dependent acquisition. We collected sciatic nerves from five postnatal day-21 mice for each of the three studied models and their respective wild-type (Wt) controls, with 2 to 3 males and females in each group. *PMP22* overexpressing (C22) and *PMP22* (Leu16Pro) mutant Trembler J (TrJ) mice

are bread on the C57/Bl6 background, while the HNPP model is maintained on the 129S background. The resulting raw sequenced data was used to identify the original protein contents of each sciatic nerve sample. A linear model was utilized to determine statistically significant differences between strain-specific wild-type (WT) and neuropathic samples. Proteins with an adjusted p-value of 0.05 were deemed statistically significant. The untargeted proteomics approach of these samples resulted in 3,752 quality quantified protein groups. Clear separations were identified between the nerves of PMP22-knockout (KO) and TrJ mice compared with their respective Wt, indicating that over 1,000 proteins are significantly up or down-regulated within the samples. Impact analysis of these proteins showed relevant biological pathways affected in the neuropathic nerves. Differences in the steroid biosynthesis pathway were identified between the two most affected models, HNPP and CMT1E. Myelin sheath and compact myelin components were differentially affected by the studied *PMP22* mutations. Additionally, discrepancies in cholesterol-related biological processes within the affected nerves diverge amongst the diseased models. Overall the unbiased approach taken in this proteomic study reveals a divergence of cholesterol-related functions among the CMT1E and HNPP models.

Disclosures: V. Defilippi: None. **J. Petereit:** None. **Y. Woo:** None. **L. Notterpek:** None.

Poster

374. CMT and Other Degenerative Diseases

Location: SDCC Halls B-H

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Program #/Poster #: 374.07

Topic: C.06. Neuromuscular Diseases

Title: Chitinase Expression and Distribution in Neurodegenerative Diseases

Authors: *C. TRAN[1,2], V. VENUGOPAL[1], L. VU[1], R. BOWSER[1];
[1]Translational Neurosci., Barrow Neurolog. Inst., Phoenix, AZ; [2]Sch. of Life Sci., Arizona State Univ., Tempe, AZ

Abstract: Neuroinflammation is widely regarded as a contributing factor to the pathogenesis of Amyotrophic Lateral Sclerosis (ALS) as well as other neurodegenerative disorders. This inflammatory response can be measured through a host of protein-based biomarkers that are secreted into body fluids or using immunohistochemistry of post-mortem tissue. Chitinase proteins, specifically, chitotriosidase-1 (Chit-1), chitinase-3-like protein 1 (CHI3L1), and chitinase-3-like protein 2 (CHI3L2) are a group of proteins that have been investigated as markers of neuroinflammation by our lab and others. In addition to ALS, these chitinase proteins have also been implicated in other neurodegenerative disorders, including frontotemporal dementia (FTD) and Alzheimer's Disease (AD). However, the expression pattern of these chitinase proteins in various cell types as well as the spatial distribution of these proteins has not been well characterized across neurodegenerative diseases. We will test the hypothesis that, in each neurological disease condition, CHI3L1 will be expressed in a subset of astrocytes, CHI3L2 will be expressed in a subset of microglia and infiltrating macrophages, and Chit-1 is expressed

in a subset of microglia. We also hypothesize that expression of CHI3L1, CHI3L2, and Chit-1 are a histopathological feature associated near cytoplasmic TDP-43 inclusions in TDP proteinopathies. Our aim is to characterize the spatial distribution of these chitinase proteins relative to pathological hallmarks of disease in post-mortem tissue samples across multiple brain regions, as well as identify which cell types express these proteins across various neurodegenerative diseases, including ALS, C9-ALS, FTD, AD, and non-neurologic disease controls.

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Poster

374. CMT and Other Degenerative Diseases

Location: SDCC Halls B-H

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Program #/Poster #: 374.08

Topic: C.06. Neuromuscular Diseases

Support: Associazione Girotondo ONLUS donation
Italian Ministry of Health RC L1.3

Title: An in-vitro model to study the role of survival motor neuron (SMN) protein isoforms in neuronal differentiation.

Authors: *C. CAGNOLI[1], B. CIPELLETTI[1], F. COLCIAGHI[1], P. SCALMANI[1], M. COSTANZA[2], A. REPISHTI[1], A. CIOTTI[1], M. DE CURTIS[1];
[1]Pre-Clinical Neurosci. Laboratories, Clin. and Exptl. Epileptology Unit, [2]Dept. of Clin. Neuroscience, Mol. Neuro-Oncology Unit, Fondazione IRCCS Inst. Neurologico Carlo Besta, Milano, Italy

Abstract: Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder, caused by mutations in survival motor neuron (SMN) 1 gene. SMA has an incidence of 1 in 6,000 live births, and it is characterized by motor neuron degeneration, leading to progressive amyotrophic paralysis, respiratory deficiency and, in more severe cases, death. Recently approved SMN-targeted therapies have revolutionized the approach to SMA, but their efficacy is only partial, emphasizing the need for understanding the mechanisms of SMA pathogenesis, in order to find targets for additional therapies. We have identified a new isoform of the SMN gene, preferentially expressed in the axon, called a-SMN. SiRNA silencing of either the full length FL- or the a-SMN isoform in rat hippocampal neurons led to loss of normal neuronal polarity with abnormal ankyrin G (AnkG) labelling, found in none or in multiple neurites, including those positive for the dendrite-specific protein MAP2 or in the terminal portion of the axon. We aim to study the molecular mechanisms that link the two SMN isoforms and AnkG functions in axon initial segment (AIS) formation. In neurons where FL-SMN or a-SMN siRNA was co-

transfected with a plasmid for the expression of FL-SMN or a-SMN, respectively, the ratio of cells displaying abnormal AnkG labelling was significantly lower than in silenced neurons transfected with empty cDNA vector, and similar to non-silenced control. a-SMN seemed more effective than FL-SMN in rescuing the phenotype. As siRNA isoform specificity is only partial, we characterized a new model of hippocampal neurons prepared from a murine SMA model (SMA Δ7 mice). Direct interaction between the proteins is unlikely, given that immunofluorescence (IF) analysis of WT mouse neurons showed AnkG highly localized in the proximal AIS, while a-SMN is distributed along the axon but absent from AIS as well as growth cone and dendrites. The percentage of neurons displaying abnormal AnkG IF labeling was significantly higher in cultures from SMA mice *vs*. WT. Similar results were obtained when the localization of sodium voltage-gated channels was analyzed, confirming the role of SMN in neuronal polarization and AIS formation. Na^+ and K^+ current densities recorded by patch clump showed a trend to increase in SMA neurons *vs.* WT, while spontaneous synaptic activity (sEPSC frequency and peak amplitude) seemed not changed. Our results indicate that hippocampal neurons from SMA Δ7 mice are a suitable *in-vitro* model to study the role of SMN isoforms in neuronal differentiation. Evidence of a differential effect of the FL- and a-SMN isoforms on axon polarization would give new significance to neuronal susceptibility to SMN loss.

Disclosures: C. Cagnoli: None. **B. Cipelletti:** None. **F. Colciaghi:** None. **P. Scalmani:** None. **M. Costanza:** None. **A. Repishti:** None. **A. Ciotti:** None. **M. de Curtis:** None.

Poster

374. CMT and Other Degenerative Diseases

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Program #/Poster #: 374.09

Topic: C.06. Neuromuscular Diseases

Support: NIH Grant GM103653
NIH Grant NS120154

Title: Spinal muscular atrophy (SMA) causes gliosis and structural and cell type defects within the cerebellum of SMND7 mice

Authors: *N. COTTAM[1], M. HARRINGTON[1], C. CHARVET[1,2], J. SUN[1];
[1]Biol., Delaware State Univ., Dover, DE; [2]Auburn Univ., Auburn, AL

Abstract: Spinal Muscular Atrophy (SMA) is a disease that affects 1 in every 6,000-10,000 individuals at birth, making it the leading genetic cause of infant mortality. SMA is best defined by motor neuron dysfunction due to a deletion or mutation in transcripts of survival motor neuron protein (SMN), which leads to degeneration and dysfunction in the anterior horn of the spinal cord. However, due to the key roles of SMN in a broad range of cellular functions, a growing number of studies support the notion that SMA is a multi-system disease. The cerebellum has received little attention, even though it plays an important role in motor function

and widespread pathology has been reported in the cerebellum of SMA patients. Previously, using the SMNΔ7 mouse model, we used T2-weighted and DTI scans from MRI of ex-vivo P12 mouse brains to acquire volumetric measurements and connectivity information. We found a disproportionate loss in cerebellar volume and an overall decrease in white matter connectivity, with spinocerebellar tracts being the main tract diminished by SMA. We also documented diffusivity metrics that can represent axonal health. Fractional anisotropy and radial diffusivity were most affected by SMA, indicating altered axonal structure in the cerebellum. In this study, analysis of cerebellar layer thicknesses showed a decrease in all cellular layers, yet only in posterior lobes. Additionally, migrating granule cell density increased in the ML of both affected and unaffected lobes in SMA cerebella. Staining of Purkinje cells (PC) revealed PC loss and structural degeneration localized to posterior lobules. Astrocytic structural integrity was analyzed across the cerebellum; white matter regions of posterior lobes and peduncles displayed decreased astrocytic robustness, molecular and granular layers of posterior lobes and the deep cerebellar nuclei (DCN) displayed gliosis. Lastly, electrophysiological recordings of DCN neurons revealed decreased spontaneous firing and increased action potential firing frequencies. We conclude that several cell types in SMA-affected mouse cerebella show distinct structural and functional defects, indicating that defects within the cerebellum may need to be addressed in SMA treatment and therapy.

Disclosures: **N. Cottam:** None. **M. Harrington:** None. **C. Charvet:** None. **J. Sun:** None.

Poster

374. CMT and Other Degenerative Diseases

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Program #/Poster #: 374.10

Topic: C.06. Neuromuscular Diseases

Support: NIH Grant R01NS085164

Title: Mitochondrial Complex I deficiency affects NMJ function through distinct pre- and postsynaptic mechanisms, including a form of homeostatic plasticity

Authors: B. MALLIK, ***C. FRANK**;
Univ. of Iowa, Iowa City, IA

Abstract: Neurons are highly polarized cells with immense energy demands. Healthy pools of mitochondria primarily fulfill those energy demands. In response to altered energy states of the neuron, mitochondria can adapt to maintain energy homeostasis and nervous system function. This adaptation, also called mitochondrial plasticity, can be observed as changes in morphology, function, or localization of mitochondria at synapses. Through an RNAi-mediated genetic screen in *Drosophila melanogaster*, we identified Mitochondrial Complex I subunits as essential for maintaining mitochondrial morphology and synapse function. In humans, mutations affecting Mitochondrial Complex I subunits have been reported to be involved in severe mitochondrial

diseases, like Parkinson's Disease, Leigh Syndrome, and cardiomyopathy - as well as forms of ataxia and epilepsy. Yet the neuronal and synaptic mechanisms by which Complex I dysfunction results in disease remain elusive. Here, we found a *Drosophila melanogaster* model of Complex I deficiency caused by a nuclear DNA-encoded NADH dehydrogenase subunit 20 (*ND-20L*) gene. We show that *ND-20L*-depleted larval mutants exhibit phenotypes that resemble symptoms of mitochondrial disease, including progressive degeneration of muscle and presynaptic cytoskeleton, enhanced reactive oxygen species (ROS) formation and alteration in mitochondrial morphology. Our genetic and electrophysiological analyses at the neuromuscular junction (NMJ) suggest that a form of homeostatic synaptic plasticity is mobilized to stave off presynaptic dysfunction when ND-20L is lost. Namely, active zone protein enhancements, as well as ER-mediated calcium release and calcium import into mitochondria all contribute to maintaining evoked neurotransmission when Complex I is lost in neurons. Consistent with our genetic and electrophysiological evidence, chelating calcium with membrane-permeable BAPTA-AM showed a similar phenotype when intracellular calcium stores were blocked in *ND-20L*-depleted larvae. By contrast, postsynaptic depletion of Complex I disrupted the Dlg-Spectrin scaffold that maintains active zone-receptor apposition. This synaptic degeneration and neurodegeneration phenotype may explain the defects in the evoked release. We are also testing if muscle-to-nerve signaling paradigms also play a role. Finally, loss of additional Complex I members phenocopies *ND-20L* loss, as does pharmacological blockade of Complex I activity by rotenone. Our findings support a model in which diminished Complex I activity and consequent energy deficiency are responsible for specific synaptic defects in *Drosophila*.

Disclosures: B. Mallik: None. **C. Frank:** None.

Poster

374. CMT and Other Degenerative Diseases

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 374.11

Topic: C.06. Neuromuscular Diseases

Support: NSF grant 20116438

Title: Simulations of active zone structure and function at mammalian NMJs predict that loss of calcium channels alone is not sufficient to replicate LEMS effects

Authors: *S. MERINEY[1], S. P. GINEBAUGH[1], R. LAGHAEI[2], G. MERSKY[3], C. WALLACE[1], T. B. TARR[1], C. KAUFHOLD[1], S. W. REDDEL[4], Y. BADAWI[1];
[1]Univ. of Pittsburgh, Pittsburgh, PA; [2]Carnegie Mellon Univ., Pittsburgh, PA; [3]Univ. of PIttsburgh, Pittsburgh, PA; [4]Concord Hosp., Sydney, Australia

Abstract: Lambert-Eaton myasthenic syndrome (LEMS) is an autoimmune-mediated neuromuscular disease thought to be caused by autoantibodies against P/Q-type voltage-gated calcium channels (VGCCs), which attack and reduce the number of VGCCs within transmitter

release sites (active zones; AZs) in the presynaptic terminal of the neuromuscular junction (NMJ), resulting in neuromuscular weakness. However, LEMS patients also have antibodies to other neuronal proteins, and up to 15% of LEMS patients are seronegative for antibodies against VGCCs. We hypothesized that a reduction in the number of P/Q-type VGCCs alone is not sufficient to explain LEMS effects on transmitter release. Here, we used a computational model to study a variety of LEMS-mediated effects on AZ organization and transmitter release based on prior electron microscopic, pharmacological, immunohistochemical, and electrophysiological observations from mouse models of LEMS. We show that healthy AZs can be modified to predict the transmitter release and short-term facilitation characteristics of LEMS, and that disruption in the organization of AZ proteins, a reduction in AZ number, reductions in synaptotagmin and the compensatory expression of L-type channels outside the remaining AZs are important contributors, in addition to a decrease in number of AZ VGCCs, to LEMS-mediated effects on transmitter release. Furthermore, our models predict that antibody-mediated removal of synaptotagmin in combination with disruption in AZ organization could mimic LEMS effects without removal of VGCCs (a seronegative model). Overall, our results suggest that LEMS pathophysiology may most likely be caused by a collection of pathological alterations to the presynaptic AZ at the NMJ, rather than by a simple loss of VGCCs.

Disclosures: **S. Meriney:** None. **S.P. Ginebaugh:** None. **R. Laghaei:** None. **G. Mersky:** None. **C. Wallace:** None. **T.B. Tarr:** None. **C. Kaufhold:** None. **S.W. Reddel:** None. **Y. Badawi:** None.

Poster

374. CMT and Other Degenerative Diseases

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 374.12

Topic: C.06. Neuromuscular Diseases

Title: Functional characterization of KY021 genetic variant in a rare neurological disorder using zebrafish disease model

Authors: *S. KI, S. PARK, B. HWANG, Y. KEE;
Kangwon Natl. Univ., Chuncheon, Korea, Republic of

Abstract: Tens of millions of people suffer from rare diseases worldwide. Model organism research is essential to investigate the pathophysiology of rare diseases. The genetic variants of the patients with global developmental delay, no acquisition of independent walking, and Rett symptoms were clinically detected by the *Trio*-Based Whole-Genome Sequencing study. This study aims to validate the function of an undiagnosed genetic variant and investigate the mechanism underlying the pathophysiology of the rare neurodevelopmental disorder that affects the way the brain develops. Here, we obtained the zebrafish knockout animal (KO) for the KY021 gene and analyzed the anatomical and behavioral phenotypes in early zebrafish development. The results show the early development phenotypes of the homozygous mutants

recapitulate the patient symptoms: the abnormal motility of the homozygous mutants and the cell death in the developing brain. We performed rescue experiments for the phenotypes by injecting the mRNA of the genetic variants into 1-cell zebrafish embryos. mRNA of the patient variant was not efficient in rescuing the KO abnormalities compared to wild-type mRNA. The present study implicates that the KY021 de novo variant is a loss-of-function mutation causing the patient's developmental and neurological symptoms. The further analysis explores the mechanism underlying the neurodevelopmental pathology of the variant in the undiagnosed disease.

Disclosures: **S. Ki:** None. **S. Park:** None. **B. Hwang:** None. **Y. Kee:** None.

Poster

374. CMT and Other Degenerative Diseases

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Program #/Poster #: 374.13

Topic: C.06. Neuromuscular Diseases

Support: MDF Fellowship
NIH NS048843

Title: Choroid plexus spliceopathy in myotonic dystrophy type 1 and Alzheimer's disease

Authors: *B. KIDD[1], C. NUTTER[2], H. CARTER[2], P. MACKIE[1], N. KUMBKARNI[2], D. TUYN[2], J. SAMPSON[4], L. SZNAJDER[2], L. RANUM[2], E. WANG[2], H. KHOSHBOUEI[1], J. HAMEL[5], S. PROKOP[3], J. DAY[4], M. SWANSON[2];
[1]Univ. of Florida Dept. of Neurosci., Gainesville, FL; [2]Mol. Genet. and Microbiology, [3]Pathology, Univ. of Florida, Gainesville, FL; [4]Stanford Sch. of Med., Stanford, CA; [5]Neurol., Univ. of Rochester, Rochester, NY

Abstract: *Dmpk* CTG^{exp} knockin (KI) mouse models for myotonic dystrophy type 1 (DM1) demonstrated the choroid plexus (ChP) is particularly susceptible to DM1 spliceopathy. The ChP is important for neurodevelopment, brain homeostasis, circadian rhythms, and sleep via its production and regulation of cerebrospinal fluid (CSF). To clarify how the ChP is affected in DM1, we investigated transcriptomic changes characteristic of the DM1 pathomechanism. Interestingly, the ChP has also been suggested to play a role in Alzheimer’s disease (AD) pathology. Since DM1 has been characterized as a disease with impairments in developmental mRNA splicing transitions regulated by MBNL family of RNA binding proteins (RBP), we performed RNAseq on wild-type mice ChP from late embryogenesis to adults and characterized the ChP developmental mRNA splicing transitions with corresponding changes in regulatory RBP expression. *Dmpk* CTG^{exp} KI mice ChP show a spliceopathy that primarily reverts splicing patterns to an earlier developmental pattern. We identified DM1 ChP specific transcriptional changes, including robust and concordant splicing changes. Next, we determined that DM1 ChP mis-splicing is driven by MBNL2 loss and this spliceopathy recapitulates embryonic splicing

patterns. Additionally, *Mbnl2* KO, *Dmpk* CTG^{480} KI mice and DM1 present mis-splicing of key ion channels and secreted proteins. Our preliminary evidence indicates that the AD ChP transcriptome is also affected, but in unique ways compared to DM1. In summary, we established the choroid plexus is affected in DM1, and ChP mis-splicing is a factor likely underlying DM1 CNS symptoms, MBNL2 is the major upregulated RBP during ChP development in mice, and MBNL2 loss leads to mis-splicing predicted to affect brain CSF composition.

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Poster

374. CMT and Other Degenerative Diseases

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Program #/Poster #: 374.14

Topic: C.06. Neuromuscular Diseases

Support: NINDS R21NS116936
NINDS R37NS054154

Title: Sipa1l2, a candidate modifier of CMT1A from human GWAS, does not alter neuromuscular phenotypes in C3-PMP22 mice when deleted

Authors: *G. C. MURRAY[1], T. J. HINES[2], A. L. D. TADENEV[2], R. W. BURGESS[2]; [1]Res., The Jackson Lab. & The Univ. of Maine, Bar Harbor, ME; [2]The Jackson Lab., Bar Harbor, ME

Abstract: Charcot-Marie-Tooth disease (CMT) is a heterogeneous group of inherited peripheral neuropathies that cause muscle atrophy and loss of sensation in the distal limbs. CMT1A is the most common form of CMT, comprising over one-third of all CMT cases, and is caused by a genetic duplication that includes *Peripheral Myelin Protein 22* (*PMP22*), leading to its pathogenic overexpression. In a recent patient-only genome-wide association study, *Signal Induced Proliferation Associated 1 Like 2* (*SIPA1L2*) was identified as a putative modifier of ankle dorsiflexion in CMT1A patients. Accompanying *in vitro* studies revealed that knocking down *Sipa1l2* in rat Schwann cell cultures decreased expression of *Pmp22*, making reduction of *SIPA1L2* a potential therapeutic target for CMT1A. Here we tested whether knocking out *Sipa1l2* would improve the neuromuscular disease phenotypes in the C3-PMP22 mouse model of CMT1A, thereby validating the patient GWAS hit. We performed neuromuscular phenotyping for muscular endurance, electrophysiology to assess nerve conduction, nerve histopathology, and gene expression analyses in male and female C3-PMP22 mice that were bred into a *Sipa1l2* mutant background to produce mice that were wild-type, heterozygous, or homozygous for the

knockout. These genotypes will allow us to better understand the normal function of SIPA1L2 and to determine if *Sipa1l2* knockout alters neuromuscular phenotypes in our disease model. We have reproduced previously reported CMT1A-relevant phenotypes in C3-PMP22 mice, but these outcomes were not changed by the *Sipa1l2* knockout, which caused significant sex-dependent changes in body weight but no alteration of neuromuscular phenotypes on its own. RNASeq studies are underway, and analyses will examine the normal function of SIPA1L2 in the peripheral nervous system of mice, which is not known, and address whether the *Sipa1l2* knockout alters the expression of *Pmp22* and other myelin-associated genes, as suggested by *in vitro* studies. Our findings to date suggest that decreasing *SIPA1L2* expression may not be an effective therapeutic strategy for CMT1A; however, analysis of ongoing gene expression studies is needed to help determine whether this lack of effect stems from insufficient *Sipa1l2*-induced changes in levels of myelin-related genes in our mouse models or other causes.

Disclosures: G.C. Murray: None. **T.J. Hines:** None. **A.L.D. Tadenev:** None. **R.W. Burgess:** None.

Poster

374. CMT and Other Degenerative Diseases

Location: SDCC Halls B-H

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Program #/Poster #: 374.15

Title: WITHDRAWN

Poster

374. CMT and Other Degenerative Diseases

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 374.16

Topic: C.06. Neuromuscular Diseases

Support: 1R03-NS116433-01

Title: Exercise has no effect in the development or intensity of Charcot-Marie-Tooth Type 2 associated phenotypes in heterozygous ATP1A1 knockout mice

Authors: *R. SWEAZEY, K. SPONTARELLI, A. PADRO, J. BAILOO, P. ARTIGAS; Texas Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX

Abstract: Charcot-Marie-Tooth disease (CMT) is a common inheritable peripheral neuropathy that presents with distal muscle atrophy and weakness, loss of sensation, absent reflexes, and *pes cavus*. the two major types of CMT are due to demyelination (CMT1) or axonal degeneration

(CMT2). CMT2 has been linked to heterozygous germline mutations in *ATP1A1* (the gene encoding the α_1 subunit of the Na^+/K^+-ATPase) which are reported to cause loss-of-function. To further investigate the role of *ATP1A1* haploinsufficiency in CMT2 pathophysiology, heterozygous *ATP1A1* knockout mice, (*ATP1A1*$^{+/-}$) were evaluated by comparing the performance of *ATP1A1*$^{+/-}$ and WT littermates mice in a series of behavioral tests performed regularly up to 18 months old to evaluate strength, coordination, balance, and endurance; as well as identify other neuropathy-like phenotypes. No significant differences between WT and *ATP1A1*$^{+/-}$ mice were observed throughout their lifespans. This suggests the loss of function of one *ATP1A1* allele is not solely responsible for CMT2 pathophysiology. We evaluated if exercise, and environmental conditions increasing the usage of sensory-and/or motor-neurons, may elicit CMT2-like phenotypes in *ATP1A1*$^{+/-}$ mice. Baseline motor performance of *ATP1A1*$^{+/-}$ and WT littermates was evaluated at one months of age. The mice were then made to run on an electrically driven treadmill five days a week. Exercise intensity and duration were increased during the first month, after which a consistent speed and duration (14 m/min for 30 min) was used for the next 5 months. Behavioral tests performed up to 9 months old revealed no significant differences relative to their baseline performance in *ATP1A1*$^{+/-}$ or WT mice. Likewise, no significant differences in performance were observed at any time point between exercised *ATP1A1*$^{+/-}$ and WT mice when compared to non-exercised ATP*1A1*$^{+/-}$ and WT mice (sedentary group). The absence of CMT2 phenotypes in *ATP1A1*$^{+/-}$ mice suggests additional research is needed regarding the mechanisms of CMT2 induction.

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Poster

374. CMT and Other Degenerative Diseases

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Program #/Poster #: 374.17

Topic: C.06. Neuromuscular Diseases

Support: Ohio State University Fellowship (Pino)
Ohio State University Center for RNA Biology Fellowship (Hall)
Ohio State University Undergraduate Research Scholarship (Hall)
Ohio State University Undergraduate Research Scholarship (Jones)

Title: Exon skipping: The molecular mechanism underlying KIF5A-linked ALS pathogenesis?

Authors: *M. G. PINO[1], N. J. HALL[2], M. L. JONES[2], A. J. BLATNIK, III[3], A. H. BURGHES[3], S. J. KOLB[4];
[1]Neurology, Med. Scientist Training Program, Neurosci. Grad. Program, [2]Neurol., [3]Biol. Chem. & Pharmacology, Mol. Genet., The Ohio State Univ., Columbus, OH; [4]Neurol., The Ohio State Univ. Wexner Med. Ctr., Columbus, OH

Abstract: Background: Single nucleotide variants in the cargo-binding domain of Kinesin Family Member 5A (KIF5A), a neuronal motor protein involved in transport along microtubules, have been associated with amyotrophic lateral sclerosis (ALS). ALS is a rapidly progressive and fatal neuromuscular disease that primarily affects motor neurons. Interestingly, ALS-associated *KIF5A* variants are clustered near splice-site junctions of the penultimate exon 27. Thus, we sought to determine whether mis-splicing of *KIF5A* RNA, specifically of exon 27, is the key mechanism underlying *KIF5A*-associated ALS pathogenesis.
Methods: To characterize pre-mRNA splice defects in each of the 12 *KIF5A* variants, we first developed an *in vitro* minigene splicing assay in HEK293 cells to detect full-length and mis-spliced *KIF5A* mRNA isoforms. We performed multiplexed droplet digital PCR (ddPCR) to quantify mRNA expression, then calculated the rate of exon 27 inclusion for each *KIF5A* variant. To address potential differences in cell type-specific splicing, we next genetically edited human stem cells using CRISPR-Cas9 to generate stable lines of human motor neurons that endogenously express *KIF5A*. We chose representative variants at the 5' and 3' splice-sites of exon 27, as well as wildtype and C-terminus truncation controls. A 14-day viability assay was performed in addition to the same ddPCR quantification outlined above.
Results: In both HEK293 cells and human stem cell-derived motor neurons, we showed that 5' splice-site variants selectively result in exon 27 exclusion from spliced *KIF5A* mRNA. Additionally, viability of the 5' splice-site motor neurons is reduced compared to wildtype and C-terminus truncation motor neurons.
Conclusions: Though all of the *KIF5A* variants were reported to cause the same ALS phenotype, they do not have the same effect on RNA splicing and in fact result in drastically different rates of exon 27 inclusion. We believe that 5' splice-site variants in *KIF5A* selectively disrupt consensus splice sequences where crucial ribonuclear proteins - such as U1 RNP - bind, leading to exon exclusion from mature RNA, altered protein, and eventually ALS.
Ongoing Work and Implications: Currently, we are performing Western blot quantification and cycloheximide chase experiments to quantify KIF5A protein levels and the rate of protein degradation, respectively. Updated findings will be presented at the annual SfN conference. Through these studies, we aim to achieve a comprehensive evaluation of the regulatory mechanisms governing *KIF5A* transcription and translation, with the future potential to develop therapeutics to ameliorate the devastating symptoms of ALS.

Disclosures: M.G. Pino: None. **N.J. Hall:** None. **M.L. Jones:** None. **A.J. Blatnik:** None. **A.H. Burghes:** None. **S.J. Kolb:** None.

Poster

374. CMT and Other Degenerative Diseases

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 374.18

Topic: C.06. Neuromuscular Diseases

Support: CMTA
NIH / NINDS

Title: Exploring SARM1 inhibition in mouse models of CMT

Authors: *C. L. HATTON, A. L. D. TADENEV, M. PRESA, M. TERREY, C. LUTZ, R. W. BURGESS;
The Jackson Lab., Bar Harbor, ME

Abstract: Activation of Sterile Alpha and Toll/Interleukin-1 Receptor motif-containing 1 (SARM1) is responsible for the degradation of NAD+ and promotes Wallerian degeneration of axons. Inhibition of SARM1 delays axon degeneration, similar to the spontaneous mouse mutation Wallerian Degeneration Slow (*Wld*^{S}). Inhibiting SARM1 has been shown to be axon protective in mouse models following challenges including injury, chemotherapy-induced neuropathy, and diabetic/metabolic neuropathy. Here we tested whether the loss of SARM1 would be beneficial in Charcot-Maire-Tooth disease (CMT), which is a collection of inherited peripheral neuropathies that result in demyelination and/or axon degeneration. We have tested inhibiting SARM1 as a treatment for three forms of CMT using mouse models. *Sarm1* knock-out (KO) mice were bred to models of *Kif1A*/HSN2C, *Fig4*/CMT4J, and *Gjb1*/CMT1X. Mice were evaluated through lifespan, body weight, grip strength, histopathology, and neurophysiology. The knockout of *Sarm1* did not change the phenotype in *Kif1A* or *Fig4* mutant mice, based on body weight, life span, and other outcomes, such as plasma neurofilament light chain levels, which remain elevated in Kif1A$^{-/-}$, Sarm1$^{-/-}$ double mutant mice. *Gjb1* studies are on-going, but preliminary data show no improvements in NCVs, body weight, grip strength or femoral motor nerve histology. In preliminary studies using a dominant-negative SARM1 delivered by AAV9 (dn-SARM1 AAV), wild-type mice were treated at birth and the sciatic nerve was crushed at 8 weeks. Histologically, axon integrity was protected 5 days after injury, but functionally, there was no EMG following nerve stimulation distal to the crush site, raising the possibility that SARM1 inhibition preserves axon anatomy, but not function. Thus far deleting *Sarm1* in mouse models of HSN2C, CMT4J, and CMT1X has shown no benefit in disease pathophysiology. Studies with dn-SARM1 AAV in other models of CMT and injury are on-going.

Disclosures: C.L. Hatton: None. **A.L.D. Tadenev:** None. **M. Presa:** None. **M. Terrey:** None. **C. Lutz:** None. **R.W. Burgess:** None.

Poster

374. CMT and Other Degenerative Diseases

Location: SDCC Halls B-H

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Program #/Poster #: 374.19

Topic: C.06. Neuromuscular Diseases

Support: 270949263/GRK2162
Bavarian Ministry of Science and the Arts in the framework of the ForInter network
TreatHSP consortium BMBF 01GM1905B

Title: Efficient and easy conversion of human iPSCs into functional induced microglia-like cells as a great tool to elucidate the involvement of microglia in Hereditary Spastic Paraplegia type 11

Authors: *L. KRUMM[1], J. LANFER[1], J. KAINDL[1], Z. V. ARSHAD[1], T. BÖRSTLER[1], F. KRACH[1], B. WINNER[1,3], M. REGENSBURGER[1,3,2];
[1]Dept. of Stem Cell Biol., [2]Dept. of Mol. Neurol., Univ. Hosp., Erlangen, Germany; [3]Ctr. of Rare Dis. Erlangen (ZSEER), Erlangen, Germany

Abstract: Current protocols converting human-induced pluripotent stem cells (iPSCs) into induced microglia-like cells (iMGL) are either dependent on overexpression of transcription factors or require substantial experience in stem-cell technologies. Recently, we developed an easy-to-use two-step protocol to convert iPSCs into functional iMGL via highly efficient differentiation of hematopoietic progenitor cells (HPCs) from iPSCs, and optimized maturation of HPCs to iMGL. A sequential harvesting approach led to an increased HPC yield. The protocol implemented a freezing step, thus allowing HPC biobanking and flexible timing of differentiation into iMGL. Our iMGL responded adequately to the inflammatory stimuli LPS, and iMGL RNAseq analysis matched those of other frequently used protocols. Comparing three different coating modalities, we increased the iMGL yield by culturing on uncoated glass surfaces, thereby retaining differentiation efficiency and functional hallmarks of iMGL. In summary, we provide a high-quality, easy-to-use protocol, rendering generation and functional studies on iMGL an accessible lab resource. In a wide spectrum of neurodegenerative disorders, microglia with altered transcriptome, morphology, and functional properties are detectable. These pathologically activated microglia were determined as disease-associated microglia and are detectable in the brain of patients with Alzheimer's disease, Parkinson's disease and Huntington's disease. Hereditary Spastic Paraplegia (HSP) is a heterogeneous group of inherited neurological disorders with a common feature of prominent lower-extremity spasticity due to an axonopathy of corticospinal motor neurons. HSP type 11 (SPG11-HSP) is linked to pathogenic variants in the *SPG11* gene and it represents the most frequent form of complex autosomal recessive HSP. In a mouse model of SPG11-HSP, pathogenic neuroinflammation including microgliosis was demonstrated in distinct compartments of the diseased CNS. Furthermore, a human study revealed similar RNA expression levels of *SPG11* in peripheral blood mononuclear cells (PBMCs), lymphoblasts, and iPSC-derived cortical neurons, indicating an important role for *SPG11* encoded spatacsin also within the immune system. To further elucidate the interplay between microglia and neurodegeneration in SPG11-HSP, our recently developed conversion protocol from human iPSC to functional iMGL provides an excellent tool.

Disclosures: L. Krumm: None. **J. Lanfer:** None. **J. Kaindl:** None. **Z.V. Arshad:** None. **T. Börstler:** None. **F. Krach:** None. **B. Winner:** None. **M. Regensburger:** None.

Poster

374. CMT and Other Degenerative Diseases

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 374.20

Topic: C.06. Neuromuscular Diseases

Title: Calotropis procera leaf extract expedites sensorimotor function recovery in the mouse model of peripheral nerve injury.

Authors: S. ZAFAR[1], *R. HUSSAIN[2], G. HUSSAIN[1];
[1]Neurochemical biology and Genet. Lab. (NGL), Dept. of Physiology, Fac. of Life Sciences, Government Col. Univ., Faisalabad, Pakistan; [2]Ctr. for Translational Neuromedicine, Univ. of Rochester, Rochester, NY

Abstract: Peripheral nerve injury results in sensorimotor function loss with ultimate life-long disability and compromised quality of life. In the current study, the influence of ethyl acetate extract of Calotropis procera (CP) leaves on augmenting sensorimotor function recovery was investigated in BALB/C male mice induced with sciatic nerve crush. Following sciatic nerve injury induction, mice were grouped equally into two; Crush-non treated (n=8) and Crush-treated (n=8). Both sensory and motor behavior analyses were performed at different time points to measure the extent of functional recovery in them. After an injury (day 12), all animals were sacrificed, serum was collected, and skeletal muscles from hind limbs were harvested for molecular markers. Significant Sensorimotor function revival (as investigated through grip strength, sciatic functional index, hotplate test, and pinprick analysis) in the treated group implicates the beneficial effect of CP ethyl acetate extract in enhancing functional recovery.Moreover, a higher value of total antioxidant capacity, less level of total oxidant status and higher systemic level of Arylesterase and Peroxinase-1 enzymes in treated mice implicate the oxidative stress attenuating ability of the CP extract. While down-regulation of Acetylcholine receptor alpha (ACHR-α) and muscle-specific kinase (MUSK) and up-regulation of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1-α) in ipsilateral gastrocnemius muscles of treated group points out towards it effect in enhancing the axonal regeneration following nerve injury.

Disclosures: S. Zafar: None. **R. Hussain:** None. **G. Hussain:** None.

Poster

375. Blood Brain Barrier

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 375.01

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: VA BLR&D Grant BX003486

Title: Endotoxemia promotes blood-brain barrier permeability and neuroinflammation via thrombospondin-1-dependent transforming growth factor beta 1 activation

Authors: S. BHATTARAI[1], G. FRAMPTON[1], A. JHAWER[1], E. TROYANOVSKAYA[2], *M. A. MCMILLIN[1];

[1]Intrnl. Med., Univ. of Texas at Austin, Austin, TX; [2]Central Texas Veterans Hlth. Care Syst., Austin, TX

Abstract: Endotoxemia induces systemic inflammation, blood-brain barrier (BBB) permeability, dysregulation of tight junction proteins and neuroinflammation. We have shown that transforming growth factor beta-1 (TGFβ1) is upregulated during systemic inflammation and promotes BBB permeability. Therefore, we hypothesize that TGFβ1 is upregulated during endotoxemia and compromises BBB integrity by disrupting tight junction proteins. Endotoxemia was induced in C57Bl/6 mice by intraperitoneal administration of lipopolysaccharide (LPS, 3 mg/kg) and were subsequently monitored for neurological decline. In a second set of mice 6 hours after LPS administration, an antagonist of TGFβ1 activation, LSKL, or SLLK as a control, was injected into the peritoneum (1 mg/kg). Mice were euthanized 24 hours after LPS injection and tissue was collected. In a subset of mice, 3 hours prior to euthanasia, Evans Blue (8%) was injected into the peritoneum to assess BBB function. TGFβ1, claudin-5, occludin, IL-1β, IL6 and TNFα expression assessed by RTPCR, immunoblotting and immunofluorescence. Mouse brain endothelial cells (bEnd.3 cells) were treated with LPS (10 μg/ml) or rTSP1 (1 μg/ml) to activate TGFβ1. TGFβ1, TSP1, claudin-5 and occludin expression was assessed by immunofluorescence or RTPCR. Monolayer permeability was determined in bEnd.3 cells using TEER or 10-kDa FITC-dextran diffusion. TGFβ1 expression was increased in the cortex and cerebellum of LPS-treated mice. This was associated with decreased claudin-5 and occludin mRNA expression and immunofluorescence staining. Treatment of bEnd.3 cells with LPS increased TGFβ1 and TSP1 expression, decreased TEER, and increased FITC-dextran diffusion. Supplementation of bEnd.3 cells with rTSP1 decreased claudin-5 and occludin mRNA expression, decreased TEER and increased FITC-dextran diffusion across the transwell. LSKL treatment in LPS-treated mice reduced sickness behavior, reduced Evan's blue penetrance into the brain, restored tight junction expression and alleviated neuroinflammation when compared to SLLK-treated mice. In conclusion, strategies to target TGFβ1 signaling may prove effective at alleviating endotoxin-induced BBB dysfunction.

Disclosures: S. Bhattarai: None. **G. Frampton:** None. **A. Jhawer:** None. **E. Troyanovskaya:** None. **M.A. McMillin:** None.

Poster

375. Blood Brain Barrier

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 375.02

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: R00DA044838
R01DA052859

Title: Cocaine's impact on treating the brain during HIV: drug metabolism and transport at the blood brain barrier

Authors: *L. FRIDMAN, R. COLON ORTIZ, S. KNERLER, A. MERCADO, A. PRICE, A. GAUSEPOHL, E. EISS, J. ALVAREZ, B. FLORES, D. WILLIAMS;
Mol. and Comparative Pathobiology, Johns Hopkins Univ., Baltimore, MD

Abstract: HIV enters the brain within the first two weeks of infection. Despite potent antiretroviral therapies (ART), the blood-brain barrier (BBB) restricts therapeutic access to the brain, rendering the CNS under treated. This is particularly important for those with comorbid disorders that impact the brain, including substance abuse disorders. While chemical and physical properties of the BBB are well characterized in normal conditions, much less is understood about how HIV and substances of abuse impact the BBB. Our goal was to evaluate the impact of cocaine on two key aspects of the BBB that play an integral role in treating the brain: drug metabolism and drug transport. To accomplish this, we first employed our *in vitro* model of the human BBB comprised of a transwell coculture system utilizing primary brain microvascular endothelial cells (BMVEC) and primary astrocytes. Three first line ART drugs, dolutegravir (DTG), tenofovir (TFV) and emtricitabine (FTC), were added to the apical portion of our BBB model in the presence and absence of cocaine or vehicle control for 24 hours (5 independent experiments each containing four technical replicates). After this time, media in the basal lateral portion was collected and DTG, TFV and FTC were quantified by mass spectrometry. To our surprise, both TFV and TFC, readily migrated across the BBB whereas DTG did not. Interestingly, cocaine had a variable effect; it significantly decreased TFV migration, increased FTC migration and did not change DTG migration. To investigate the mechanism by which this occurred, we evaluated CYP3A4-mediated metabolism and drug influx (OATP2A1 and OATP1A2) and efflux transporters (BCRP and P-gp). BMEVC were grown to confluency, treated with cocaine or vehicle control for 24 hours, and utilized in downstream assays including qPCR (n=11 independent experiments), Western blot (n=12 independent experiments), and immunofluorescence (n=5 independent experiments with over 200 cells imaged per experiment). Cocaine decreased CYP3A4, which was mediated by regulation of its primary transcription factor CAR. Protein levels of BCRP and P-gp decreased with cocaine, while OATP2A1 and OATP1A2 remained unchanged. Our findings identify a previously unknown role of cocaine in impacting BBB function and demonstrate the need to evaluate adverse drug:drug interactions that may impact dosing of the brain during HIV.

Disclosures: L. Fridman: None. **R. Colon Ortiz:** None. **S. Knerler:** None. **A. Mercado:** None. **A. Price:** None. **A. Gausepohl:** None. **E. Eiss:** None. **J. Alvarez:** None. **B. Flores:** None. **D. Williams:** None.

Poster

375. Blood Brain Barrier

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Program #/Poster #: 375.03

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: CONACYT Scholarship No. 580359
FODECIJAL-8237-2019

Title: Moderate hyperglycemia disrupts differentially the blood-brain barrier in various brain regions

Authors: *N. A. POSADAS-RAMIRO, J. L. CASTAÑEDA-CABRAL, S. J. LÓPEZ-PÉREZ, M. E. UREÑA-GUERRERO;
Biología Celular y Mol., Univ. de Guadalajara, CUCBA, Zapopan, Mexico

Abstract: Type 2 diabetes mellitus (T2DM) is a highly prevalent degenerative metabolic disease, characterized mainly by hyperglycemia and vascular endothelial dysfunction. In this study, blood-brain barrier (BBB) permeability was evaluated in a murine model of T2DM. Six-week-old C57BL/6 male mice were randomly distributed into two groups: 1) control (n=58): treated with Nicotinamide (NA)+vehicle; and 2) experimental (n=65): treated with NA+streptozotocin (STZ). NA (120 mg/kg) was applied 15 min before vehicle (0.1 M $Na_3C_6H_5O_7$, pH 4.5) or STZ (100 mg/kg dissolved in vehicle). Treatments were applied intraperitoneally (i.p.) on days 0 and 2. Blood glucose levels were measured at 4 h of fasting, before the treatments, and every week for 16 weeks after treatment. BBB permeability through fluorescein extravasation to the brain (2, 4, 8, 12, and 16 weeks) and claudin-5 protein expression (12 weeks) were evaluated in the cerebral motor cortex, striatum, hippocampus, entorhinal cortex, and hypothalamus by western-blot. Data were represented as median ± 95% CI and analyzed using the Mann-Whitney U test to establish the significant differences at $p<0.05$. Treatment with NA+STZ significantly increased the blood glucose levels from week 1 to 16 (≈214 mg/dL) relative to the control group (≈130 mg/dL). Although hyperglycemia could be considered as moderate, we found that glucose cell uptake was significantly altered in experimental animals with levels two-fold higher in the tolerance glucose test. Fluorescein extravasation to the brain parenchyma was significantly increased and claudin-5 protein expression decreased at 12 weeks in all studied brain regions of hyperglycemic animals. We conclude that the prolonged and moderate hyperglycemia compromises the functionality of the BBB, which could be related to the neuronal alterations found in patients with T2DM.

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Poster

375. Blood Brain Barrier

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 375.04

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

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NSF NRT-UtB 1735252

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Yale Stem Cell Center
2021 Beckman Institute Graduate Fellows Program
Beckman Institute for Advanced Science and Technology

Title: Circadian rhythmicity of blood-brain interface permeability in vitro

Authors: *A. WEISS[1], Q. NGUYEN[1], J. W. MITCHELL[2], K. E. WEIS[1], W.-C. KAO[1], S. FOK[1], H.-Y. M. CHENG[3], K. H. OBRIETAN[4], B. HAN[5], H. KONG[1], M. U. GILLETTE[6]; [1]Univ. of Illinois at Urbana-Champaign, Urbana, IL; [2]Dept. of Cell and Developmental Biol., Univ. of Illinois At Urbana-Champaign, Urbana, IL; [3]Univ. of Toronto Mississauga, Mississauga, ON, Canada; [4]Dept Neurosci, Ohio State Univ. Dept. of Neurosci., Columbus, OH; [5]Purdue Univ., Chicago, IL; [6]Dept. of Cell & Developmental Biol., Univ. of Illinois, Urbana-Champaign Neurosci. Program, Urbana, IL

Abstract: The Blood-Brain Interface (BBI) facilitates protection of the brain from pathogens, the removal of waste products, brain homeostasis, and the permeation of nutrients and drugs. Its dysfunction contributes to the onset of neurological diseases, including strokes and dementia. Both BBI permeability and human susceptibility to strokes fluctuate according to time of day. However, more *in vitro* studies are required to isolate the effects of circadian rhythms from the effects of sleep and other homeostatic regulators on BBI permeability. To address this, we are using an approach featuring multiple BBI models. We isolate and culture primary rat cerebral neuroendothelial cells on Transwell inserts, semi-permeable membranes that separate the environment into 2 compartments. We then perform Transepithelial/Transendothelial Electrical Resistance (TEER) assays. As electrical resistance across cells is a function of the tightness of cell-cell contacts, these values measure BBI integrity. The synchronized neuroendothelial cells exhibit a fluctuation in permeability that is both significant and oscillatory with a period of approximately 24 hr. Using a mouse model expressing a VENUS reporter driven by the circadian clock gene, Period 1 (Per1), we demonstrate that neuroendothelial cells express the clock protein, PER 1. From these images and our TEER assays, we conclude that cerebral neuroendothelial cells express Per1, and their permeability changes with a circadian rhythmicity. Our goal is to humanize a BBI model on a microfluidic chip.

Disclosures: A. Weiss: None. **Q. Nguyen:** None. **J.W. Mitchell:** None. **K.E. Weis:** None. **W. Kao:** None. **S. Fok:** None. **H.M. Cheng:** None. **K.H. Obrietan:** None. **B. Han:** None. **H. Kong:** None. **M.U. Gillette:** None.

Poster

375. Blood Brain Barrier

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 375.05

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: CIHR Grant

Title: The liver and muscle secreted Hfe2-protein maintains blood-brain barrier integrity

Authors: *M. SYONOV[1,3], X. WANG[3,2], R. VIGOUROUX[3], P. P. MONNIER[4,3,1];
[1]Physiol., [2]Inst. of Biomed. and Biomaterial Engin., Univ. of Toronto, Toronto, ON, Canada;
[3]Univ. Hlth. Network, Toronto, ON, Canada; [4]Toronto Western Res. Inst., Toronto, ON, Canada

Abstract: Liver failure causes blood-brain-barrier (BBB) breakdown leading to central nervous system damage, however the mechanisms whereby the liver influences BBB-integrity remain elusive. One possibility is that the liver secretes an as-yet to be identified molecule(s) that directly promotes BBB integrity. We developed light-sheet imaging for three-dimensional study of BBB function. We show that liver- or muscle-specific knockout of Hemojuvelin (Hfe2) induces BBB breakdown, leading to accumulation of toxic-blood-derived fibrinogen in the brain, lower cortical neuron numbers, and behavioral deficits. In healthy animals, soluble Hfe2 competes with its homologue Repulsive Guidance Molecule A (RGMa) for binding to Neogenin, thereby blocking RGMa-induced downregulation of Platelet derived growth factor subunit B (PDGF-B) and Claudin-5 in endothelial cells and BBB disruption. Hfe2 administration in an animal model of multiple sclerosis prevented paralysis and immune cell infiltration by inhibiting RGMa-mediated BBB alteration. This study has implications for the pathogenesis and potential treatment for diseases associated with BBB dysfunction.

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Poster

375. Blood Brain Barrier

Location: SDCC Halls B-H

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Program #/Poster #: 375.06

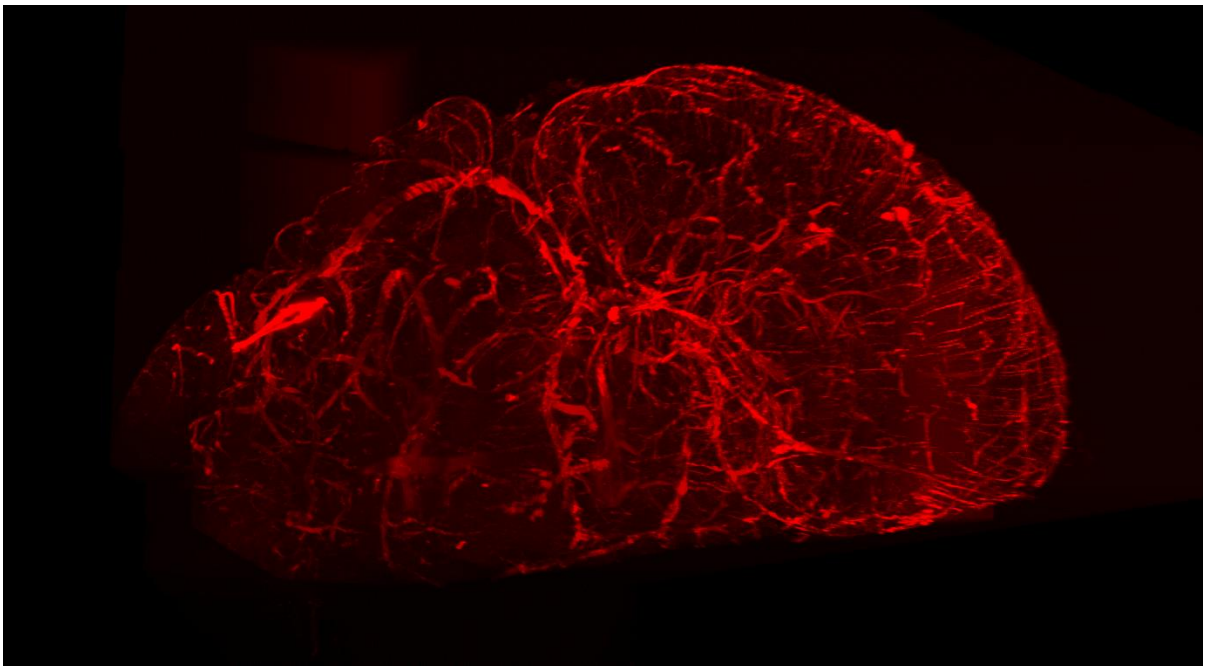
Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Title: Mapping inflammatory receptors as drivers of blood-brain barrier permeability with 3D vascular imaging

Authors: *A. XIANG, B. DIAMOND;
Feinstein Inst. for Med. Res., Donald and Barbara Zucker Sch. of Med. at Hofstra/Northwell, Manhasset, NY

Abstract: Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by production of autoantibodies to nuclear antigens and cytokine dysregulation. Disease presentation, particularly that of neuropsychiatric lupus (NPSLE), is highly heterogenous; NPSLE pathology can initiate before clinical manifestations of systemic disease are apparent. Over 80% of SLE patients are estimated to have some neurological manifestation of their disease. As there is strong evidence that NPSLE is often associated with a breach in blood-brain barrier (BBB) integrity, it is important to understand both the regulation of the BBB and how its

dysfunction permits transit of molecules into the brain.
Here, we assess the expression of key cytokine and complement receptors on the brain microvascular endothelium known to modulate BBB integrity, such as IL-1R1, IL-6R, TNFR, and C5aR, in wild-type C57BL6/J mice. We combined a novel method of *in-situ* immunostaining with whole-mount clearing and visualization to generate a 3D brain microvascular reconstruction **(Fig. 1)**. We will use this method to determine if a correlation exists between receptor densities and regional neurologic dysfunction. Additionally, we are mapping in 3D antibody distribution across the BBB following various immunological insults, such as TNF or IL-1, as we have previously shown that the same autoantibody can cause different symptoms depending on where it penetrates the BBB. In brief, following administration of inflammatory molecules to mice, reconstruction of the 3D brain microvasculature will be used to examine the correlation between BBB disruption, as identified by antibody accumulation, and the expression of associated receptors.
A better understanding of the BBB in the chronic systemic inflammation of SLE will drive a more comprehensive understanding of how dysfunction in the central nervous system is regulated by systemic inflammation. Thus, identifying key signaling pathways involved in regional BBB permeability will permit focused studies of both initiating events and factors driving chronicity of NPSLE.



Disclosures: **A. Xiang:** None. **B. Diamond:** None.

Poster

375. Blood Brain Barrier

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 375.07

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Title: The synthetic cannabinoid WIN,5512-2 effects on adolescence pro and mature BDNF in peripheral blood and the periaqueductal gray area in rats

Authors: *A. TORRES[1,2], C. PIERCE[2];
[2]Anat. and Cell Biol., [1]Oklahoma State Univ., Tulsa, OK

Abstract: Cannabinoids are compounds that bind to endocannabinoid receptors CB1 and CB2 present in the central nervous system. The synthetic CB1 receptor agonist WIN 55212-2 (WIN) emulate the effects of delta-9-tetrahydrocannabinol (THC), the psychoactive component in cannabis. Interestingly, endocannabinoids and neurotrophins, play critical roles in homeostasis and stress/anxiety response. To that extent, the periaqueductal gray (PAG) is a brain area not only involved in stress and pain modulation during adolescence, but also is a neurotrophin-rich brain area, abundant in brain derived neurotrophic factor (BDNF) which plays a role in synaptic plasticity and neuroprotection. However, the roles of BDNF and its precursor proBDNF after synthetic cannabinoid exposure during this critical period is not clearly understood. Therefore, we determine the effect of adolescent exposure to WIN on BDNF levels in the PAG and blood concentrations in the adolescent rat. Methods: adolescent rats received 5 twice-daily injections of saline (1 mL/kg i.p.) or WIN55,212-2 (0.8 mg/kg i.p.) every other day. Brains and truncal blood were collected, and ELISA immunoassay was used to determine pro and BDNF levels.Results: One way ANOVA revealed that WIN55 increased proBDNF [$F_{1,11} = 12.57$, $p<0.05$]and mBDNF levels [$F_{1,11} = 2.63$, $p<0.05$]in the dorsolateral PAG and periphery [$F_{1,11} = 5.15$, $p<0.05$]. Conclusion: The chronic exposure of synthetic cannabinoid WIN during adolescence modified the proBDNF/mBDNF ratio in the dorsal PAG and periphery, suggesting that proBDNF/mBDNF ratio are involved in endocannabinoid-mediated adolescence brain plasticity.

Disclosures: A. Torres: None. **C. Pierce:** None.

Poster

375. Blood Brain Barrier

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Program #/Poster #: 375.08

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: BRFSG-2022-07

Title: Revealing novel architectures in the extracellular matrix of the brain

Authors: *I. STERIN, A. NIAZI, J. PARK, S. PARK;
Neurobio., Univ. of Utah, Salt Lake City, UT

Abstract: The extracellular matrix (ECM) of the brain not only provides a structural scaffold, but also regulates neuronal plasticity, and has been implicated in many neurological disorders. Unlike collagen-rich peripheral ECMs, the brain parenchymal ECM lacks fibrous collagens and is scaffolded by hyaluronic acid (HA), which is bound by chondroitin sulfate proteoglycans (CSPGs), tenascins and link proteins (HAPLN). This sugar scaffold has an innate flexibility, and the brain ECM is known to be structurally dynamic. Interestingly, net-like clustering of ECM proteins (called perineuronal nets: PNNs) are formed around the soma and proximal processes of neurons. W*isteria floribunda agglutinin* (WFA), which binds to aggrecan CS chains, is widely used to label PNNs that surround mainly parvalbumin interneurons. Since digestion of brain ECM sugar chains with hyaluronidase or chondroitinase, which dissembles PNNs, alleviates memory decline in aged animals and induces plasticity, assembly of PNNs is thought to locally restrict neuronal plasticity in the surrounding region. However, the field lacks tools to study the diversity and dynamics of these PNNs. To monitor the architectures of individual PNNs assembled around different types of neurons, we recently developed a novel genetic probe using Halotag-fused HAPLN1 (we termed H-Link). AAV-mediated sparse expression of H-Link allowed us to visualize different populations of PNNs including WFA(+) PNNs, WFA(-) PNNs, and PNNs around excitatory neurons at different developmental stages (P14, P21 and P70). PNNs revealed by H-Link show a remarkable contrast in organizational patterns depending on the cellular compartment: PNNs are densely clustered around the soma and proximal axon while being dispersed but punctated in the dendrites. By injecting a fluorescent Halo-tag ligand between P3 and P5 and sacrificing at P14 or P23, we have shown the ECM is stable through this stage of development. Overall, H-link revealed novel populations of PNNs and their organizational pattern and developmental profiles. H-Link will be a useful tool to characterize the dynamic changes in brain ECM architectures during development and activity.

Disclosures: I. Sterin: None. **A. Niazi:** None. **J. Park:** None. **S. Park:** None.

Poster

375. Blood Brain Barrier

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 375.09

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NEI EY029227-01A1
NIH T32GM132057

Title: Sex-specific analysis of opioid transporter expression at the blood-retina barrier

Authors: *C.-T. BEREZIN[1], G. M. TORRES-LOPEZ[2], N. BERGUM[2], J. VIGH[2];
[1]Cell & Mol. Biol., [2]Biomed. Sci., Colorado State Univ., Fort Collins, CO

Abstract: Sex differences in the antinociceptive, as well as adverse, effects of opioid analgesics may be underscored by sex differences in the metabolism and pharmacokinetics of opioids. We

have recently shown that morphine accumulates in the mouse retina, but not hypothalamus, upon chronic systemic injection. This persistence appeared to be negatively related to the expression of P-glycoprotein, a major opioid extruder present at both the blood-brain barrier (BBB) and blood-retina barrier (BRB). In male mice, P-glycoprotein mRNA expression was higher in the hypothalamus than the retina. Previous studies have suggested that P-glycoprotein expression may be regulated by sex hormones (e.g. testosterone and progesterone). Here, we expand on our work by using immunohistochemistry and quantitative reverse-transcription PCR to interrogate the expression of three opioid transporters (P-glycoprotein, breast cancer resistance protein and multidrug resistance protein 2) in the retina and hypothalamus. We compare transporter expression in males, females with a high estrogen/progesterone ratio (i.e. estrus/proestrus) and females with a low estrogen/progesterone ratio (i.e. metestrus/diestrus).

Disclosures: **C. Berezin:** None. **G.M. Torres-Lopez:** None. **N. Bergum:** None. **J. Vigh:** None.

Poster

375. Blood Brain Barrier

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 375.10

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: Neurelis, Inc.
BioAxone BioSciences, Inc.
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Royal Netherlands Academy of Arts and Sciences Van Leersum Grant 2017 (to I.A.M.)
The Dutch Heart Foundation 2021 E. Dekker Grant (03-006-2021-T019 to I.A.M.)

Title: Nrl-1049, a selective rho-associated protein kinase 2 inhibitor, preserves the blood brain barrier in models of cold injury and ischemic stroke

Authors: I. E. MULDER[1,2], S. A. WOLLER[3], M. D. ABBINANTI[3], J. M. COUTINHO[2], H. E. DE VRIES[2], E. VAN BAVEL[2], K. M. ROSEN[3], ***M. A. LOPEZ-TOLEDANO**[4], J. GUTIERREZ[4], S. N. MISRA[4], A. L. RABINOWICZ[4], E. CARRAZANA[4,5], L. MCKERRACHER[3,6], C. AYATA[1];
[1]Massachusetts Gen. Hospital, Harvard Med. Sch., Boston, MA; [2]Amsterdam Univ. Med. Centers, Amsterdam, Netherlands; [3]BioAxone BioSciences Inc, Cambridge, MA; [4]Neurelis, Inc., San Diego, CA; [5]Univ. of Hawaii John A. Burns Sch. of Med., Honolulu, HI; [6]McGill Univ., Montreal, QC, Canada

Abstract: The vascular endothelium plays a vital role in maintenance of the blood-brain barrier (BBB). The BBB can become dysfunctional under pathological conditions (stroke, injury, seizure, vascular lesions [cerebral cavernous malformations]). Overactivation of rho-associated

protein kinase (ROCK) has been observed in vascular pathologies and can lead to endothelial/BBB dysfunction, and ROCK2 is the predominant isoform in the central nervous system. We examined the effects of a selective ROCK2 inhibitor, NRL-1049, to attenuate pathological alterations to the BBB in models of acute brain injury and stroke. ROCK2 activation was greater in a mouse model of brain injury (cold injury; n=5) demonstrated by Western blots showing increased phosphorylated cofilin (downstream marker of ROCK2) at perilesional sites 1 hour after injury (vs contralesional homotopic tissue). NRL-1049 vs vehicle was studied in brain injury and stroke models. Brain water content, a measure of BBB dysfunction, increased in the ipsilateral hemisphere 24 hours following cold injury in mice treated with vehicle. NRL-1049 significantly attenuated the increase in water content (P =0.003, 2-way ANOVA, Sidak; n=10/group). Interestingly, 6 mice in the vehicle group had developed seizures within 24 hours after cold injury. None of the NRL-1049-treated mice had seizures. Extravasation of Evans Blue, indicative of a loss of BBB integrity, was greater in the ipsilateral hemisphere 24 hours following cold injury in vehicle-treated mice (n=6) compared with sham-operated mice (n=3; P<0.001, 2-way ANOVA, Sidak), which did not receive cold injury or treatment. NRL-1049 treatment reduced Evans Blue extravasation (n=5; vs sham-operated mice; P=0.188, 2-way ANOVA, Sidak). In spontaneously hypertensive rats, transient middle cerebral artery occlusion (tMCAO) increased Evans Blue extravasation in the ischemic hemisphere 9 hours after tMCAO in the vehicle arm (n=18), an effect that was attenuated in rats that received NRL-1049 (n=16; P=0.047, Welch's t-test). The proportion of rats with intracerebral hemorrhage following tMCAO was dramatically lower with NRL-1049 treatment compared with vehicle (25% vs 78%; P=0.004, Chi square). The severity (grade) of hemorrhage was also lower with NRL-1049 (P=0.007, Mann Whitney). In summary, we show that NRL-1049, a ROCK2 inhibitor, attenuates BBB dysfunction in animal models of acute cerebral injury and stroke. Future studies are needed to explore the effectiveness and safety of NRL-1049 in humans for treatment of both acute (ischemic stroke, traumatic injury) and chronic (vascular lesions) conditions that involve disruption of normal BBB structure and function.

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Poster

375. Blood Brain Barrier

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 375.11

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH - R01NS113912

Title: Regulation of extracellular vesicles and iron transport from human blood-brain barrier endothelial cells

Authors: *K. PALSA[1], S. L. BARINGER[1], G. SHENOY[1], I. A. SIMPSON[2], J. R. CONNOR[1]; [1]Neurosurg., Penn State Col. of Med., Hershey, PA; [2]Neural and Behavioral Sci., Penn State Univ. Col. of Med., Hershey, PA

Abstract: Iron is essential for normal brain development and function. Hence, understanding the mechanisms of iron efflux at blood-brain barrier and their regulation is critical for the establishment of brain iron homeostasis. Here-in we investigated the role of extracellular vesicles (EVs) in mediating the transfer of H-Ferritin (FTH1) or Transferrin (Tf) across the blood-brain barrier endothelial cells (BBBECs). The study used ECs derived from human-induced pluripotent stem cells (hiPSC) that are grown in bicameral chambers. When cells were exposed to ^{55}Fe -Tf or ^{55}Fe-FTH1-, the ^{55}Fe activity in the EV fraction in the basal chamber was significantly higher, compared to the supernatant (non-EV) fraction. Furthermore, the release of endogenous Tf, FTH1, and EVs number is regulated by the iron concentration of the endothelial cells. Moreover, the release of exogenously added Tf or FTH1 to the basal side via EVs was significantly higher when ECs were iron loaded compared to when they were iron deficient. GW4869, a potent inhibitor of EVs blocked the EVs released from the ECs and also Tf, and FTH1 bound iron within the ECs. The release of EVs containing iron bound to Tf or FTH1 was independent of hepcidin regulation indicating this mechanism by-passes a major iron regulatory pathway. We further demonstrated that the EVs, containing FTH1 were taken up by co-cultured human astrocytes identifying a new pathway for these cells to obtain iron. These results indicate that iron transport across the BBB is mediated via the EVs pathway and is modified by iron status of the ECs, providing evidence for novel alternate mechanisms of iron transport into the brain.

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Poster

375. Blood Brain Barrier

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 375.12

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Title: An experimentally validated simulation method to predict brain exposure of chemicals and drugs

Authors: *M. ULMSCHNEIDER[1,2], N. A. BERGLUND[3];
[1]King's Col. London, London, United Kingdom; [2]Eve BioTek Ltd, London, United Kingdom; [3]Aarhus Univ., Aarhus, Denmark

Abstract: Drug development for the treatment of central nervous system (CNS) diseases is extremely challenging, in large part due to the difficulty in crossing the blood-brain barrier (BBB). Here we develop and experimentally validate a new in silico method to predict quantitatively the BBB permeability for small-molecule drugs. We show accurate prediction of solute permeabilities at physiological temperature using high-temperature unbiased atomic detail molecular dynamics simulations of spontaneous drug diffusion across BBB bilayers. These simulations provide atomic detail insights into the transport mechanisms, as well as converged kinetics and thermodynamics. The method is validated computationally against physiological temperature simulations for fast-diffusing compounds, as well as experimentally by direct determination of the compound permeabilities using a transwell assay as an in vitro BBB model. The overall agreement of the predicted values with both direct simulations at physiological temperatures and experimental data is excellent. This new tool has the potential to replace current semi-empirical in silico screening and in vitro permeability measurements in CNS drug discovery.

Disclosures: M. Ulmschneider: None. **N.A. Berglund:** None.

Poster

375. Blood Brain Barrier

Location: SDCC Halls B-H

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Program #/Poster #: 375.13

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: R01NS113912-01

Title: Regulation of iron uptake via transferrin at the blood-brain barrier

Authors: *S. BARINGER[1], E. NEELY[1], K. PALSA[2], I. A. SIMPSON[3], J. R. CONNOR[1];
[2]Neurosurg., [1]Penn State Col. of Med., Hershey, PA; [3]Penn State Univ. Col. of Med., Hershey, PA

Abstract: While iron plays an important role in many bodily functions, it is especially crucial to the highly metabolically active brain. As such, maintaining a proper balance of brain iron levels is vital to cellular function. Excessive amounts of brain iron can lead to neurodegenerative

diseases and inadequate amounts can lead to cognitive impairment and Restless Legs Syndrome. Regulation of iron uptake into the brain is required, however the mechanism of such regulation is largely unknown. We have previously shown that brain iron uptake is mediated at the level of the endothelial cells (ECs) of the blood-brain barrier (BBB) and regulation is modulated by apo (iron poor) and holo (iron rich) transferrin (Tf) in the cerebrospinal fluid (CSF). Here we aimed to address the mechanism by which apo- and holo-Tf control iron release from ECs. Using an in vivo steady state ventricular infusion, we demonstrate that increasing brain-side apo- (iron poor) transferrin (Tf) significantly increases 55Fe-Tf into the brain and microvasculature ($p<0.05$). Additionally, we note that females do not have the same immediate 55Fe-Tf uptake response as males do. To investigate the mechanism of Tf's regulation on iron release from endothelial cells (ECs) of the BBB, we employed hiPSC-derived ECs. ECs cultured on bi-chamber plates were exposed to apo- or holo (iron rich)-Tf in the basal (brain side) chamber. After cell were collected for western blotting, we found that holo-Tf reduced ferroportin (Fpn) levels by 50% ($p<0.05$) and this decrease was prevented when inhibiting Fpn's degradation. Collectively, these results demonstrate that apo- and holo-Tf modulate iron release from ECs through interactions with Fpn and its complex proteins. These data establish the mechanism for regulation of iron uptake into the brain that can be explored for drug delivery and potential treatment of iron dysregulation disorders.

Disclosures: S. Baringer: None. **E. Neely:** None. **K. Palsa:** None. **I.A. Simpson:** None. **J.R. Connor:** None.

Poster

375. Blood Brain Barrier

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 375.14

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant 2RF1AG039452-06
NIH Grant AG023084
NIH Grant NS034467

Title: iPSC-derived human CNS pericyte-like cells protect blood brain barrier integrity and neuronal function in pericyte-deficient mice

Authors: *A. BOSWORTH, A. P. SAGARE, K. KISLER, Y. WANG, A. CHAKHOYAN, A. R. NELSON, A. MONTAGNE, M. T. HUUSKONEN, C. TORRES-SEPULVEDA, J. B. STANLEY, B. V. ZLOKOVIC;
Zilkha Neurogenetic Inst., Keck Sch. of Med. of USC, Los Angeles, CA

Abstract: Brain vascular dysfunction contributes to the development of neurodegenerative disorders, including Alzheimer's disease (AD). We and others have shown that pericytes, which are vascular mural cells embedded in the wall of brain microvessels, play an essential role in the

central nervous system (CNS) in regulating blood-brain barrier (BBB) integrity, controlling cerebral blood flow, clearing neurotoxins, providing neurotrophic support, and angiogenesis. Previous studies by our group have shown that pericyte degeneration and loss occurs in AD and that pericyte loss in the AD mouse model results in a significant increase in BBB leakage and neuronal loss. The goal of the present investigation is to generate and characterize induced pluripotent stem cell (iPSC)-derived cranial pericyte-like cells (iPSC-PCs), then to assess their potential as a cell therapy treatment. First, we show that iPSC-PCs express pericyte specific proteins at similar levels as primary human adult forebrain pericytes. Then, we show that iPSC-PCs home to microvessels in live brain tissue slices from pericyte-deficient mice. Finally, we use dynamic contrast enhanced-MRI to show that transplantation of iPSC-PCs into hippocampi of pericyte-deficient mice leads to restoration of BBB integrity and improved neuronal retention. These fully-characterized iPSC-PCs that share molecular and phenotypic similarities with endogenous human adult brain microvascular pericytes hold potential as a future non-neuronal pericyte-based cell therapy for AD and related neurodegenerative disorders.

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Poster

376. Neurovascular Coupling

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 376.01

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

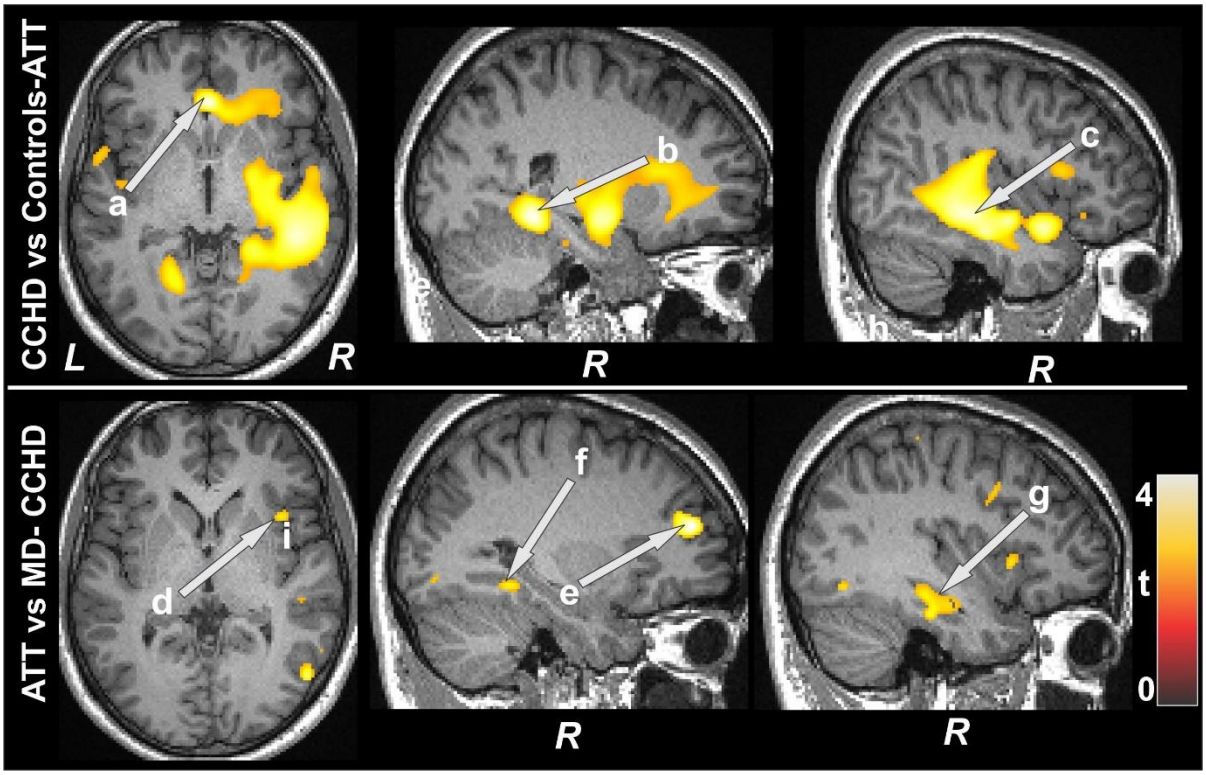
Support: R01NR 013930
R01NR016463

Title: Cerebral Artery Integrity Evaluation and Associations with Brain Tissue Status in Adolescents with Complex Congenital Heart Disease

Authors: *B. ROY[1], N. A. PIKE[2], C. CABRERA-MINO[2], X. SHAO[7], N. J. HALNON[3], A. B. LEWIS[8], D. J. J. WANG[7], R. KUMAR[1,4,5,6];
[1]Anesthesiol., [2]UCLA Sch. of Nursing, [3]Pediatric Cardiol., [4]Radiological Sci., [5]Bioengineering, [6]Brain Res. Inst., Univ. of California Los Angeles, Los Angeles, CA; [7]USC, Los Angeles, CA; [8]Pediatric Cardiol., Children's Hosp. Los Angeles, Los Angeles, CA

Abstract: Introduction: Adolescents with complex congenital heart disease (CCHD) show brain changes in regions associated with mood, cognition, and autonomic functions. However, the underlying etiology for tissue changes in CCHD is unknown. Alteration in cerebral artery integrity (CAI; measured by arterial transit time [ATT]) can be a potential cause of tissue changes in CCHD. However, no published reports are available examining whole-brain CAI and associations between CAI and brain tissue changes in CCHD. Methods: 70 subjects (37 CCHD /

33 controls), 14-18 years of age, undergone surgical palliation, and no contraindications for a magnetic resonance imaging (MRI). Healthy controls were age- and gender-matched to CCHD subjects. Participants completed a brain MRI using 3.0 Tesla scanner. ATT values (via diffusion-weighted pseudo-continuous arterial spin labeling [pCASL] procedures) and regional mean diffusivity [MD] (measure of tissue integrity) were measured, and ANCOVA and partial correlations (covariates, age and sex) were computed to examine ATT differences between CCHS and controls, and correlations between ATT and MD values in CCHD patients, respectively.Results: ATT was significantly increased in CCHD in the parahippocampal gyrus, mid temporal cortices and white matter, anterior cingulate, indicating impaired CAI, compared to controls (Fig 1; q-FDR corrections, $p<0.05$). Partial correlations between ATT and MD values in CCHS patients showed positive correlations in the right hippocampus, bilateral prefrontal cortices, parahippocampal gyrus, frontal white matter, right anterior and posterior cingulate, right insula, right frontal cortex, left temporal cortices, and bilateral midbrain (Fig 1). Conclusion: Adolescents with CCHD have impaired CAI and show associations with tissue changes, indicating role of CAI to brain tissue integrity.



Disclosures: **B. Roy:** None. **N.A. Pike:** None. **C. Cabrera-Mino:** None. **X. Shao:** None. **N.J. Halnon:** None. **A.B. Lewis:** None. **D.J.J. Wang:** None. **R. Kumar:** None.

Poster

376. Neurovascular Coupling

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 376.02

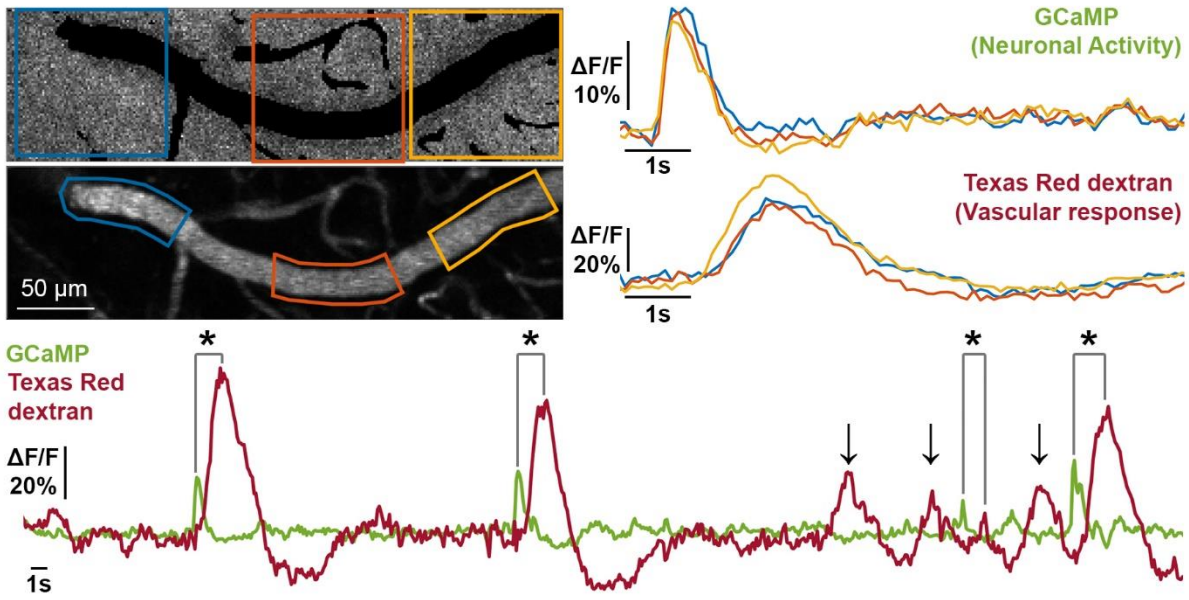
Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH U19-NS123717

Title: Fast, volumetric 2-photon imaging of pial arteriolar dynamics and surrounding neuronal activity

Authors: K. KILIC, J. T. GIBLIN, I. A. CHEN, J. X. JIANG, S.-W. PARK, M. THUNEMANN, D. A. BOAS, A. DEVOR;
Biomed. Engin., Boston Univ., Boston, MA

Abstract: Pial cortical arterioles integrate local neuronal activity with the intrinsic arteriolar vasomotion as well as subcortical and ascending inputs. Previously, we hypothesized that this integration could produce dynamic patterns of coherent oscillations in the arteriolar diameter effectively parcellating the cortical mantle potentially offering a biophysical explanation for '*functional connectivity networks*' observed with fMRI [1]. In mice, 2-photon microscopy with an axially extended Bessel focus is perfectly suited for addressing this hypothesis providing large-scale, simultaneous measurements of the arteriolar diameter [2] and neuronal activity. Combined with high-speed galvanometer scanners, this technology allows continuous monitoring of dilation along hundreds of microns of arteriolar length simultaneous with measurements of surrounding neuronal activity. We used awake GCaMP transgenic mice with 'crystal skull' optical windows [3]. Vasculature was labeled with Texas Red dextran. Data were acquired by scanning a Bessel focus with 1.0 NA and 100-μm axial FWHM. ~350x100x100 μm^3 volumes with ~1.35 μm resolution in xy were acquired at ~10Hz. With Bessel 2-photon imaging, the fluorescence signal arising from the pial vascular lumen is proportional to the vessel diameter [2]. GCaMP transients reflecting brief epoch of increased neuronal activity preceded arteriolar dilation events (marked with *). With our temporal resolution of ~100 ms, the GCaMP signal increased simultaneously across the imaged field of view. Despite this synchronous onset of neuronal activity, some locations along the arteriole were leading some other locations by up to ~200 ms. In addition, we observed slow fluctuations in the arteriolar diameter (< 1Hz) that were independent from the GCaMP signal (marked with ↓). Experiments are underway to determine the underlying mechanisms for this vasodilation dynamics. References: [1] Mächler, P. *et al*. *Curr Opin Biomed Eng* **18** (2021). [2] Fan, J. L. *et al. Nat Commun* **11**, 6020 (2020). [3] Kim, T. H. *et al. Cell Rep* **17**, 3385-3394 (2016).



Disclosures: **K. Kilic:** None. **J.T. Giblin:** None. **I.A. Chen:** None. **J.X. Jiang:** None. **S. Park:** None. **M. Thunemann:** None. **D.A. Boas:** None. **A. Devor:** None.

Poster

376. Neurovascular Coupling

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 376.03

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant R01-HL-102457

Title: The effects of one-year aerobic exercise on cerebral blood flow regulation in cognitively normal older adults

Authors: *A. VERMA[1,2], T. TOMOTO[2,3], R. ZHANG[2,3];
[1]Sch. of Behavioral and Brain Sci., Univ. of Texas At Dallas, Richardson, TX; [2]Inst. of Exercise and Envrn. Med., Texas Hlth. Presbyterian Hosp. Dallas, Dallas, TX; [3]Dept. of Neurol., UT Southwestern Med. Ctr., Dallas, TX

Abstract: Age-related decline in cerebrovascular function is associated with cognitive impairment and may lead to neurodegenerative diseases. Aerobic exercise may improve cerebral blood flow (CBF) regulation; however, the impacts of aerobic exercise on CBF and cerebrovascular resistance (CVR) in older adults are inconclusive. The purpose of this study was to investigate the effects of aerobic exercise on CBF and CVR in cognitively normal older adults. Seventy-three cognitively normal older adults were randomized to 12-months of progressive, moderate-to-vigorous aerobic exercise training (AET, n=37) or stretching-and-toning (SAT,

active control, n=36). Cardiorespiratory fitness was assessed by peak oxygen uptake ($\dot{V}O_{2peak}$). CBF was measured as the sum of volumetric blood flow from both the internal carotid and vertebral arteries using ultrasonography and divided by total brain tissue mass measured by magnetic resonance imaging to obtain normalized CBF (nCBF). Mean arterial pressure (MAP) was calculated by the area under the curve of the brachial pressure waveform that was obtained using applanation tonometry. CVR was calculated as MAP divided by total CBF. 56 subjects (AET, n=28, SAT, n=28) completed the one-year intervention. One-year AET improved $\dot{V}O_{2peak}$, increased CBF, and decreased CVR. Increases in $\dot{V}O_{2peak}$ were associated with increased nCBF (r=0.621, p=0.001) and decreased nCVR (r=-0.412, p=0.037) in the AET group but not in the SAT group. These results suggest that aerobic exercise improves cerebrovascular function, which may benefit brain health and delay cognitive impairment in older adults.

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Poster

376. Neurovascular Coupling

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Program #/Poster #: 376.04

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: 1R01AG066645
5R01NS115401
1DP2NS121347-01
5T32GM008181-33
19IPLOI34660108

Title: Neuronal reprogramming of endothelial calcium signaling

Authors: *A. VIGDERMAN[1], C. A. CALARCO[2], R. R. CAMPBELL[2], M. LOBO[2], T. LONGDEN[3];
[1]Univ. of Maryland Sch. of Med. Program In Neurosci., Baltimore, MD; [2]Anat. and Neurobio., [3]Univ. of Maryland Baltimore, Baltimore, MD

Abstract: Neuronal network activity is associated with an increase in the delivery of glucose and oxygen via increased blood flow through nearby vascular networks. This process is known as functional hyperemia (FH) and is enacted by a collection of blood flow control mechanisms termed neurovascular coupling (NVC). In rodents, environmental exploration via whisking engages NVC mechanisms and FH in the barrel cortex. Plasticity in response to chronic whisker stimulation in neuronal networks is well established, but whether this is accompanied by plasticity in endothelial cells (ECs), the cellular constituents of local capillaries, to adjust blood flow control mechanisms and reshape FH to meet changing energy requirements has not been examined. To explore this possibility, we characterized changes in activity at the level of neurons and ECs following environmental enrichment designed to produce a large increase in input to the

barrel cortex. Using two-photon laser scanning microscopy, we analyzed calcium (Ca^{2+}) transients in Thy1-expressing neurons engineered to express the Ca^{2+} biosensor GCaMP6f and concurrent local blood flow responses in mice housed in an enriched environment over 7 days. We also imaged Ca^{2+} transients in GCaMP8-expressing capillary ECs in a separate group of mice and observed profound alterations in Ca^{2+} transients in environmentally enriched animals. This is accompanied by a dramatic increase in Ca^{2+}-permeable TRPV4 channel current density in the membranes of isolated capillary ECs. Taken together, our data suggest that neuronal activity reshapes the molecular composition of the EC membrane to augment Ca^{2+}-based blood flow control mechanisms, which likely contributes to continual reprogramming of the blood flow response to optimize blood delivery. These experiments lay the foundation for further exploration of the mechanisms of this 'vascular signaling plasticity' phenomenon and its role in optimization of energy usage and delivery in the brain.

Disclosures: A. Vigderman: None. **T. Longden:** None.

Poster

376. Neurovascular Coupling

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Program #/Poster #: 376.05

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant R01NS078168
NIH Grant R01NS079737

Title: A cellular substrate for ultra-slow bilateral hemodynamic correlations

Authors: *K. L. TURNER[1], M. HOSSAIN[1], D. I. GREENAWALT[2], Q. ZHANG[3], K. W. GHERES[3], P. J. DREW[3];
[1]Dept. of Biomed. Engin., [2]Grad. Program in Molecular, Cellular, and Integrative Biosci., [3]Dept. of Engin. Sci. and Mechanics, The Pennsylvania State Univ., University Park, PA

Abstract: Hemodynamic signals in the brain are used to infer local neural activity. Understanding which neurons modulate the arousal-state-dependent strength of neurovascular coupling (NVC) and functional correlations in the hemodynamic signals between brain regions is important for understanding these signals. Previous work has shown that the activity of neuronal nitric oxide synthase (nNOS) expressing neurons play an important role in NVC and setting the basal diameter of arterioles. To explore the role of these neurons in NVC and bilateral hemodynamic correlations, we selectively removed a subset of neuronal nitric oxide synthase (nNOS) expressing interneurons, known as type-1 nNOS neurons, from the whisker-related region of somatosensory cortex of mice using a targeted ablation technique. We concurrently measured cerebral blood volume and neural activity in these mice during sleep and wake states. We also measured the dynamics of pial and penetrating arteriole diameter changes using 2-photon microscopy. Ablation of these neurons had little effect on sensory-evoked and fidgeting-

evoked neural and hemodynamic responses but did decrease the bilateral correlations in gamma-band power and blood volume in the somatosensory cortex at ultra-low frequencies (~0.003-0.1 Hz). Ablation of type-1 nNOS neurons reduced the coherence between gamma-band power and hemodynamic signals in the ultra-low frequency band. Our results suggest that type-1 nNOS neurons may play a role in synchronizing neural and vascular signals across hemispheres at ultra-low frequencies.

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Poster

376. Neurovascular Coupling

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 376.06

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Title: An atlas of the developing post-natal cerebral vasculature

Authors: *S. SKRIABINE[1], E. DE LAUNOIT[2], A. VIEITES PRADO[3], T. DUPONT[5], S. MECHAUSSIER[5], O. POSTAL[5], M. GAGLIARDINI[5], F. DE VICO FALLANI[4], N. MICHALSKI[5], N. RENIER[4];
[1]Paris Brain Inst., [2]Neurosciences, [3]Lab. of Structural Plasticity, [4]ICM Inst. du Cerveau et de la Moelle Epinière, Paris, France; [5]Inst. Pasteur, Paris, France

Abstract: The brain is densely perfused by the vascular network, which provides nutrients and oxygen to support neuronal function. The architecture of the cerebral vasculature addresses specific constrains of the neural tissues, including the near absence of energy storage and very high metabolic demand. Despite the clear observation that both the vascular density and the metabolic demands are heterogenous across the brain, whether and how neuronal activity controls the vascular topology is still debated for several reasons: first, there is no correlation between the densities of neurons and blood vessels. Second, the organization of the vascular and neuronal networks don't match closely. Third, there is disagreement in the literature on whether modulating neuronal activity levels can lead to a remodeling of the vasculature or not. To better understand the relationship between the metabolic need of the different neural cell types and the topology of the adult vascular network, we built a 3D developmental atlas of the brain vasculature. For this, we generated the annotation maps and templates for the developing mouse brain to align vascular datasets onto. We next optimized a series of computational tools to measure and classify the organization of the different brain regions. We used these tools to generate a system's view of the developmental trajectories for the various brain regions. Finally, we tested in different models of neuronal activity modulation its impact on the development and maintenance of the network. This work reveals how the vascular network can cater differently to the metabolic needs of both the developing and adult brain, and how cerebral networks shape the development and maintenance of the cerebral vasculature.

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Poster

376. Neurovascular Coupling

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Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

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JSPS KAKENHI

Title: Liver-secreted fluorescent blood plasma markers enable chronic imaging of microcirculation

Authors: X. WANG[1], C. DELLE[1], ***A. ASIMINAS**[1], S. AKTHER[1], M. VITTANI[1], P. BRØGGER[1], P. KUSK[1], C. T. VO[1], A. KONNO[2,3], H. HIRAI[2,3], M. FUKUDA[4,5], P. WEIKOP[1], S. A. GOLDMAN[6,1], M. NEDERGAARD[1,6], H. HIRASE[6,1];
[1]Ctr. for Translational Neuromedicine, Univ. of Copenhagen, Copenhagen, Denmark; [2]Gunma Univ. Initiative for Advanced Res., Viral Vector Core, Japan; [3]Dept. of Neurophysiol. & Neural Repair, Gunma Univ. Grad. Sch. of Med., Gunma, Japan; [4]Program in Neurosci. and Behavioral Disorders, Duke-Nus Grad. Med. Sch. Singapore, Singapore, Singapore; [5]Intl. Res. Ctr. for Med. Sci., Kumamoto Univ., Kumamoto, Japan; [6]Ctr. for Translational Neuromedicine, Univ. of Rochester Med. Ctr., Rochester, NY

Abstract: Studying blood microcirculation is vital for gaining insights into vascular diseases. Blood flow imaging in deep tissue of animal models is typically achieved by introducing fluorescent dyes in the blood plasma. Acute administration of fluorescent tracers is invasive, and the plasma fluorescence decreases within an hour of administration. Here, we report a novel approach for the longitudinal study of vasculature. Using a single intraperitoneal or intravenous administration of viral vectors, we express fluorescent secretory albumin-fusion proteins in the liver to chronically label the blood circulation in mice. All segments of the vasculature in brain and peripheral tissue are observable by two-photon microscopy within two weeks of vector administration. This approach allows for longitudinal observation of circulation for over four months without the need for repeated administration of fluorescent dyes. We demonstrate the chronic assessment of vascular functions, including functional hyperaemia and vascular plasticity, in micro- and mesoscopic scales. This genetic plasma labelling approach represents a

versatile and cost-effective method for the chronic investigation of vasculature functions across the body in health and disease animal models.

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Poster

376. Neurovascular Coupling

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Title: Two-photon imaging showed serotonin modulates sensory-induced functional hyperemia and astrocyte Ca2+ transients in awake, behaving mice

Authors: R. RENDEN, K. SHARMA, ***C.-H. TRAN**;
Physiol. and Cell Biol., Univ. of Nevada Reno, Reno, NV

Abstract: The brain possesses control mechanisms to regulate blood flow to meet moment-to-moment metabolic demands. Functional hyperemia is an essential process in which cerebral blood flow is increased in response to increased neural activity. Astrocytes, which form an associated network with both neurons and blood vessels, have been shown to play a significant role in controlling blood flow accompanying changes in neural activity. Significant progress has been made in understanding the essential role of localized synaptic glutamatergic signaling in regulating local cerebral blood flow (CBF) increase in response to increased neuronal activity, a process known as neurovascular coupling (NVC). Nevertheless, little is currently known about the integration between the neural (e.g., neurons and/or astrocytes) and vascular network and the broader cellular and molecular mechanisms underlying the spatiotemporal coordination of local and global vascular responses within the cortical angioarchitecture and among different brain regions. In particular, the role of serotonergic signaling in controlling cerebral blood flow remains to be determined. Our objective was to uncover the contributions of serotonin to NVC in fully awake, behaving mice. A craniotomy over the barrel cortex with the dura removed was performed. Two-photon microscopy was used to image the vasculature and astrocytes Ca2+ from Aldh1l1Cre-ERT2 x GCaMP6f mice. We found that the state of alert and wakefulness associated with the release of long-range modulatory neurotransmitters altered vascular responses and astrocyte Ca2+ dynamics. Additionally, manipulations of serotonin release and blockage of its receptors using neurotoxin (i.e., 5,7DHT) and other pharmacological reagent (e.g., Ketanserin)

modulated sensory-induced vasodilation and endfoot Ca2+. Our data introduce a modulatory role of serotonergic signaling in NVC.

Disclosures: **R. Renden:** None. **K. Sharma:** None. **C. Tran:** None.

Poster

376. Neurovascular Coupling

Location: SDCC Halls B-H

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Program #/Poster #: 376.09

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Title: Astrocytic endothelial nitric oxide synthase responds to cholinergic stimulation in mouse somatosensory cortex

Authors: *B. LE GAC[1,2,3], X. WANG[1,2], D. VALLERAND[1,2,3], A. LESSARD[4], H. GIROUARD[1,2,3];
[1]Physiologie et pharmacologie, Univ. de Montéal, Montréal, QC, Canada; [2]Groupe de Recherche sur le Système Nerveux Central (GRSNC), Montréal, QC, Canada; [3]Ctr. interdisciplinaire de recherche sur le cerveau et l'apprentissage (CIRCA), Montréal, QC, Canada; [4]Univ. of Maryland, Baltimore, MD

Abstract: Neurovascular coupling (NVC) is the close relationship between neuronal activity and the local increase in blood perfusion, essential for brain function. Among vasoactive messengers involved in NVC, nitric oxide (NO) is a major vasodilator produced by neurons and the endothelium through the so-called neuronal NO synthase (nNOS) and endothelium NO synthase (eNOS), respectively. Astrocytes also participate to the NVC but their capacity to produce NO remains uncertain. We therefore sought to determine the sources and activators of astrocytic NO production in mice. To test this hypothesis, we used electronic microscopy, immunohistochemical and fluorophore labelling in astrocytes primary culture and brain slices in wild-type (WT) and transgenic mice. We show that astrocytes express eNOS preferentially distributed in perineuronal (71 ± 5 %, mean ± sem) compared to perivascular processes (29 ± 5 %). More specifically, astrocytic eNOS is spatially connected to cholinergic neurons. To determine if astrocytes can produce NO in response to acetylcholine (ACh), we detect NO with the fluorescent NO indicator DAF-FM *in vitro* and *ex vivo* of WT mice and knock-out mice for eNOS and nNOS ($eNOS^{-/-}$ and $nNOS^{-/-}$ respectively). We show *in vitro* that Ach induces 21.8 % increase in NO production that mostly relies on eNOS signalling as there was no production in $eNOS^{-/-}$ mice. In brain slices, Ach induces localized NO synthesis in astrocytes. The number of NO hot spots after cholinergic stimulation were identical in WT (8.00 ± 1.53), $nNOS^{-/-}$ (7.67 ± 1.20) and $eNOS^{-/-}$ (8.00 ± 2.00) mice but their intensity was reduced in $eNOS^{-/-}$ (11.5 %) and $nNOS^{-/-}$ (11.1 %), compared to WT group (22.0 %). We also eliminated pools of thiol-bounded NO using a thiol remover. After thiol-bound NO removing, there was no significant difference between WT and $nNOS^{-/-}$ groups in the number (4.67 ± 0.33 vs. 4.67 ± 0.33) and strength of NO hot spots (0.198 ± 0.033 vs. 0.185 ± 0.040), while in $eNOS^{-/-}$, no new hot spot was induced in

response to ACh. These results show that astrocytes can produce NO from eNOS in response to Ach. This project will allow us to better understand the role of astrocytic NO in NVC during cholinergic activations. Knowing that cholinergic neurons are more vulnerables in neurodegenerative diseases, this pathway may help to better understand how NVC become impaired in neurodegenerative diseases.

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Poster

376. Neurovascular Coupling

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Program #/Poster #: 376.10

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant U19 NS123717

Title: Competing vascular and neuronal inputs shape pial vasomotor dynamics across the cortical mantle

Authors: T. BROGGINI[1,5], K. CHHABRIA[1], ***J. DUCKWORTH**[1], C. HERNÁNDEZ[6], X. JI[1], R. LIU[1], X. XIA[2], B. FRIEDMAN[3], C. MATÉO[1], I. SHAKED[1], M. KOTLIKOFF[7], G. MISHNE[2], M. VERGASSOLA[1,6], D. KLEINFELD[1,4];
[1]Dept. of Physics, [2]Halıcıoğlu Data Sci. Inst., [3]Dept. of Computer Sci. and Engin., [4]Section of Neurobio., Univ. of California San Diego, La Jolla, CA; [5]Universitätsklinikum Frankfurt, Frankfurt, Germany; [6]Dept. de Physique, l'Ecole Normale Supérieure, Paris, France; [7]Dept. of Biomed. Sci., Cornell Univ. Col. of Vet. Med., Ithaca, NY

Abstract: The pial arteriole network distributes blood across the surface of neocortex to nourish underlying brain cells. These pial arterioles and the vessels they source, the penetrating arterioles, intrinsically dilate at vasomotor frequencies (~ 0.1 Hz), interact with neighboring arterioles, and can be modulated by underlying neuronal activity. An interpretation of resting-state fMRI data suggests that oscillations of the pial network can parcellate into phase-locked regions. To explore this interpretation biophysically, we used optical imaging to simultaneously measure arteriole diameter and pan-neuronal activity across the murine cortical mantle. We found that the spatial extent of neurovascular entrainment sharply peaks when sensory-driven and vasomotor frequencies match. This resonance implies that the pial network may be abstracted as a two-dimensional network of coupled oscillators. Moreover, using localized optogenetic activation, we found that subcortical regions that modulate blood flow can mask sensory-driven responses. We conclude that parcellation is dependent on the frequency of intrinsic vasomotion, competition between neighboring pial branch inputs, and underlying neural input. Our data reveal the importance of intrinsic vasomotion in arterial parcellation dynamics, indicates that only part of the pial vasodynamics are explained by neuronal activity, and suggests

that manipulating the frequency of a stimulus relative to the vasomotion frequency can alter the fMRI signal.

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Poster

376. Neurovascular Coupling

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Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH T32 NS007220
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Title: Optogenetic activation of potassium channels in cortical neurons of awake mice increases local blood flow despite inhibiting neuronal activity

Authors: ***B. HOLLOWAY**[1], A. DESOUZA[2], B. L. BLOODGOOD[4], D. KLEINFELD[3]; [1]Univ. of California San Diego, San Diego, CA; [2]Dept. of Neurosci., [3]Univ. of California San Diego, La Jolla, CA; [4]Divsion of Biol. Science, Neurobio., UCSD Dept. of Neurosciences, La Jolla, CA

Abstract: Cerebral blood flow is coupled to neuronal activity. However, changes in blood flow proceed tissue oxygen and glucose depletion. Neuronal activation has multiple consequences that could facilitate neurovascular coupling, and interruption of several pathways mitigates the blood flow response. Here, we isolated the accumulation of neuronal potassium in tissue from other consequences of neuronal activation, and test whether a transient potassium efflux from cortical neurons is sufficient to increase local blood flow despite inhibition of neuronal activity. We injected AAV-mCherry-PAC-K into the superficial layer of mouse cortex (23nL; -200µm cortical surface). After six weeks this led to expression of a light-sensitive adenylyl cyclase, a cyclic nucleotide-gated potassium channel, and an mCherry tag in neurons. To test the functionality of the photoactivatable adenylyl cyclase-potassium channel system, we performed whole-cell patch clamp electrophysiology on neurons expressing mCherry in slice. In voltage clamp, illumination (445nm; 0.01, 0.1, or 1s) of mCherry+ neurons generated an outward current with a reversal potential near E_K (-93mV). In current clamp, we injected current until neurons fired regularly (2-10Hz). Illumination hyperpolarized and silenced neurons for extended periods (>60s). To measure red blood cell velocity, we performed a craniotomy and installed a cranial window (4x4mm) above the injection site in a subset of injected mice. After a week of recovery, we labeled blood via FITC dextran (2MDa, 50uL) injected intravenously via the retro-orbital

sinus. We then used two photon laser scanning microscopy to measure red blood cell velocity in vessels near the injection site before, during, and after laser pulses (445nm; 0.01, 0.1, or 1s; OBIS-LX). Illumination of mCherry+ neurons resulted in an increase in velocity of red blood cells in vessels near the injection site in awake mice. These results suggest that increases in neuronally-derived interstitial potassium is sufficient to drive neurovascular coupling. The threshold level of potassium needed to initiate this process is under study.

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Poster

376. Neurovascular Coupling

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Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

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Title: Public volume electron microscopy resources to study the brain microvasculature

Authors: *A. Y. SHIH[1], S. K. BONNEY[1], V. COELHO-SANTOS[1], S.-F. HUANG[2], M. M. TAKENO[3], J. KORNFELD[4], A. KELLER[2];
[1]Seattle Children's Res. Inst., Seattle, WA; [2]Dept. of Neurosurg., Univ. Hosp. Zürich, Univ. of Zürich, Zürich, Switzerland; [3]Neural Coding, Allen Inst. For Brain Sci., Seattle, WA; [4]Max Planck Inst. of Neurobiology,, Planegg, Germany

Abstract: Electron microscopy is the primary approach to study ultrastructural features of the cerebrovasculature. However, 2D snapshots of a vascular bed capture only a small fraction of its complexity. Recent large-scale efforts to map neuronal circuitry using volume electron microscopy have also sampled the brain microvasculature in 3D. These data sets span different species and brain regions, including two data sets from the MICrONS consortium that have made efforts to segment vasculature in addition to all parenchymal cell types in mouse visual cortex. Exploration of these data have revealed rich information for detailed investigation of the cerebrovasculature. Neurovascular cell types (including, but not limited to, endothelial cells, mural cells, perivascular fibroblasts, microglia, and astrocytes) could be discerned across broad microvascular zones. Image contrast was sufficient to identify subcellular details, including endothelial junctions, caveolae, peg-and-socket interactions, mitochondria, Golgi cisternae,

microvilli and other cellular protrusions of potential significance to vascular signaling. Additionally, non-cellular structures including the basement membrane and perivascular spaces were visible and could be traced between arterio-venous zones along the vascular wall. These explorations revealed structural features that are important for vascular functions, such as blood-brain barrier integrity, blood flow control, brain clearance, and bioenergetics. We will introduce these valuable community resources and provide: (1) guidance on how to access and navigate the data sets online, (2) examples of unique cerebrovascular biology that can be explored in 3D, (3) information on ways to extract and analyze data for use in independent research projects, and (4) information on current limitations of the data.

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Poster

376. Neurovascular Coupling

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Title: Neurovascular coupling is preserved in healthy, aged mice

Authors: *Q. ZHANG[1], H. C. BENNETT[2], Y. KIM[2], P. J. DREW[1,3];
[1]Dept. of Engin. Sci. and Mechanics, The Pennsylvania State Univ., University Park, PA; [2]Dept. of Neural and Behavioral Sci., The Pennsylvania State Univ., Hershey, PA; [3]Dept. of Biomed. Engin. and Neurosurg., The Pennsylvania State Univ., University Park, PA

Abstract: Cerebrovascular malfunction has been implicated in age-related cognitive decline and dementia, but the underlying vascular mechanisms are not well understood. An improved understanding of the nature of normal cerebrovascular aging is needed to help establish the role that vascular dysfunction might play in cognitive decline and dementia. Here, we investigated how the vascular morphology changes accompanying healthy aging impacts brain hemodynamics during rest, and in response to voluntary locomotion in awake, head-fixed mice, using intrinsic optical signal (IOS) imaging and two-photon laser scanning microscopy (2PLSM). We focused our analysis in two functionally distinct cortical regions, the forelimb/hindlimb representation of the somatosensory cortex (FL/HL) and a frontal cortical region (FC) including anterior lateral motor cortex (ALM). We targeted ALM because it is involved in motor planning and performs “higher-order” cognitive functions in mouse, which makes it analogous to human prefrontal cortex. We tested mice at 2 months (young), 18 months and 24 months (aged) of age.Using 2PLSM, we found that healthy aging increased vessel

tortuosity. To understand how the vasculature morphology change during aging affects cerebral hemodynamics, we first assessed the spatial extent of cortical hemodynamic responses and their relationship to voluntary locomotion using IOS imaging. During locomotion, we observed region-specific changes in total hemoglobin concentration (ΔHbT). In young mice, there was a pronounced increase in ΔHbT (corresponding to an increase in cerebral blood volume) in FL/HL, while in FC there was no change, or a slight decrease in ΔHbT. Healthy aging does not affect locomotion-evoked responses of ΔHbT. As the proper vasculature function is important for oxygen delivery, we further compared the aging effects on brain tissue oxygenation responses, using the difference between oxy- and deoxy-hemoglobin concentration (ΔHbO-HbR) as an indicator. Healthy aging does not affect locomotion-evoked global increase of oxygenation.To test how the brain hemodynamics change with aging during resting-state, we compared the variance of the ΔHbT and ΔHbO-HbR. In aged mice, the variance of ΔHbT and ΔHbO-HbR is significantly smaller than young adult mice in FC, but similar in FL/HL. These results suggest that aging brain vasculature has smaller buffering capacity of brain hemodynamic oscillations in FC.In conclusion, the results of this study suggest that neurovascular coupling is preserved in healthy aging. However, healthy aging may weaken the brain vasculature's capacity to combat sudden changes of blood supply.

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Poster

376. Neurovascular Coupling

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Title: In vivo single cell optical ablation to study brain pericyte function in health and disease

Authors: *C. D. NIELSON[1,2], A.-A. BERTHIAUME[2,3], V. COELHO-SANTOS[2], S. K. BONNEY[2], A. Y. SHIH[2,4,5];
[1]Univ. of Washington Grad. Program In Neurosci., Seattle, WA; [2]Ctr. for Developmental Biol. and Regenerative Med., Seattle Children's Res. Inst., Seattle, WA; [3]Dept. of Neurosci., Med. Univ. of South Carolina, Charleston, SC; [4]Dept. of Pediatrics, [5]Dept. of Bioengineering, Univ. of Washington, Seattle, WA

Abstract: Capillary pericytes serve myriad functions in cerebrovascular regulation and are lost at an accelerated rate in Alzheimer's disease and related dementias. However, the effects of pericyte loss on vascular and neuronal function remain incompletely understood. To dissect pericyte roles *in vivo*, prior studies have used genetic approaches to induce global pericyte loss in the murine brain. However, widespread loss of pericytes leads to complex outcomes, making it challenging to disentangle the physiological roles of pericytes from the pathophysiological effects of their depletion *en masse*. We describe a protocol to optically ablate individual pericytes of the mouse cerebral cortex *in vivo* for fine-scale studies of pericyte function. The strategy utilizes multiphoton microscopy and cranial window-implanted transgenic mice with mural cell-specific expression of fluorescent proteins. Single pericyte somata are precisely targeted with pulsed infrared laser light to induce selective pericyte death. The approach has been used to study pericyte roles in capillary blood flow regulation and the remodeling of pericytes during restoration of endothelial coverage after pericyte loss. Additionally, we will demonstrate how this method was used to reveal that pericyte remodeling is diminished in the aged mouse brain, leading to long-lasting blood flow changes in the capillary network. Further, we will show how focal pericyte loss has minimal effects on endothelial tight junction structure and blood-brain barrier integrity in triple transgenic mice (PDGFRβCre-tdTomato;Claudin5-eGFP). We envision that optical ablation will be useful for studying how pericytes contribute to neurovascular coupling, release of trophic factors for neuronal survival, and brain clearance of metabolic waste, among many other possibilities. Finally, the approach will be useful to understand the consequences of pericyte loss in different disease conditions, dissect mechanisms of pericyte remodeling, and identify therapeutic strategies for augmentation of pericyte coverage.

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Poster

376. Neurovascular Coupling

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Program #/Poster #: 376.15

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH HL135562

Title: Cerebral oxygenation responses to valsalva maneuver in obstructive sleep apnea

Authors: *L. H. FURTICK[1], F. MARTINEZ[1], B. KRAUSE-SORIO[1], R. S. AYSOLA[2], P. M. MACEY[1];
[1]UCLA Sch. of Nursing, UCLA, Los Angeles, CA; [2]Dept. of Medicine, David Geffen Sch. of Med. UCLA, Univ. of California at Los Angeles, Los Angeles, CA

Abstract: Obstructive sleep apnea (OSA) is associated with atonic episodes of the upper airway muscles and cardiovascular sequelae, resulting in lower peripheral oxygen saturation (SpO_2).

The vagus nerve enervates upper airway musculature and is a main component of the parasympathetic nervous system, a mediator of heart rate and respiratory rate. Altered neural-vagal responses and lower SpO_2 in OSA patients suggest vagal autonomic challenge will also reduce brain tissue oxygenation. Cerebral perfusion can be inferred from oxygenated hemoglobin (HbO_2) measures in brain tissue, which can be measured quickly and noninvasively with functional near-infrared spectroscopy (fNIRS). We determined changes in cerebral oximetry using fNIRS. Readings were recorded during baseline and three Valsalva maneuvers in 5 OSA patients and 5 healthy controls. Data were analyzed from the left lateral prefrontal cortex region of interest associated with fNIRS optodes #1-4. This region, associated with the left dorsolateral prefrontal cortex (DLPFC), is thought to have functional connectivity to deeper brain regions that mediate functions of the vagus nerve. Both groups showed changes in cerebral oxygenation during the Valsalva maneuver. There was a significant decrease in HbO_2 signal during the Valsalva task period in OSA [mean±SD: 0.977±0.164μM mm; one sample t-test, $t(4)=9.787$, $p<0.000$, Cohen's $d=0.597$]. These findings indicate reduced brain tissue oxygenation in response to the Valsalva maneuver in OSA patients. This suggests vagal pathways mediating autonomic processes are affected in OSA. Neural hypoxia and autoregulation abnormalities in OSA may contribute to neural injury and increases the risk for additional cardiovascular, neurological and metabolic comorbidities. These preliminary findings identify autonomic pathways as a potential target for therapeutic intervention for improvement in OSA conditions.

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Poster

376. Neurovascular Coupling

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 376.16

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: Canadian Institutes of Health Research
Hotchkiss Brain Institute

Title: Neurovascular coupling to sustained sensory cortex activation is modulated by locomotion activity, and by baseline calcium levels of mural cells and astrocytes

Authors: *G. PERINGOD[1], A. INSTITORIS[1], L. YU[2], K. MURARI[3], G. R. GORDON[1]; [1]Physiol. and Pharmacol., Hotchkiss Brain Institute, Univ. of Calgary, Calgary, AB, Canada; [2]Wellman Ctr. for Photomedicine, Massachusetts Gen. Hosp., Boston, MA; [3]Electrical and Software Engin., Univ. of Calgary, Calgary, AB, Canada

Abstract: Sensory-related neural activity drives regional blood flow changes to optimize oxygen supply to respiring brain cells. Although head-fixed, awake-animal imaging has become the gold-standard for mechanistic investigations of neurovascular coupling, many studies neglect to

monitor and control for several factors – like location relative to center of neuronal activation, ongoing/spontaneous brain activity, animal's behavioral/arousal state, systemic influences like respiration rate – which limits the classification and interpretation of stimulus-evoked neuroimaging data, and also limits comparison of data across laboratories. Integrated spatial and temporal assessments of the blood flow response, as well as multiscale comparisons, are also lacking – which limits the amount and quality of information we can gain from these experiments.
Here, we performed widefield intrinsic optical signal (WF-IOS)-guided two-photon imaging of the neurovascular unit in awake, behaving transgenic mice reporting calcium signals in mural cells and astrocytes. We conducted detailed behavior analyses (of locomotion, natural whisking, and pupil diameter) and we applied multiple spatiotemporal analysis techniques (markerless pose estimation, optical flow analyses, seed-based correlation, graph-based clustering, massive feature extraction, and frequency-domain analyses) to extract a wealth of information from these data. We find that cortical blood flow and tissue oxygenation responses to sustained (30-second) whisker stimulation is sensitive to: 1) context and level of locomotion, 2) pharmacological blockade/depletion of noradrenergic input, and 3) reduction of astrocyte calcium signaling using the recently developed silencing tool, CalEx. We also find that driving a canonical vasoconstriction pathway using mural cell Gq-chemogenetics dramatically disrupts the temporal and spatial properties of the evoked blood flow response.
These data and analyses provide new insights into our understanding of cerebral hemodynamics in the awake state and improve our ability to extract and understand information contained in multiscale imaging datasets. Our findings reaffirm the need to develop more nuanced transfer functions linking fMRI-BOLD signals to the underlying neural activity – especially when the neural activity is prolonged and occurs in the presence of confounding natural behaviors.

Disclosures: G. Peringod: None. **A. Institoris:** None. **L. Yu:** None. **K. Murari:** None. **G.R. Gordon:** None.

Poster

376. Neurovascular Coupling

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 376.17

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: BBSRC funded SoCoBio DTP

Title: Effects of a mild decrease in blood and oxygen supply on hippocampal neuronal and neurovascular function.

Authors: *L. MCMULLAN[1], K. SHAW[2], D. M. GRIJSEELS[4], M. VARGAS-CABALLERO[5], C. N. HALL[3];
[2]Psychology, [3]Sch. of Psychology, [1]Univ. of Sussex, Brighton, United Kingdom; [4]Univ. of California San Diego, San Diego, CA; [5]Univ. of Southampton, Southampton, United Kingdom

Abstract: Baseline blood flow, oxygen saturation, red blood cell velocity (RBCV) and capillary density are lower in the hippocampus than other brain regions with similar neuronal activity; likely making it especially vulnerable to even a mild decrease in blood/ oxygen supply, as seen in early Alzheimer's Disease (AD) (~10-25%). However, little is known about how such a mild decrease in blood/ oxygen supply affects hippocampal neuronal and neurovascular function, and whether this may lead to subsequent hippocampal dysfunction and cognitive decline in AD. In 5 adult male and female mice (>10 weeks) of a C57BL/6J background, we modelled this using a systemic injection of the vasoconstrictive drug L-NG-Nitro arginine methyl ester (L-NAME) to reduce hippocampal blood flow. Cortex overlaying the hippocampus was surgically ablated and custom-made cannula were implanted over mouse hippocampus. Net haemodynamic measures, including blood flux and oxygen saturation (SO2), were recorded using a combined laser doppler flowmetry/haemoglobin spectroscopy probe (Oxy-CBF probe). The cerebral metabolic rate of oxygen consumption (CMRO2), a proxy for neuronal activity, was calculated. Changes in response to 75mg/kg subcutaneous L-NAME injection, an equivalent volume of saline, or no injection, were recorded in awake mice. Subcutaneous L-NAME reduced resting mouse hippocampal blood flow and tissue oxygenation (~30%), suppressed hyperaemic responses to locomotion, and increased vasomotion (spontaneous oscillations in blood vessel walls at a peak frequency of 0.25Hz) 30-45 minutes post-injection. These data demonstrate that it is possible to produce and continuously monitor a mild decrease in mouse hippocampal blood and oxygen supply and oxygen consumption in awake animals in vivo. To test the impact of mild hypoxia on hippocampal and cognitive function without the confound of neuronal effects that comes with using broad-spectrum nitric oxide synthase (NOS) inhibition, we are developing methods to manipulate inspired oxygen while measuring central and peripheral blood oxygenation, alongside neuronal and vascular function. We will also investigate the impacts of a mild decrease in oxygen availability on hippocampal neuronal function using ex vivo field potential recordings in acute hippocampal slices.

Disclosures: L. McMullan: None. **K. Shaw:** None. **D.M. Grijseels:** None. **M. Vargas-Caballero:** None. **C.N. Hall:** None.

Poster

376. Neurovascular Coupling

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 376.18

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: IRSC: #MOP-451610)
FRQS: # 33237

Title: Il-17a contributes to the angiotensin ii-induced neurovascular coupling impairment through oxidative stress

Authors: *J. YOUWAKIM[1,2,3], D. VALLERAND[1,2], H. GIROAURD[1,2,3,4];
[1]Dept. of pharmacology and physiology, Univ. de Montréal, Montréal, QC, Canada; [2]Ctr. interdisciplinaire de recherche sur le cerveau et l'apprentissage, Montréal, QC, Canada; [3]Groupe de Recherche sur le Système Nerveux Central, Montreal, Montréal, QC, Canada; [4]Ctr. de recherche de l'Institut Universitaire de Gériaterie de Montréal, Montréal, QC, Canada

Abstract: Hypertension, a multifactorial chronic inflammatory condition, is known as a risk factor for neurodegenerative diseases including stroke and Alzheimer's disease. We have recently demonstrated that angiotensin II, a hormone involved in the development and maintenance of arterial hypertension, induces brain inflammation. This inflammation can be induced by modulation of T cell activity involving an increase in the production of the proinflammatory cytokine interleukin (IL)-17A. Interestingly, neurogeneratives diseases have been associated with higher concentration of blood interleukin (IL)-17A. However, the role that IL-17A plays in the relationship between hypertension and brain remains misunderstood. Cerebral blood flow regulation may be the crossroads of these conditions. Hypertension alters cerebral blood flow regulation including neurovascular coupling (NVC). Since cerebral vasculature is at the crossroads of the effects of hypertension on the brain, we hypothesized that hypertension alters cerebral blood flow regulation including NVC through IL-17A.The objective of the present study is to examine the effects of IL-17A on NVC in the context of hypertension induced by angiotensin (Ang) II. Therefore, Ang-II induced hypertensive C57BL/6J mice (600 ng/kg/min, for 14 days) were injected with a neutralizing anti-IL-17A antibody, an IL-17RA receptor antagonist or with an IgG isotype control (0.5 μg/μL). NADPH oxidase 2 depleted mice and mice treated with the Tempol, a superoxide dismutase mimetic agent, received a chronic administration of IL-17A recombinant (50 pg/kg/h) in order to further investigate the mechanism behind its effect on NVC. NVC was assessed by monitoring cerebral blood flow in response to whiskers stimulations by laser-Doppler flowmetry in anesthetized mice. Oxidative stress was assessed *ex-vivo* with dihydroethidium immunostaining. Our results show that the neutralization of IL-17A or the specific inhibition of its receptor IL-17RA prevent the Ang II-induced NVC impairment. These treatments also reduce the Ang II-induced cerebral oxidative stress. Moreover, Tempol and NADPH oxidase 2 depletion prevent NVC impairment, and the increased superoxide anion production induced by chronic IL-17A recombinant administration. These findings suggest that IL-17A, through superoxide anion production, is an important mediator of cerebrovascular dysregulation induced by Ang II. Therefore, targeting this cytokine in hypertension is a promising approach to prevent cerebrovascular dysfunctions and neurodegenerative diseases.

Disclosures: J. Youwakim: None. **D. Vallerand:** None. **H. Giroaurd:** None.

Poster

376. Neurovascular Coupling

Location: SDCC Halls B-H

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Program #/Poster #: 376.19

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH U19 NS123717-02

Title: Cerebral blood flow and neuronal activity regulation via rostral ventrolateral medulla

Authors: *K. CHHABRIA[1], L. E. MCELVAIN[1], T. BROGGINI[1], P. YAO[1], D. KLEINFELD[1,2];
[1]Physics, [2]Neurobio., UCSD, La Jolla, CA

Abstract: Cerebral blood flow (CBF) is regulated by multiple pathways. These include intrinsic arteriole vasomotion, local neuronal activity, global control related to midbrain neuromodulatory structures, and extracortical regulation via a sympathetic regulator in the rostral venterolateral medulla (RVLM). Early studies (Golanov and Reis, J Physiol, 1996) reported CBF regulation via neurons in RVLM. However, the mechanism(s) and anatomical pathway(s) for this action are yet to be established. Here we aim, firstly, to establish if whether the C1 neurons in RVLM drive CBF and electrocorticogram (ECoG) regulation, and, secondly, to determine the mechanism of this regulation. Thirdly, we aim to establish neuroanatomical pathway(s) from C1 neurons to cortex. We specifically targeted the C1 neurons of RVLM in dopamine-beta-hydroxylase cre-expressing (DBH-Cre) mice animals. The centroid of RVLM was established by microstimulation mapping experiments in which increased blood flow in cortex, as measured by Doppler spectrometry, served as the metric. We injected AAV.DIO.hSyn.mcherry for anterograde tracing experiments. We injected AAV.DIO.hSyn.ChR-EYFP for optogenetic experiments in awake animals. In response to optogenetic activation in awake animals, CBF was measured over primary vibrissa somatosensory cortex through a thinned-skull cranial window via laser-doppler-flowmetry and arteriole diameter was measured via two-photon microscopy. As a control, the electrocardiogram was acquired. Our functional studies show that optogenetic stimulation of C1 neurons led to a 15% increase in CBF and a similar fractional increase in vessel diameter; note that for flow in a network a change in flux can be linear in the change in diameter. We further see a decrease in spectral (<10 Hz) power in the ECoG, indicating involvement of cortical neurons. Trains of stimulation at near the vasomotor frequency of 0.1 Hz could entrain vasomotion, suggesting a specific role for RVLM in brain homeostasis. In ongoing neuroanatomical work, we injected AAV.DIO.hSyn.mcherry in DBH-Cre mice for anterograde tracing of C1 neurons neuroanatomical observations show labeling of and observed axonal labeling in diverse target regions, including the locus coerulus, zona incerta, lateral hypothalamus, paraventricular nucleus of the hypothalamus, and paraventricular nucleus of the thalamus. The contribution of each pathway is under investigation.

Disclosures: K. Chhabria: None. **L.E. McElvain:** None. **T. Broggini:** None. **P. Yao:** None. **D. Kleinfeld:** None.

Poster

376. Neurovascular Coupling

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Program #/Poster #: 376.20

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH grant R01AG033106

Title: Wavelet Coherence Analysis of Dynamic Cerebral Autoregulation during Stepwise Blood Pressure Changes

Authors: *D. CARDIM[1], T. KURAZUMI[1], T. TOMOTO[1], J. WON[1], H. LIU[2], R. ZHANG[1]; [1]Neurol., Univ. of Texas, Southwestern Med. Ctr., Dallas, TX; [2]Bioengineering, Univ. of Texas at Arlington, Arlington, TX

Abstract: Cerebral autoregulation (CA) is a fundamental vascular regulatory mechanism to protect brain perfusion against fluctuations in systemic blood pressure (ABP). An adequate and relatively constant cerebral blood flow (CBF) is critical for a continuous supply of oxygen and nutrients to the brain and for metabolic waste clearance. Cerebrovascular dysfunction, particularly brain hypoperfusion, has been proposed as an important pathophysiological mechanism for the development of neurodegenerative diseases such as Alzheimer's disease (AD). However, whether CA, especially its dynamic properties, is altered in older adults at greater risk of developing neurodegenerative diseases is not completely elucidated. CA assessment during dynamic ABP changes, referred to as dynamic CA (dCA), has been traditionally studied with analytic methods, such as transfer function analysis (TFA), of beat-to-beat variations of both CBF velocity (CBFV) (using transcranial Doppler), and mean ABP (MAP). TFA assumes that ABP and CBFV changes are stationary, i.e., their statistical properties do not change in the time domain. However, ABP and cerebral hemodynamics are fundamentally non-stationary signals, particularly under pathophysiological conditions, therefore revealing a methodological challenge to assess dCA in most studies previously reported. Wavelet coherence analysis (WCA), which is a time-frequency domain approach that makes no assumption about the stationarity of input signals, was used to characterize dCA in this study. The wavelet-based metric of gain was derived to quantify dCA in older individuals (N=25, age: mean 66.3 SD 6.7 years, 40% women) during pharmacologically-induced stepwise decreases and increases in MAP, respectively promoted by continuous intravenous infusion of sodium nitroprusside (SNP) and phenylephrine (PE). Compared to initial baseline values, MAP was significantly reduced by 16 mm Hg during SNP infusion and increased by 12 mm Hg during PE infusion, whereas dCA gain did not change significantly during either study phases indicating functional integrity of dCA. However, a linear mixed-models analysis revealed a significant effect of the interaction "MAP * end-tidal CO_2 ($ETCO_2$)" on dCA gain (β=0.003, p=0.03, conditional R^2=0.69) during SNP infusion. These results suggest that the dynamic regulation of CBF during sustained hypotension was related to both changes in MAP and $ETCO_2$ in older adults. Furthermore, WCA can track dCA changes under non-stationary conditions and has the potential to be used for continuous monitoring of cerebral hemodynamics.

Disclosures: D. Cardim: None. **T. Kurazumi:** None. **T. Tomoto:** None. **J. Won:** None. **H. Liu:** None. **R. Zhang:** None.

Poster

376. Neurovascular Coupling

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Program #/Poster #: 376.21

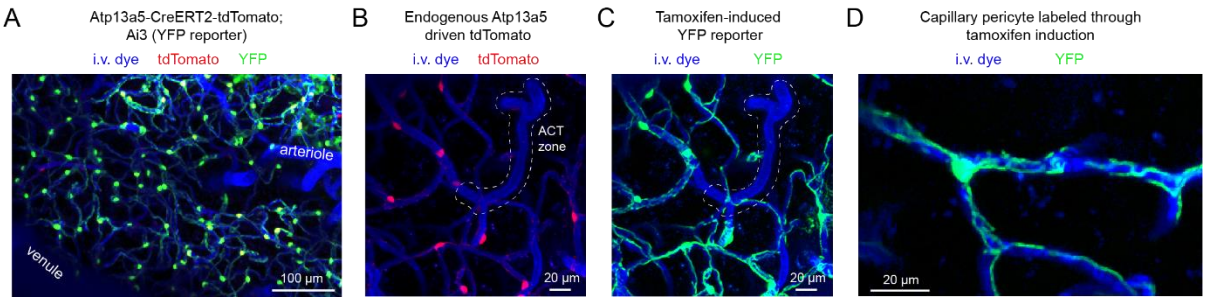
Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH grant NS106138
NIH grant NS097775
NIH grant AG063031
NIH grant AG062738
NIH grant AG077731

Title: Characterization of brain capillary pericyte-specific targeting using the Atp13a5-2A-CreERT2-IRES-tdTomato mouse line

Authors: *L. T. SULLIVAN[1], S. K. BONNEY[1], Z. ZHAO[2], A. Y. SHIH[1];
[1]Ctr. for Developmental Biol. and Regenerative Med., Seattle Childrens Res. Inst., Seattle, WA;
[2]Zilkha Neurogenetic Inst., USC, Los Angeles, CA

Abstract: Single cell transcriptomics have revealed that the Atp13a5 gene is preferentially expressed by capillary pericytes of the central nervous system. Atp13a5-2A-CreERT2-IRES-tdTomato knock-in mice (aka Atp13a5-CreERT2) were generated by the Zhao lab, and further tested for Cre functionality and pericyte specificity. We crossed Atp13a5-CreERT2 mice with Ai3 floxed yellow fluorescent protein (YFP) reporter mice, and administered a 5-day regimen of tamoxifen (75 mg/kg) to 3 month old mice, and waited 7 days before cranial window implantation and in vivo two-photon imaging. YFP reporter expression was robust in capillary pericytes, but not seen in pial/penetrating arterioles or arteriole-capillary transition (ACT) zones, both of which are covered by a-SMA-positive mural cells. We also noted high penetrance of recombination using this tamoxifen regimen, with most tdTomato-positive cells also expressing YFP (see Figure). Genetic targeting to the ACT zone (upstream of capillaries) would be a confounding factor for studies of blood flow regulation by capillary pericytes. Thus, we carefully examined microvascular zone-specific expression of YFP reporter in optically cleared, alpha-smooth muscle actin (a-SMA) immunostained brain tissues from tamoxifen-treated Atp13a5-CreERT2;Ai3 mice. Consistent with specificity for the capillary zone, we found a-SMA and YFP expression to be nearly mutually exclusive along the microvasculature. While YFP+/a-SMA+ mural cells of the ACT zone were not completely absent, their presence was far lower compared to YFP+/a-SMA- capillary pericytes. Overall, these data provide confidence that the Atp13a5-CreERT2 line is quite specific for brain capillary pericytes, opening the door to studies that require CNS-wide genetic manipulation of capillary pericytes in vivo.



Disclosures: **L.T. Sullivan:** None. **S.K. Bonney:** None. **Z. Zhao:** None. **A.Y. Shih:** None.

Poster

376. Neurovascular Coupling

Location: SDCC Halls B-H

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Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant NS110069
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NIH Grant NS123644
NIH Grant AG070507

Title: Vasculo-glia-neuronal coupling in vivo

Authors: *P. O'HERRON, K. XIE, K. KIM, J. A. FILOSA;
Physiol., Augusta Univ., Augusta, GA

Abstract: It has long been known that neuronal activity drives increases in local blood flow. This process - termed neurovascular coupling - is thought to play an important metabolic role by providing neurons with the energy substrates needed to sustain high activity levels. However, much less is known about how changes in vascular reactivity can affect neuronal activity. Previous work from our group has shown that, in an *ex vivo* brain slice preparation, a pressure-induced increase in the vascular tone (myogenic constriction) of parenchymal arterioles caused an increase in astrocyte Ca^{2+} events, followed by a reduction in the firing activity of pyramidal neurons - a process termed *vasculo-neuronal coupling (VNC)*. We also showed that a subset of GABAergic inhibitory interneurons, namely, somatostatin neurons, increased their activity during the constriction. VNC was hypothesized to serve a protective role by reducing activity when decreases in blood flow limit metabolic substrates. We recently developed a mouse line expressing a depolarizing opsin in smooth muscle cells. Using light to constrict parenchymal arterioles *ex vivo* (brain slices), we replicated the phenomenon of constriction-induced pyramidal neuron inhibition. We sought to determine if vascular constriction *in vivo* led to similar effects in neurons and astrocytes. Using two-photon microscopy, we imaged arteriole diameter simultaneously with astrocyte or neuronal (excitatory and inhibitory) Ca^{2+} events. We used pharmacological vasoactive agonists and optogenetics to constrict arteries in the cerebral cortex of mice. As in the brain slice, constriction of arteries *in vivo* led to a robust astrocyte Ca^{2+} increase. In addition, excitatory neurons frequently showed reductions in Ca^{2+} transients, suggestive of reduced action potential frequency. Notably, a large proportion of excitatory neurons exhibited a small increase in baseline Ca^{2+} following the constriction. We explored multiple potential explanations, including 1) an imaging artifact (increased brightness due to a reduction in vessel shadows during constriction); 2) a subthreshold depolarization resulting in an

intracellular Ca^{2+} increase but not large enough to evoke an increase in spike frequency or 3) an active inhibitory process involving an intracellular Ca^{2+} rise leading to the suppression of action potential frequency. Understanding the mechanisms by which dynamic arteriole diameter changes impact neuronal network excitability will increase our understanding of the bi-directional interaction between vessels and neurons, shedding light on how vascular dysfunction can contribute to neurological disease.

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Poster

376. Neurovascular Coupling

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Program #/Poster #: 376.23

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

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NIH grant 1DP2NS121347-01
NIH grant R01HL136636
AHA grant 19IPLOI34660108

Title: Vascular Signaling Plasticity Reprograms Blood Delivery Mechanisms to Meet Fluctuating Neuronal Energy Needs

Authors: *N. WEIR[1], L. XIANG[2], D. C. G. GARCIA[2], H. QADIR[2], M. S. PATTON[2], B. N. MATHUR[2], F. DABERTRAND[3], T. A. LONGDEN[2];
[1]Univ. of Maryland, Baltimore, Baltimore, MD; [2]Univ. of Maryland Baltimore, Baltimore, MD; [3]Univ. of Colorado Anschutz Med. Campus, Aurora, CO

Abstract: Neuronal computation is metabolically expensive and depends on precision delivery of energy substrates—oxygen and glucose—via tightly coordinated blood flow to ensure that energy demands are continually met. This is facilitated by neurovascular coupling (NVC)—a range of mechanisms matching neural activity to local blood flow. These NVC mechanisms are typically considered invariant, and whether they are reshaped according to ever-changing neuronal metabolic needs has not been examined. We present evidence that neural activity continually reprograms the blood flow control mechanisms embedded in the local vasculature, a process we refer to as 'vascular signaling plasticity' (VSP). Using an established enrichment paradigm, we find that animals housed in conditions that increase input to the barrel cortex for 7 days exhibit profound neuronal plasticity in this region. This resculpts local vascular reactivity to elevate blood delivery. Indeed, VSP results in increased basal capillary blood flow and augmented activity of vasodilatory agents *in vivo* manifesting as increased red blood cell (RBC) flux responses to capillary stimulation and upstream diameter changes measured using 2-photon laser scanning microscopy. Moreover, the sensitivity of capillaries to stimulation is also

dramatically elevated in an *ex vivo* isolated capillary-arteriole preparation. Using K^+-mediated capillary-to-arteriole electrical signaling as a springboard to study the molecular underpinnings of VSP, we find that this process induces an ~70% increase in the density of strong inward rectifier K^+ (Kir2.1) currents in capillary endothelial cells, which profoundly augments the retrograde hyperpolarization generated by these channels to control upstream diameter at the level of the penetrating arteriole. Our data thus recast the vasculature as a plastic, brain-wide activity sensing network that is continually reshaped at the molecular level by local neuronal input, leading to precisely-tuned blood delivery to meet continually fluctuating neural energy needs. VSP represents a new dimension to brain plasticity that may underpin a range of vital physiological processes and is likely disrupted in numerous brain pathologies with a blood flow component.

Disclosures: **N. Weir:** None. **L. Xiang:** None. **D.C.G. Garcia:** None. **H. Qadir:** None. **M.S. Patton:** None. **B.N. Mathur:** None. **F. Dabertrand:** None. **T.A. Longden:** None.

Poster

376. Neurovascular Coupling

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 376.24

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Title: Neuronal activity regulates brain endothelial cholesterol metabolism as a feedback mechanism for neurovascular coupling

Authors: *C. P. PROFACI[1], V. F. COELHO-SANTOS[2], K. BAJC[1], S. S. HARVEY[1], A. Y. SHIH[3], F. DABERTRAND[4], R. DANEMAN[1];
[1]UCSD, La Jolla, CA; [3]Ctr. for Developmental Biol. and Regenerative Med., [2]Seattle Children's Res. Inst., Seattle, WA; [4]Univ. of Colorado, Aurora, CO

Abstract: Current dogma considers brain and systemic cholesterol to be different pools, with brain cholesterol synthesized de novo, primarily by astrocytes and oligodendrocytes. Brain endothelial cells—which sit at the border of these two separate pools—are not considered to be significant producers or transporters of cholesterol, and thus not much is known about the regulation or function of brain endothelial cholesterol metabolism. To our surprise, we found that treatment with PLX5622, a CSF1R inhibitor widely used to deplete microglia, causes brain endothelial cells to upregulate cholesterol synthesis enzymes and the cholesterol uptake receptor, low-density lipoprotein receptor (LDLR). This effect was restricted to CNS endothelial cells, occurred throughout the vascular tree, and was independent of microglial depletion. We further show that brain endothelial cholesterol metabolism is bi-directionally regulated by neuronal activity, with increased activity upregulating cholesterol synthesis and uptake machinery, and decreased activity downregulating the same machinery. Furthermore, brain endothelial cholesterol inhibits arteriole dilation in response to capillary K+ stimulation, suggesting that

brain endothelial cholesterol synthesis and uptake act as a negative feedback mechanism for the processes of neurovascular coupling.

Disclosures: C.P. Profaci: None. **V.F. Coelho-Santos:** None. **K. Bajc:** None. **S.S. Harvey:** None. **A.Y. Shih:** None. **F. Dabertrand:** None. **R. Daneman:** None.

Poster

376. Neurovascular Coupling

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

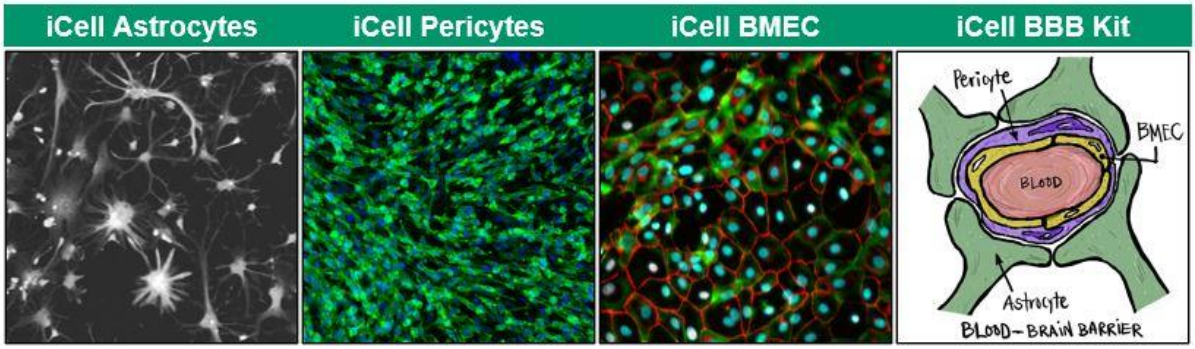
Program #/Poster #: 376.25

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Title: Establishment of a blood-brain barrier model using iPSC-derived human cells

Authors: C. A. MUNN, M. E. GOEDLAND, M. K. LIVINGSTON, R. K. FIENE, K. TOMOTOSHI, J. LIU, S. A. HILCOVE, R. VAIDYNATHAN, *C. B. CARLSON; FUJIFILM CDI, Madison, WI

Abstract: The blood-brain barrier (BBB) is a specialized network of cells that function to maintain a tightly controlled microenvironment around the brain. A robust and human relevant BBB model is needed to evaluate barrier function, test drug permeability, and study the diseases that affect it. While the power of induced pluripotent stem cell (iPSC) technology provides access to the specialized cell types of the brain required to assemble such a model system, the field has been challenged with generating cells that contain the appropriate markers and relevant functionality, manufacturing a consistent supply of cells at-scale, and cryopreserving the material for subsequent on-demand use. As a leader in iPSC technology and innovation, FUJIFILM Cellular Dynamics, Inc. will present data on the generation, characterization, and utilization of three unique human iPSC-derived cell types for use in BBB model development, including astrocytes, brain microvascular endothelial cells (BMEC), and pericytes. Most notably, differentiation of iPSC into BMEC yields a cell type with distinctive cellular structures (tightly packed, cobblestone morphology, proper organization of tight junctions), appropriate marker expression (transporters: GLUT1, CD98hc and efflux/influx proteins: BCRP, P-gp, MRP1, transferrin receptor), and functional assay performance (effective barrier formation, low permeability). These features separate BMEC from other vascular endothelial cells lining peripheral blood cells. Importantly, establishment of a reliable BBB model requires optimization of media and supplements to enable long-term survival of all three cell types in co-culture and to promote high transendothelial electrical resistance (TEER) signal in assays using Transwell inserts. The potential to integrate this cellular BBB system with emerging organ-on-a-chip (OoC) technologies and other 3D culture platforms offers an exciting new capability for the drug discovery community to advance the understanding of BBB function with respect to human health and disease.



Disclosures: **C.A. Munn:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics, Inc. **M.E. Goedland:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics, Inc. **M.K. Livingston:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics, Inc. **R.K. Fiene:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics, Inc. **K. Tomotoshi:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics, Inc. **J. Liu:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics, Inc. **S.A. Hilcove:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics, Inc. **R. Vaidynathan:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics, Inc. **C.B. Carlson:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics, Inc..

Poster

376. Neurovascular Coupling

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 376.26

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: P01DK113954
R01DK115761
R01DK117281
R01DK125480
R01DK120858
2020AHA000POST000204188

Title: Anoctamin 4 Defines Glucose-Inhibited Neurons in the Ventromedial Hypothalamus

Authors: *L. TU, Y. XU;
Baylor Col. of Med., Houston, TX

Abstract: Glucose levels are strictly controlled in a narrow range as glucose provides the basic fuel for the brain. Extreme drops in glucose levels, due to energy deprivation (e.g., fasting) or therapeutic agents (e.g., insulin), can therefore be life threatening. In normal subjects, drops of glucose levels are counterbalanced by a variety of defensive responses that involve increased

hunger. In many type 1 diabetic (T1D) patients, however, this protection is often impaired. The mechanisms for defending hypoglycaemia remains largely unknown. Here we showed that electrical currents mediated by anoctamin 4 (Ano4) channel are exclusively present in glucose-inhibited (GI) neurons in the ventromedial hypothalamic nucleus (VMH) and are functionally required for their activation in response to low glucose. Genetic disruption of the *Ano4* gene in VMH neurons reduces blood glucose, impairs counterregulatory responses during hypoglycemia and glucopenia, and contributes to hypoglycemia associated autonomic failure in mice. Specific activation of VMH^{Ano4} neurons increases food intake and blood glucose, while chronic inhibition of VMH^{Ano4} neurons ameliorates hyperglycemia in a T1D mouse model. Finally, we showed that VMH^{Ano4} neurons represent a unique orexigenic VMH population and transmit a positive valence, while non-Ano4 neurons in the VMH suppress feeding and transmit a negative valence. Together, our results indicate that the Ano4 channel and VMH^{Ano4} neurons are potential therapeutic targets for human diseases with abnormal feeding behavior or glucose imbalance.

Disclosures: L. Tu: None. **Y. Xu:** None.

Poster

376. Neurovascular Coupling

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 376.27

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: K99AG061231
1R01NS102272
R01AG064902

Title: Aqp4 stop codon readthrough generates an elongated version of AQP4 with neurobiological significance

Authors: K. M. WHITE[1], C. FLORIAN[1], B. M. DOHERTY[1], K. REARDON[1], C. M. YUEDE[1], J. R. CIRRITO[1], J. D. DOUGHERTY[1], ***D. SAPKOTA**[2];
[1]Washington Univ. in St. Louis, Saint Louis, MO; [2]Biol. Sci., Univ. of Texas, Dallas, Richardson, TX

Abstract: Aquaporin 4 (AQP4) is an astrocyte-specific water channel with roles in the homeostasis of brain water, clearance of brain wastes, and pathophysiology of neurological diseases. We have previously identified a novel C-terminally extended variant of AQP4 (termed AQP4X) that is generated by *Aqp4* stop codon readthrough, where some of the translating ribosomes neglect the stop codon and continue into the 3' untranslated region. And we showed that this elongated AQP4X is exclusively perivascular, while the normal-length AQP4 is localized away from the blood vessels. Here we investigated if AQP4X has neurobiological significance. Using AQP4 readthrough-specific knockout mice that still express normal AQP4, we determine this variant mediates Aβ clearance. Further, with high-throughput screening, we

identify small molecules that enhance *Aqp4* readthrough and find that these compounds enhance brain Aβ clearance *in vivo*. Our data suggest that modulation of *Aqp4* readthrough can be a viable pharmaceutical approach to enhance clearance of Aβ and potentially other aggregating proteins in neurodegenerative disease.

Disclosures: K.M. White: None. **C. Florian:** None. **B.M. Doherty:** None. **K. Reardon:** None. **C.M. Yuede:** None. **J.R. Cirrito:** None. **J.D. Dougherty:** None. **D. Sapkota:** None.

Poster

377. Mechanisms of Neurodegeneration and Neuroprotective Therapies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 377.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: KMDF_PR_20200901_0088
NRF-2016R1D1A3B03932649
NRF-2019R1I1A3A01043477

Title: Focused ultrasound-induced endogenous neural stem cells activation

Authors: *Y. SEO[1,2], S. HAN[1], J. CHANG[1,2], Y. NA[3], W. CHANG[1];
[1]Dept. of Neurosurg., Yonsei Univ. Col. of Med., Seoul, Korea, Republic of; [2]Brain Korea 21 Project for Med. Science, Yonsei Univ. Col. of Med., Seoul, Korea, Republic of; [3]Catholic Kwandong Univ. Col. of Med., Catholic Kwandong Univ. Col. of Med., Incheon Metropolitan City, Korea, Republic of

Abstract: Introduction The finding of neural stem cells (NSCs) in the adult brain, as well as their potential to self-renew and specialize into tissue-appropriate functional cell types, has raised new expectations for neurological diseases therapy. Endogenous NSCs are distributed in subgranular zone (SGZ) of dentate gyrus in the hippocampus and subventricular zone (SVZ) of lateral ventricles, and are important role in endogenous neurogenesis of adult brain. Meanwhile, some studies have found that when focused ultrasound (FUS), which is used to penetrate the blood-brain barrier, is applied to the SGZ in the hippocampus, neurogenesis occurs. In this study, FUS stimulation was given to the hippocampus to investigate the activation and lasting effects of endogenous NSCs. **Materials and methods** In this study, male Sparague-Dawley rats (230-250g) were used. Low intensity focused ultrasound (LIFUS) was applied to parameters of 0.25Mpa, 120s (Targeted hippocampal region: AP -3.5, ML +2.5). Before the sonication, microbubble was injected into the tail vein of the rats. Each group was divided into 5 groups, which were normal (non-treat control) group, sonication after 3 days, 1, 2 and 4 week-groups. Endogenous NSC activity through FUS and the duration of maintaining its effectiveness were confirmed through immunohistochemistry (IHC), Western blot (WB) and Positron emission tomography (PET) using 3'-deoxy-3'[^{18}F]fluoro-L-thymidine ([^{18}F]FLT) tracer to track stem cells. **Results** As a result of confirming through IHC with Nestin and Sox-2, which are markers

of stem cells, significant activity values could be verified in 1 week-group. Comparing the sacrificed group at each time point after LIFUS with WB, 1 week-group had the highest degree of activation rate, the 2 and 4 week-groups showed a pattern of decreasing sequentially, and the 4 week-group was similar rate to the normal group. Compared with the normal group, FLT-PET imaging showed higher activity of stem cells in the 1 week-group. **Conclusion** LIFUS is a way to activate endogenous NSCs and cause neurogenesis. The results of this study show that able to visualize the degree of activation after FUS treatment and lasting effects through histological, WB evaluation and PET imaging. Based on this, LIFUS treatment can be provided effective treatments for patients suffering neurological damage or neurological disorders due to external factors, in clinical fields. However, further studies are needed a more diverse and detailed study of the mechanism of cell differentiation.

Disclosures: **Y. Seo:** None. **S. Han:** None. **J. Chang:** None. **Y. Na:** None. **W. Chang:** None.

Poster

377. Mechanisms of Neurodegeneration and Neuroprotective Therapies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 377.02

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: FAPESP#2020/15892-8
FAPESP#2018/05006-0

Title: Neuroprotection and immunomodulation by the major histocompatibility complex class I (MHC I) upregulation after IFNβ treatment in SOD1G93A mice

Authors: *A. M. R. TOMIYAMA, L. P. CARTAROZZI, G. B. CHIAROTTO, A. L. OLIVEIRA;
Structural and Functional Biol., Univ. of Campinas - Lab. of Nerve Regeneration, Campinas, Brazil

Abstract: Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease that affects motoneurons, resulting in muscle weakness and atrophy. ALS can be familial or sporadic. In both cases, the clinical signs are a consequence of mutations that cause excitotoxicity in the neuronal environment. Furthermore, it has been demonstrated that, during the disease, astrocytes inhibit the expression of MHC-I by neurons, thus contributing to further motoneuron degeneration and A1 polarization. The present study aimed to verify the influence of interferon beta-1b (IFNβ), a pro-inflammatory cytokine that induces increased expression of MHC-I, in $SOD1^{G93A}$ mice. Two periods of disease were analyzed, the pre-symptomatic (90 days of age) and the initial symptomatic phase (100 days of age). Thus, the pre-symptomatic groups were treated with IFNβ at doses of 0IU (vehicle), 250IU, 1,000IU, and 10,000IU. Subsequently, the groups in the initial symptomatic period were treated only with the most effective dose (250IU). All mice were euthanized and the lumbar spinal cords were collected for motoneuron survival,

reactive gliosis, and synaptic coverage evaluation. Quantification of gene transcripts was carried out by RT-qPCR. The motor behavior was monitored with a rotarod device, and weight monitoring and neurological score were obtained throughout the experiment, as well as the analysis of the compound muscle action potential (CMAP) of the tibialis cranialis muscle. The results, for the pre-symptomatic groups, support that IFNβ upregulates MHC-I expression, even at the lowest dose, which was coupled with a twenty percent preservation of the lumbar spinal motoneurons ($p<0.001$). IFNβ preserved synapses at all doses tested ($p<0.01$) although there was a decrease in excitatory inputs (250IU and 1,000IU - $p<0.01$ and 10,000IU - $p<0.0001$). Astrogliosis decreased in the IFNβ-treated groups compared to the vehicle group ($p<0.0001$). In contrast, microglial reactivity significantly increased in the group that received the 1,000 IU dose ($p<0.001$). Of note, the disease progression led to upregulation of gene transcripts for pro and anti-inflammatory cytokines and, of these, treatment with IFN β significantly decreased IL4. The Rotarod motor tests, score, and body weight did not show significant differences in the two periods analyzed, although disease progression was apparent due to a significant loss of amplitude and increase in the latency of CMAP of the tibialis cranialis muscle. Overall, a low dosage of IFNβ shows therapeutic potential by increasing MHC-I expression, resulting in neuroprotection and immunomodulation in the pre-symptomatic period.

Disclosures: A.M.R. Tomiyama: None. **L.P. Cartarozzi:** None. **G.B. Chiarotto:** None. **A.L. Oliveira:** None.

Poster

377. Mechanisms of Neurodegeneration and Neuroprotective Therapies

Location: SDCC Halls B-H

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Program #/Poster #: 377.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: This work was supported by the European Regional Development Fund: Project "PharmaBrain" (no. CZ.CZ.02.1.01/0.0/0.0/16_025/0007444).

Title: 7-Phenoxytacrine and its derivatives effectively inhibit the GluN1/GluN2B subtype of the NMDA receptor

Authors: M. HORAK[1,2], A. MISIACHNA[1,2], O. SOUKUP[3], J. KORABECNY[3];
[1]Inst. of Physiol. of the Czech Acad. of Sci., Prague, Czech Republic; [2]Inst. of Exptl. Med. of the Czech Acad. of Sci., Prague, Czech Republic; [3]Biomed. Res. Centre, Univ. Hosp. Hradec Kralove, Hradec Kralove, Czech Republic

Abstract: *N*-methyl-D-aspartate receptors (NMDARs) are a subclass of ligand-activated ion channels that play an essential role in mediating excitatory neurotransmission. Functional dysregulation of NMDARs plays a key role in the etiology of many neuropsychiatric disorders; therefore, NMDARs are a promising pharmacological target. We have recently shown that the 7-methoxy derivative of tacrine (7-MEOTA) is a potent "foot-in-the-door" blocker of

GluN1/GluN2 receptors, exerting neuroprotective efficacy *in vivo.* However, 7-MEOTA is characterized by a higher selectivity towards the GluN1/GluN2A subtype of the NMDARs. Therefore, we have developed 7-phenoxytacrine (7-PhO-THA) and characterized its mechanism of action using whole-cell patch-clamp recordings from the HEK293 cells expressing NMDARs (at membrane potentials of -60 mV, -20 mV, and +40 mV, using 0.3-100 μM concentrations). Our experiments showed that i) 7-PhO-THA inhibited GluN1/GluN2A receptors, but not GluN1/GluN2B receptors, in a voltage-dependent manner, and ii) 7-PhO-THA exhibited profoundly lower IC_{50} values at the GluN1/GluN2B receptors compared with the GluN1/GluN2A receptors. Next, we observed that 7-PhO-THA inhibited the GluN1/GluN2B receptors lacking the amino-terminal domains (ATDs) containing the ifenprodil-binding site (GluN1-ΔATD/GluN2B-ΔATD) as well as the mutated GluN1/GluN2B receptors with the disrupted ifenprodil-binding site (GluN1-Y109C/GluN2B) in a voltage-dependent manner, and with similar IC_{50} values to the GluN1/GluN2A receptors. These findings showed that 7-PhO-THA acts via the ifenprodil-binding site at the GluN1/GluN2B receptors, in addition to its voltage-dependent inhibitory effect at both GluN1/GluN2A and GluN1/ GluN2B receptors. Finally, we observed that two different derivatives of 7-PhO-THA, K1958, and K1959, exhibited similar interaction with the ifenprodil-binding site of the GluN1/GluN2B receptor when compared with 7-PhO-THA, but K1959 had a more profound voltage-dependent inhibitory effect at both GluN1/GluN2A and GluN1/GluN2B receptors. Together, our experimental data showed that 7-PhO-THA and its derivatives are promising compounds for further development.

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Poster

377. Mechanisms of Neurodegeneration and Neuroprotective Therapies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 377.04

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH/NIA: R01AG057555
NIH: 5T32HL135465-02
Graduate Center (Biochemistry Program) at City University of New York

Title: Timapiprant an antagonist of the prostaglandin D2 receptor DP2 ameliorates cognitive deficits and hippocampal pathology in a transgenic rat model of Alzheimer's disease

Authors: *C. H. WALLACE[1], G. OLIVEROS[1], P. A. SERRANO[2], P. ROCKWELL[3,1], L. XIE[4], M. E. FIGUEIREDO-PEREIRA[5,1];
[1]Ph.D. Program in Biochem., [2]Psychology, [3]Biol. Sci., [4]Computer Sci., Hunter Col., New York, NY; [5]Hunter College, City Univ. of New York, Hunter College, CUNY, New York, NY

Abstract: Alzheimer's disease (AD) is the most common type of dementia, is highly prevalent in the ageing population, and will become more prevalent as life expectancy continues to rise.

AD is a multifactorial disease, and chronic neuroinflammation is recognized as a critical factor in its pathogenesis. A major player in inflammation is the cyclooxygenase (COX)-mediated signaling pathway, which is the principal mediator of CNS neuroinflammation. The COX pathway generates prostaglandins (PGs), which are bioactive signaling lipids responsible for many processes including inflammation. PG signaling is implicated in AD, as some PGs aggravate its pathology while others may remediate it. We investigated the relevance of the PGD2 pathway in AD because PGD2 is a major PG in the brain and given the known impact of the PGE2 pathway on AD. Thus, the role of PGD2 in AD merits attention, We used the TgF344-AD (Tg-AD) transgenic rat model of AD because it exhibits age-dependent and progressive AD pathology. PGD2 levels in hippocampi of Tg-AD and wildtype littermates were significantly higher than PGE2. PGD2 signals through DP1 and DP2 receptors. Microglial DP1 receptors were more abundant and neuronal DP2 receptors were fewer in Tg-AD than in wildtype rats. Expression of L-PGDS, the major brain PGD2 synthase, was the highest among 33 genes involved in the PGD2 and PGE2 pathways. We treated a subset of rats (wildtype and Tg-AD males) with timapiprant, a potent highly selective DP2 antagonist and currently in development for treating allergy-induced inflammation. Timapiprant significantly mitigated AD pathology and cognitive deficits in Tg-AD males. Overall, our studies provide novel insights for the development of therapeutics that target the PGD2 signaling pathway to treat neuroinflammation in AD.

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Poster

377. Mechanisms of Neurodegeneration and Neuroprotective Therapies

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Program #/Poster #: 377.05

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Twas Sandwich Fellowship Program FR number : 3240293211

Title: Ameliorative effects of aqueous leaves extract of Carissa edulis Valh (Apocynaceae) in rats D-galactose model of Alzheimer's disease: study of mechanism of action

Authors: *S. FANTA YADANG[1,2], Y. NGUEZEYE[1,2], G. SOTOING TAIWE[3], N. KOUEMOU[3], G. TEMKOU NGOUPAYE[4], G. AGBOR AGBOR[1], U.-R. NISAR[5], E. NGO BUM[2];
[1]Inst. of Med. Res. and Medicinal Plants Studies, Yaoundé, Cameroon; [2]Dept. of Biol. Sciences, Fac. of Sci., Univ. of Ngaoundere, Ngaoundere, Cameroon; [3]Dept. of Zoology and Animal Physiology, Fac. of Sci., Univ. of Buea, Buea, Cameroon; [4]Dept. of Animal Biology, Fac. of Sci., Univ. of Dschang, Dschang, Cameroon; [5]Dept. of Pharm., Comsats Inst. of Information Technol., Abbottabad, Pakistan

Abstract: Background and aim: Alzheimer's disease is a neurodegenerative disorder characterized by memory impairment and neuronal cells loss, with a significant implication of oxidative stress. The first line of treatments against Alzheimer's disease are acetylcholinesterase inhibitors; however, the antioxidant products can be used in association with acetylcholinesterase inhibitors. This study examined the therapeutic effects of an aqueous extract of a medicinal plant from Apocynaceae family, *Carissa edulis*, in experimental model of Alzheimer's disease induced by chronic administration of D-galactose in rat. Experimental procedure: Forty Wistar rats of both sexes weighing between 150 - 200 g of 2 months old were injected intraperitoneally with 100 mg/kg D-galactose for 6 weeks. From the 2 last weeks, rats were treated with *C. edulis* extract (62.8, 157, 314 and 628 mg/kg), piracetam (300 mg/kg) or vitamin C (250 mg/kg). The open field and T-maze paradigms were used to evaluated exploratory behaviour and cognitive functions, respectively. Additionally, biochemical parameters and brain histopathology were examined. Quantitative phytochemical analyses of *C. edulis* were performed as well as its *in vitro* antioxidant activity. Results and Conclusion: The results obtained showed that *C. edulis* has improved the memory performance and ameliorated locomotion of rats, attenuated brain histopathological damages, increased the activity of antioxidant enzyme and reduced the levels of oxidative damage biomarkers in D-galactose injected group. The results indicated the decrease of acetylcholinesterase activity and the increase of anti-inflammatory activity. The *in vitro* studies show that *C. edulis* have a high amount of total phenolic content, flavonoids and tannins and also have some good antioxidant properties. Our results indicate that *C. edulis* has ameliorative effects on D-galactose model of Alzheimer's disease in rat.

Disclosures: S. Fanta Yadang: None. **Y. Nguezeye:** None. **G. Sotoing Taiwe:** None. **N. Kouemou:** None. **G. Temkou Ngoupaye:** None. **G. Agbor Agbor:** None. **U. Nisar:** None. **E. Ngo Bum:** None.

Poster

377. Mechanisms of Neurodegeneration and Neuroprotective Therapies

Location: SDCC Halls B-H

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Program #/Poster #: 377.06

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NRF- 2020R1A2C2008480

Title: Administration of a phosphodiesterase 4 (PDE4) inhibitor reduces hippocampal neuronal cell death after global cerebral ischemia.

Authors: *S. PARK, S. SUH, A. KHO, S. LEE, D. HONG, B. KANG, M. PARK, S. LEE, C. LEE, H. YANG, S. WOO;
Dept. of Medicine, Physiol. 3604, Dept. of Physiology, Hallym University, Col. of Med., Chuncheon, Korea, Republic of

Abstract: Global cerebral ischemia (GCI) occurs when the blood supply to the brain is temporarily or completely interrupted. GCI initiates or exacerbates energy metabolism disturbance, lysosomal dysfunction, oxidative stress, neuronal degeneration, and cognitive impairment. The phosphodiesterase (PDE) converts cyclic adenosine monophosphate (cAMP) to adenosine monophosphate (AMP) and indirectly regulates downstream kinase activity through proteins such as protein kinase A (PKA) and AMP-activated protein kinase (AMPK). Specifically, PDE4 is a major enzyme of the PDE family and plays a central role in immunoregulatory mechanisms that govern pathological progression. For instance, PDE4 indirectly reduces lysosomal/ autophagic function via reduction of cAMP and the activity of protein kinase A (PKA). Amlexanox (AMX) is well known as an anti-inflammatory and anti-allergic immune modulator. Additionally, AMX acts as a phosphodiesterase 4 (PDE) inhibitor and can regulate lysosomal/autophagic functions. We speculated that an abnormal increase in PDE4 activity during a pathological situation could lead to a decrease in cAMP and PKA activity indirectly, resulting to intracellular accumulation of toxic substances and, finally, neuronal death occurs. So, we hypothesized that after GCI, administration of AMX enhances the lysosomal function and autophagic activity and prevents the accumulation of factors that lead to intracellular toxicity, thereby reducing neuronal cell death. To investigate whether the administration of AMX can prevent ischemic brain damage, we used the GCI-induced animal model in rat. Following GCI, we injected AMX (100 mg/kg, i.p.) for 7 days and sacrificed on the 7^{th} day post-injury. To determine the rate of degeneration of neurons that was dependent on lysosome dysfunction, we have stained against Fluoro-Jade B (FJB), Glial fibrillary acidic protein (GFAP), CD11b, and 4-hydroxynonenal (4HNE) in the hippocampal region. As a result, AMX treatment decreased markers of reactive oxidation stress, inflammation, and finally hippocampal neuronal death. Therefore, this present study revealed that the administration of AMX improved lysosomal/ autophagic functions through regulation of cAMP/ PKA activity and subsequently decreased intracellular accumulation of toxic substances, producing a clear neuroprotective effect. In conclusion, we suggest that AMX may represent a promising approach to ameliorating ischemia-induced neuronal cell death. **Key Words**: global cerebral ischemia; phosphodiesterase 4; cyclic adenosine monophosphate, protein kinase A, lysosomal dysfunction; autophagy.

Disclosures: S. Park: None. **S. Suh:** None. **A. Kho:** None. **S. Lee:** None. **D. Hong:** None. **B. Kang:** None. **M. Park:** None. **S. Lee:** None. **C. Lee:** None. **H. Yang:** None. **S. Woo:** None.

Poster

377. Mechanisms of Neurodegeneration and Neuroprotective Therapies

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Program #/Poster #: 377.07

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NRF-2020R1A6A3A01096433
NRF- 2020R1A2C2008480

Title: The effects of choline alfoscerate (α-GPC) on hypoglycemia-induced hippocampal neuronal death

Authors: *A. KHO, S. LEE, D. HONG, B. KANG, M. PARK, S. LEE, C. LEE, H. YANG, S. WOO, S. PARK, D. KIM, S. SUH;
Hallym Univ., Hallym Univ., Chuncheon, Korea, Republic of

Abstract: Type1 and type 2 diabetic patients who are prescribed drugs like insulin to control their blood glucose level could potentially experience hypoglycemia. In order to cease the severe and rapid hypoglycemic state, glucose reperfusion is often performed to recover low blood glucose levels, which, however, also acts as a secondary injury, casing activation of neuronal death signaling pathways in the brain. Choline alfoscerate (L-alpha glyceryl phosphorylcholine, α-GPC) is known as natural choline and is a parasympathomimetic acetylcholine precursor, which is important for memory and learning. Choline alfoscerate can pass through the blood brain barrier into the brain, and choline alfoscerate entering in this way is broken down into choline and glycerophosphate. Choline is used as a precursor to synthesize acetylcholine (ACh), which normalizes neurotransmitter function degraded by brain damage. Also, glycerophosphate is metabolized to phospholipids, a component of the nerve cell membrane, to normalize the function of damaged neuronal cells. For these functional reasons, choline alfoscerate is used to treat Alzheimer's disease and other forms of dementia. Hypoglycemia/glucose reperfusion itself causes abnormal functionalization of neurotransmitters and destruction of the nerve cell membrane, resulting in neuronal death or apoptosis. However, so far, to our knowledge, there have been no published studies about the effects of choline alfoscerate in hypoglycemia. So, we wanted to confirm if the diverse effects of choline alfoscerate administration could protect against neuronal death and cognitive dysfunction after hypoglycemia/glucose reperfusion. As a result, we found that the administration choline alfoscerate significantly reduced oxidative stress, excessive inflammation, neuronal death and cognitive impairment following hypoglycemia/glucose reperfusion. In conclusion, the present study suggests that choline alfoscerate may be a therapeutic option for diabetic patients to treat hypoglycemia-induced neuronal death and cognitive impairment.
Keywords: Hypoglycemia/Glucose reperfusion injury; Choline alfoscerate; Inflammation; Neuronal death; Cognitive impairment

Disclosures: A. kho: None. **S. Lee:** None. **D. Hong:** None. **B. Kang:** None. **M. Park:** None. **S. Lee:** None. **C. Lee:** None. **H. Yang:** None. **S. Woo:** None. **S. Park:** None. **D. Kim:** None. **S. Suh:** None.

Poster

377. Mechanisms of Neurodegeneration and Neuroprotective Therapies

Location: SDCC Halls B-H

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Program #/Poster #: 377.08

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NRF- 2020R1A2C2008480
HI20C0408

Title: The effects of pyruvate kinase M2 (PKM2) gene deletion in astrocyte-specific glycolysis and global cerebral ischemia-induced neuronal death

Authors: *B. KANG, S. SUH, A. KHO, S. LEE, D. HONG, M. PARK, S. LEE, C. LEE, H. YANG, S. WOO, S. PARK, D. KIM;
Hallym Univ., Hallym Univ., Chuncheon, Korea, Republic of

Abstract: Ischemic stroke is one of the most severe brain diseases and is caused by insufficient supply of nutrients and oxygen when there is a sudden decrease of blood flow toward to the brain. Astrocytes play an important role in bidirectionally converting pyruvate generated via glycolysis into lactate, and then supplying it to neurons through the astrocyte-neuron lactate shuttle (ANLS). Pyruvate kinase M2 (PKM2) is an enzyme that dephosphorylates phosphoenolpyruvate to pyruvate during astrocyte-specific glycolysis. Here, we hypothesized that a reduction of lactate supply in the astrocyte-specific PKM2 knockout (-/-) would lead to more widespread neuronal death compared to wild-type mice after global cerebral ischemia (GCI). PKM2 (-/-) mice were established by administrating tamoxifen to Aldh1l1-Cre^{ERT2}; PKM2$^{f/f}$ mice. To induce global ischemia disease, we performed blood flow restoration and resuscitation after occlusion of the bilateral common carotid artery (BCCA). Also, to confirm whether lactate administration in astrocyte-specific PKM2 (-/-) knockdown reduces neuronal death, we immediately injected sodium L-lactate (250mg/kg, i.p.) following GCI. To verify our hypothesis, we confirmed oxidative damage, microtubule disruption, disruption of lactate metabolism and neuronal death in knockdown and control subjects. As a result, PKM2 (-/-) mice demonstrated an increase degree of neuronal damage and impairment of lactate metabolism in the hippocampus region after GCI. Conversely, the lactate administration groups showed significantly reduced neuronal death and increases in neuron survival and cognitive function, compared against GCI alone. In conclusion, we suggest that lactate supply via the ANLS in astrocytes plays a crucial role in maintaining energy metabolism in neurons. Furthermore, additional lactate administration may represent a potential therapeutic strategy to treat or prevent neuronal damage following ischemic stroke.

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Poster

377. Mechanisms of Neurodegeneration and Neuroprotective Therapies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 377.09

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: HU20C0206

Title: An AMPA receptor inhibitor, L-theanine, ameliorates traumatic brain injury-induced hippocampal neuronal death in rat.

Authors: *M. PARK, A. KHO, S. LEE, D. HONG, D. KANG, S. LEE, C. LEE, H. YANG, S. WOO, S. PARK, D. KIM, S. SUH;
Hallym Univ., Hallym Univ., chuncheon, Korea, Republic of

Abstract: Traumatic brain injury (TBI) is one of the most prevalent brain diseases and is caused by physical force trauma following an accident or violent strike to the head. The primary cause of TBI-induced damage is brain edema, swelling and intracranial hemorrhage that contribute to the emergence of secondary injuries such as excitotoxicity, oxidative damage, neuroinflammation and mitochondrial dysfunction. Both primary and secondary damage from TBI lead to cognitive impairment, social and behavioral disorders, permanent disability and even death. Excitatory neurotransmitter receptors typically have a glutamate binding site, which moves extracellular calcium into the intracellular space through these receptors in neurons under certain conditions. Neuro pathological disorders such as ischemia, seizure or hypoglycemia lead to excessive calcium and zinc influx by hyper-activation of glutamate receptors including NMDA, AMPK and KA subtypes. In particular, the AMPA receptor subtype causes excessive calcium and zinc influx, leading to neuronal injury and cognitive impairment. L-theanine is known as an AMPA receptor inhibitor by functioning as an competitive antagonist and enhances glutathione formation, which has strong anti-oxidative effect. Therefore, we hypothesized that L-theanine could decrease TBI-induced hippocampal neuronal damage by reducing excessive translocation of calcium and zinc into the cells. To confirm our hypothesis, we immediately injected TBI-experienced rats with L-theanine (200mg/kg) and sacrificed them at 24 hours later. We evaluated L-theanine effects on TBI-induced brain damage through histological analysis such as Fluoro-Jade B (FJB), 4-hydroxynonenal (4HNE), ionized calcium-binding adapter molecule 1 (Iba-1), Glial fibrillary acidic protein (GFAP) staining, etc. In the present study, we confirmed that L-theanine treatment inhibited neuronal death and cognitive impairment by inhibiting the activation of AMPA receptor and increasing the production of glutathione, an antioxidant. Therefore, our findings propose that the suppression of AMPA receptor may represent a new therapeutic approach for the treatment of neuronal death after TBI.

Disclosures: **M. Park:** None. **A. Kho:** None. **S. Lee:** None. **D. Hong:** None. **D. Kang:** None. **S. Lee:** None. **C. Lee:** None. **H. Yang:** None. **S. Woo:** None. **S. Park:** None. **D. Kim:** None. **S. Suh:** None.

Poster

377. Mechanisms of Neurodegeneration and Neuroprotective Therapies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 377.10

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: HU20C0206
HI20C0408

Title: An acid sphingomyelinase inhibitor, imipramine, reduces hippocampal neuronal death after TBI

Authors: *S. LEE, A. KHO, B. CHOI, S. LEE, D. HONG, B. KANG, M. PARK, C. LEE, H. YANG, S. WOO, S. PARK, D. KIM, S. SUH;
Hallym Univ., Hallym Univ., Chuncheon, Korea, Republic of

Abstract: Traumatic brain injury (TBI), broadly, is a decline in the normal function of the brain after experiencing a bump, blow or jolt to the head. It leads to aggravation of pre- existing brain dysfunction and promotes neurotoxic cascades that involve processes such as oxidative stress, loss of dendritic arborization, and zinc accumulation. Acid sphingomyelinase (ASMase) is an enzyme that hydrolyzes sphingomyelin to ceramide in cells. Ceramide is involved in various physiological functions such as cell migration, cellular differentiation and apoptosis. However, under pathological settings such as cerebral ischemia and Alzheimer's disease, excessive ceramide production causes cell death promoting mechanisms to occur. Therefore, we hypothesized that inhibition of ASMase activity by the ASMase inhibitor, imipramine, could reduce ceramide formation and thus prevent TBI-induced neuronal death. To test our hypothesis, animals were injected with imipramine (10mg/kg, i.p.) and then sacrificed at 24 hours and 7 days following TBI. Based on the results of these experiments, we confirmed that imipramine significantly reduced ceramide formation, dendritic loss, oxidative stress and finally neuronal death in the TBI-imipramine group compared to TBI-vehicle group at 24 hours and 7 days. Additionally, we validated that imipramine prevented TBI-induced cognitive dysfunction via Morris water maze and neurological behavior testing (mNSS test). Consequently, we suggest that ASMase inhibition may be a promising therapeutic method to reduce TBI-induced hippocampal neuronal death after TBI. **Keywords:** Traumatic brain injury, Imipramine, ASMase inhibitor, Ceramide, Neuronal death

Disclosures: S. Lee: None. **A. Kho:** None. **B. Choi:** None. **S. Lee:** None. **D. Hong:** None. **B. Kang:** None. **M. Park:** None. **C. Lee:** None. **H. Yang:** None. **S. Woo:** None. **S. Park:** None. **D. Kim:** None. **S. Suh:** None.

Poster

377. Mechanisms of Neurodegeneration and Neuroprotective Therapies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 377.11

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: HU20C0206

Title: An inhibitor of phosphodiesterase 4 (PDE4), amlexanox, rescues hippocampal neuronal death after traumatic brain injury

Authors: *S. WOO, A. KHO, S. LEE, D. HONG, B. KANG, M. PARK, S. LEE, C. LEE, H. YANG, S. PARK, S. SUH;
Dept. of Physiology, Hallym University, Col. of Med., Chuncheon, Korea, Republic of

Abstract: Traumatic brain injury (TBI) is a form of severe brain damage that develops via external mechanical trauma that can then lead to cognitive, memory, behavioral and physical disability. Phosphodiesterase 4 (PDE4) is an enzyme that regulates the hydrolysis of cAMP and influences the intracellular concentration of second messengers such as cAMP and cGMP, which both play an important role in neuro-signaling. PDE4 regulates lysosomal function and autophagy via reduction of intracellular cAMP levels and the activity of protein kinase A (PKA) in pathways associated with autophagy. Under pathological conditions, the normal lysosomal and autophagic functions of protein clearance are inhibited due to a pathological decrease in cAMP levels and these toxic substances accumulate intracellularly, thus leading to dyshomeostasis and finally, cell death. Amlexanox is well-known as an anti-inflammatory drug that functions by inhibiting the synthesis and release of inflammatory mediators. Additionally, it acts as a non-selective PDE4 inhibitor that can increase intracellular cAMP levels and PKA activity. Therefore, we hypothesized that amlexanox, a PDE4 inhibitor, may prevent TBI-induced hippocampal neuronal death via anti-inflammatory effects and autophagic enhancement. To evaluate our hypothesis, after TBI, we immediately injected rats with amlexanox (100mg/kg, i.p.) once per day for 1 week and then sacrificed the subject. This present study confirmed that amlexanox administration significantly reduced the number of TBI-induced degenerating neurons and the degree of inflammation, blood-brain barrier (BBB) disruption, oxidative stress and neurologic & cognitive impairment by enhancing lysosomal and autophagic function. We anticipated that when TBI occurs, PDE4 activity will be enhanced and increased PDE4 activity will decrease both cAMP and PKA activity and demonstrated that this situation would leads to the accumulation of damaging intracellular conditions and eventually neuronal cell death due to poor lysosomal and autophagic function, after TBI. In conclusion, we suggest that amlexanox may have therapeutic potential to prevent TBI-induced hippocampal neuronal death.
Key Words: Traumatic brain injury (TBI), phosphodiesterase 4 (PDE4), cAMP, PKA, lysosomal function, autophagy

Disclosures: S. Woo: None. **A. Kho:** None. **S. Lee:** None. **D. Hong:** None. **B. Kang:** None. **M. Park:** None. **S. Lee:** None. **C. Lee:** None. **H. Yang:** None. **S. Park:** None. **S. Suh:** None.

Poster

377. Mechanisms of Neurodegeneration and Neuroprotective Therapies

Location: SDCC Halls B-H

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Program #/Poster #: 377.12

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01EY028039
R01NS114397

The Kate Sidran Family
The Giller Family Foundations

Title: Traumatic brain injury-induced progressive axonopathy is regulated by the Wallerian degeneration executor SARM1

Authors: *S. J. TRIPATHI[1], A. ALEXANDRIS[1], Y. LEE[1], M. LEHAR[1], Z. ALAM[1], D. PERDOMO[1], J. RYU[1], D. S. WELSBIE[2], D. J. ZACK[3], V. E. KOLIATSOS[1];
[1]Dept. of Pathology, Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [2]UC San Diego Sch. of Med., San Diego, CA; [3]Wilmer Eye Inst. Res. Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Traumatic axonal injury (TAI) and TAI-associated traumatic axonopathy are common consequences of traumatic brain injury (TBI), contributing to significant neurological morbidity. The mouse visual system is highly advantageous for the study of mechanisms and treatments for TAI due to its a) unique anatomical configuration allowing the separate study of perikarya, axons and terminal fields and b) its consistent degeneration in a variety of TBI models, including impact acceleration (IA-TBI). We have previously established that the perikarya and proximal, but not distal portions of injured RGCs respond to the stress MAP3K blockade. Here we explored the ultrastructural features and the time course of optic nerve TAI and the ensuing traumatic axonopathy following IA-TBI, as well as the role of the Sterile alpha and TIR-motif containing 1 (SARM1), the key executor of Wallerian degeneration, in the progressive breakdown of injured axons proximal and distal to injury. Wild type and SARM1 KO mice received IA-TBI or sham injury and were allowed to survive for 3, 7, 14 and 21 days. Morphological features of TAI and traumatic axonopathy were assessed by electron microscopy while normal and pathological axon profiles were quantified on semithin optic nerve sections using stereological methods. In both wild type and SARM1 KO mice, IA-TBI results in TAI characterized by acute and variable degrees of axonal and myelin perturbations, apparent axonal disconnection, and an evolving axonopathy and degeneration of proximal and distal segments. In both groups there is progressive loss of intact axons and an increase in pathological profiles, however the timing and the magnitude of pathology in the distal ON differ significantly: Sarm1 deletion significantly reduces pathology by 30-40 % on 7, 14 and 21 days post-injury, while it also prevents axon loss by 50% on 7 and 14 days, with a continued protective trend at 21 days. Preliminary data reveal that the effects of Sarm1 deletion extend to the protection of the retinocollicular terminal fields. Despite such strong effects on the course of distal axonopathy, Sarm1 deletion does not prevent the loss of RGC cell bodies and proximal axons. Our data indicate the molecular differentiation of axonal degeneration and axonopathy proximal and distal to the injury, with SARM1-dependent processes selectively contributing to and determining the trajectory of distal traumatic axonopathy.

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Poster

377. Mechanisms of Neurodegeneration and Neuroprotective Therapies

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CONACyT grant number: 319578
DGAPA postdoctoral fellowship
CONACYT postdoctoral fellowship

Title: In silico analysis to identify molecular mechanisms associated with Niemann-Pick disease as potential new biomarkers

Authors: T. PRADEL-BERNAL, *M. SILVA-LUCERO, L. GÓMEZ-VIRGILIO, J. RIVERA-OSORIO, M. CARDENAS-AGUAYO;
Physiol., UNAM, Ciudad de México, Mexico

Abstract: Niemann-Pick disease type C1 is a neuro-visceral lysosomal storage disorder that affects people of variable ages ranging from infancy to adulthood. The presence of mutations in either NPC1 or NPC2 leads to accumulated cholesterol and other lipids inside endosoma/lysosoma that give rise to loss of function, autophagy dysregulation and eventually death cell. The symptoms of the disease are variable and may include: enlarged liver or/and spleen, learning difficulties, seizures, gait problems, dementia, loss of muscle tone, among others. For this project we used in silico methods to identify the principal genes affected in this disease. The approach was an analysis of microarray data from GEO2, accession number GSE124283. We studied the gene expression of skin fibroblast from 45 individuals: 22 healthy controls and 23 with NPC. The raw data set were downloaded, normalized and the differential expression analysis was performed by limma package, this analysis revealed a bias (excessive variability) among subjects within the same category (control or patient) when analyzed the two sexes together, so we decided to analyze men's and women's data independently. The second approach was looking for the biological significance of the differential expressed genes with enrichments analysis: the Gene Ontology, KEGG pathway and PWMEnrich; here we got several differences between the two sexes although we found similarities that represent this pathology, some of this common genes between the two groups of the databases, that were differentially expressed between patients and controls, where enriched in the lysosome, endosome transport, lipid metabolism, Wnt signaling pathway and cell-cell interaction. Despite both data sets share just 18 genes with the same regulation, these genes participate on important pathways and we propose that some of the 18 DEGs that we found related to NPC disease could have value as potential biomarkers or targets of this disease.

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Poster

377. Mechanisms of Neurodegeneration and Neuroprotective Therapies

Location: SDCC Halls B-H

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Program #/Poster #: 377.14

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant RO1EY028039
NIH Grant R01NS114397
TEDCO Grant 2020-MSCRFD-5402

Title: Rescuing axons from Wallerian degeneration by pharmacological manipulation of NAD^+ metabolism: mechanisms and synergies of NAMPT and stress MAPK inhibitors and nicotinic acid riboside

Authors: *A. S. ALEXANDRIS[1], J. RYU[1], L. RAJBHANDARI[2], R. HARLAN[4], J. MCKENNEY[1], Y. WANG[1], S. AJA[4], D. GRAHAM[4], A. VENKATESAN[2], V. E. KOLIATSOS[1,2,3];
[1]Pathology, [2]Neurol., [3]Psychiatry and Behavioral Sci., Johns Hopkins Univ., Baltimore, MD; [4]Johns Hopkins All Children's Hosp., St. Petersburg, FL

Abstract: Wallerian degeneration (WD) is a conserved axonal self-destruction program implicated in several neurological diseases from peripheral neuropathies and traumatic brain injury to glaucoma and age-related neurodegenerative diseases. WD is driven by the degradation of the NAD^+ synthesizing enzyme NMNAT2, the buildup of its substrate NMN, and the activation of the NAD^+ degrading SARM1, eventually leading to axonal fragmentation. The amenability of these events to therapeutic interventions is still unclear. Here we explored pharmacological strategies that modulate NMN and NAD^+ metabolism, namely the inhibition of the NMN-synthesizing enzyme NAMPT, activation of the nicotinic acid riboside (NaR) salvage pathway, and inhibition of the NMNAT2-degrading stress MAPK (DLK) pathway in axotomy models. Results show that NAMPT and DLK inhibition cause a significant but time-dependent suppression of WD. NAMPT inhibition was sufficient to reduce SARM1 activity, protect axonal NAD+ levels and delay WD in a time-dependent manner. Supplementation with NaR leads to further reduction of SARM1 activity even with delayed treatment and prevention of axon fragmentation up to 4 days in vitro. Additional DLK inhibition further augments NAD^+ metabolism and extends axonal protection to 6 days. Preliminary evidence suggests that the previous pharmacological treatments are also protective in WD models using human stem cell-derived neurons in vitro and in mice in vivo. The axonal NAD^+/NMN ratio is highly predictive of cADPR levels, extending previous cell-free evidence on the allosteric regulation of SARM1. Our findings establish the protective effects of translationally relevant small molecules in the WD of injured axons that proceed via complex effects on NAD^+ metabolism and inhibition of SARM1.

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Poster

377. Mechanisms of Neurodegeneration and Neuroprotective Therapies

Location: SDCC Halls B-H

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Program #/Poster #: 377.15

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: MRC DTP Award
Gates Scholarship

Title: Effects of constitutively low NMNAT2 levels on SARM1 activation and NAD^+ metabolism in neurons

Authors: *C. ANTONIOU[1], C. ANGELETTI[2], J. GILLEY[1], A. LORETO[1], G. ORSOMANDO[2], M. COLEMAN[1];
[1]Univ. of Cambridge, Cambridge, United Kingdom; [2]Polytechnic Univ. of Marche, Ancona, Italy

Abstract: The extreme length, elaborate branching and high metabolic demand of axons makes them vulnerable to many stresses, yet they must survive and maintain their structure and function for decades. In many neurodegenerative conditions axons die long before their cell bodies, following a “dying back” mechanism from their distal ends, resulting in devastating consequences including pain, sensory and motor loss. A well-characterised signaling pathway known as Wallerian degeneration (or programmed axon death) regulates axon survival and contributes to genetic, toxic and metabolic models of disease as well as human disorders. Nicotinamide adenine dinucleotide (NAD)-related metabolism plays a central role in programmed axon death. The pathway is triggered when NMNAT2, an NAD^+-synthesising enzyme crucial for axon survival, is depleted. NMNAT2 depletion leads to activation of SARM1, an NAD(P)-consuming enzyme that kills axons. Interestingly, NMNAT2 loss has distinct effects on axon fate depending on whether it is acute or constitutive. In primary mouse neurons acute loss of a single *Nmnat2* allele kills axons whereas chronic depletion of one allele is consistent with long-term axon survival. *In vivo* mice survive and remain healthy with an expression down to 30% of wild-type NMNAT2. The aim of our study is to understand whether chronic depletion of NMNAT2 can activate SARM1 in seemingly intact axons. We have shown that superior cervical ganglion (SCG) neurons from mice expressing 30% of normal NMNAT2 have neurite outgrowth defects and significantly less NAD^+ and $NADP^+$ than wild-type neurons. This loss of NAD^+ and $NADP^+$ appears to be completely SARM1-dependent, suggesting that chronic activation of SARM1 leads to a constitutive depletion of these metabolites without causing axon degeneration. We also show that the neurite outgrowth defect can be partially rescued with application of the NAD^+ precursor Nicotinamide Riboside (NR). NR application however does cause a complementary increase in NAD^+, suggesting that NAD^+ level does not determine neurite length. Finally, it will be of great future interest to establish what compensatory mechanisms are employed to allow axons with chronically active SARM1 to survive.

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Poster

377. Mechanisms of Neurodegeneration and Neuroprotective Therapies

Location: SDCC Halls B-H

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Program #/Poster #: 377.16

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Wellcome Trust 210904/Z/18/Z

Title: Axon injury and environmental neurotoxins cause SARM1-dependent axon degeneration in human iPSC-derived dopaminergic neurons

Authors: *A. LORETO[1], K. CRAMB[2], E. MERLINI[1], L. MCDERMOTT[2], D. BENNETT[2], G. ORSOMANDO[3], M. P. COLEMAN[1], R. WADE-MARTINS[2];
[1]Univ. of Cambridge, Cambridge, United Kingdom; [2]Univ. of Oxford, Oxford, United Kingdom; [3]Universita' Politecnica delle Marche, Ancona, Italy

Abstract: Programmed axon death (Wallerian degeneration) is a well-characterised pathway of axon degeneration controlled by the antagonistic activities of two enzymes, SARM1 and NMNAT2. SARM1 is a prodegenerative enzyme with $NAD(P)^+$-consuming activity. This activity is regulated by the axonal survival and NAD^+-synthesising enzyme NMNAT2. When NMNAT2 is depleted, accumulation of its substrate NMN binds to and activates SARM1, leading to axon degeneration. Remarkably, SARM1 deletion confers strong axonal protection against many toxic insults and lifelong protection against lethality caused by NMNAT2 deficiency in mice. Many studies have shown that programmed axon death is implicated in several animal models of disease and the first links with human disease are now emerging. SARM1 is therefore a very promising therapeutic target for blocking axon degeneration in disease.
Compared to the extensive data in animal models, there are not many studies on programmed axon death in human neurons and human models of disease. It is therefore important to address this in order to move towards clinical translation. Here we report unpublished data showing that activation of programmed axon death causes axon degeneration in human iPSC-derived dopaminergic neurons. We show that deletion of *SARM1* in these neurons confers strong axon protection against injury-induced axon degeneration and neurodegeneration caused by environmental toxins and mitochondrial toxins, such as rotenone. We also demonstrate a reduction in the levels of SARM1's substrate NAD^+ and an increase in the levels of SARM1's product cADPR, confirming SARM1 activation following induction of the above stresses in iPSC-derived dopaminergic neurons. Finally, we show that activation of programmed axon death causes mitochondrial dysfunction which precedes axon degeneration and can occur also in morphologically intact axons (not destined to degenerate).
These studies demonstrate that the programmed axon death pathway regulates axonal survival in

human iPSC-derived dopaminergic neurons, validating this model to extend our molecular understanding of the pathway, its regulation, and test potential drugs for blocking axon degeneration in disease. It will be important to extend these studies in the context of disease and, among others, in iPSC lines derived from people with Parkinson's disease.

Disclosures: A. Loreto: None. **K. Cramb:** None. **E. Merlini:** None. **L. McDermott:** None. **D. Bennett:** None. **G. Orsomando:** None. **M.P. Coleman:** None. **R. Wade-Martins:** None.

Poster

377. Mechanisms of Neurodegeneration and Neuroprotective Therapies

Location: SDCC Halls B-H

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Program #/Poster #: 377.17

Title: WITHDRAWN

Poster

377. Mechanisms of Neurodegeneration and Neuroprotective Therapies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 377.18

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: VIEP-BUAP 2022
Neuropharmacology Lab´s CBD-2022

Title: The administration of cannabidiol reduces seizures and GFAP and Iba1 activation of rats with pentylentretazol

Authors: J. V. CRISANTO CUATLAYOTL[1], C. DE LA CRUZ AMADOR[5], A. GARCIA CAMARILLO[2], A. PATRICIO MARTINEZ[6], F. LUNA-MORALES[7], F. PATRICIO-MARTINEZ[3], ***I. LIMON PEREZ DE LEON**[4];
[2]FCQ Neuropharm. Lab´s, [1]Benemerita Univ. Autonoma De Puebla Neuropharm. Lab´s, San Manuel CU Puebla, Mexico; [3]Benemerita Univ. Autonoma De Puebla Neuropharm. Lab´s, San Manuel Puebla ciudad Universitaria, Mexico; [4]Benemerita Univ. Autonoma De Puebla Neuropharm. Lab´s, Puebla, Mexico; [5]Benemerita Univ. Autonoma De Puebla FCQ. Neuropharm. Lab´s, San Manuel C.U. Puebla, Mexico; [6]Facultad de Ciencias Biologicas, Benemerita Univ. Autonoma De Puebla Facucltad de Ciencias Biologicas, San Manuel C.U. Puebla, Mexico; [7]Neuroendrocrnology, Benemerita Univ. Autonoma De Puebla Neuroendocrinology ab´s, San Manuel Puebla, Mexico

Abstract: There is a gamma of treatments for the different types of epileptic seizures. Some drugs show adverse effects. In the search for drugs that contribute to treatment or that cannabidiol be a new pharmacological option, it can be a therapeutic alternative. Cannabidiol is known to exert its effect by interacting with different receptors and enzymes, regulating oxidative stress, apoptosis, mitochondrial damage. However, its effect on the inflammatory process is still unclear.The objective of this work is to show that cannabidiol modifies epileptic seizures and decreases the expression of GFAP and Iba in the hippocampus and somatosensory cortex.Male Wistar rats (sixteen rats for each group) were used, divided into two groups. One group was given cannabidiol 15 mg kg i.p. for ten days, the control group was given the vehicle. Subsequently, cannabidiol plus pentylenetetrazole (37 mg kg i.p.), a drug that produces convulsions, was jointly administered every three days. This administration was repeated four times. The experiments were videotaped and the latency time and duration of the tonic-clonic and/or myoclonic seizures were recorded. The behaviors of each animal were also analyzed. At the end, the animals were sacrificed and perfused to carry out immunofluorescence for GFAP and Iba1 (inflammatory markers) in the hippocampus and somatosensory cortex. We found that cannabidiol significantly prolonged the latency time, compared to the group with pentylenetetrazol. The duration of tonic-clonic and myoclonic seizures decreased significantly. The immunoexpression of GFAP Iba1 decreased in the hippocampus in the group of animals treated with cannabidiol plus pentylenetetrazol. These data indicate that cannabidiol can help control seizures and reduce the neuroinflammatory process.

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Poster

377. Mechanisms of Neurodegeneration and Neuroprotective Therapies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 377.19

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: HU20C0206

Title: A phosphodiesterase 4 (PDE4) inhibitor, amlexanox, reduces neuroinflammation and neuronal death after pilocarpine-induced seizure

Authors: *H. YANG[1], A. KHO[1], S. LEE[1], D. HONG[1], B. KANG[1], M. PARK[1], S. LEE[1], C. LEE[1], S. PARK[1], S. WOO[1], D. KIM[1], H. SONG[2], H. CHOI[3], S. SUH[1];
[1]Dept. of Medicine, Physiol. 3604, Dept. of Physiology, Hallym University, Col. of Med., Chuncheon, Korea, Republic of; [2]Neurology, Kangdong Sacred Heart Hosp., Seoul, Korea, Republic of; [3]Neurology, Hallym Univ. Chuncheon Sacred Heart Hosp., Chuncheon, Korea, Republic of

Abstract: Epilepsy is a neurological disorder caused by repeated seizure, which is triggered by abnormal electrical activity in the brain. When an acute neurological disease occurs, it provokes the accumulation of toxic substrates in the various cellular organelles due to defective functioning of lysosomes, leading to autophagy dysfunction. Phosphodiesterase-4 (PDE4) is an enzyme that hydrolyzes cAMP to 5'-AMP and regulates indirectly the activity of protein kinase A (PKA). Also, PDE4 is involved in lysosomal function and release of proinflammatory cytokines. Under pathological conditions, cAMP levels abnormally decreased due to high PDE4 activity. Amlexanox is used as an anti-inflammatory drug and also known as a non-selective PDE4 inhibitor. In the present study, we hypothesized that epileptic seizure induced lysosomal dysfunction via abnormally enhanced PDE4 activity, excessive neuroinflammation and finally neuronal death in the brain. Thus, we demonstrated that the administration of amlexanox, a PDE4 inhibitor, has direct neuroprotective effects by enhancing the lysosomal function. To evaluate our hypothesis, we used a pilocarpine-induced epilepsy animal model and injected amlexanox (100 mg/kg, i.p.) daily for a week after seizure. After 1 week, we obtained brain tissues and performed several histological staining to evaluate the neuroprotective effects of Amlexanox. As a result, we verified that amlexanox ameliorated lysosomal dysfunction, inflammation, hippocampal neuronal death, cognitive impairment, etc. Therefore, our findings asserted that amlexanox may be a potential therapeutic drug to treat epileptic brain disorder. **Key Words:** Epilepsy, cAMP, Phosphodiesterase4, Protein Kinase A, Lysosome, Autophagy, Cognitive dysfunction

Disclosures: **H. Yang:** None. **A. Kho:** None. **S. Lee:** None. **D. Hong:** None. **B. Kang:** None. **M. Park:** None. **S. Lee:** None. **C. Lee:** None. **S. Park:** None. **S. Woo:** None. **D. Kim:** None. **H. Song:** None. **H. Choi:** None. **S. Suh:** None.

Poster

377. Mechanisms of Neurodegeneration and Neuroprotective Therapies

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Program #/Poster #: 377.20

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NRF-2021R1A6A3A01087054

Title: An acid sphingomyelinase inhibitor, imipramine, increases the survival of newborn neurons after pilocarpine-induced seizure.

Authors: *S. LEE[1], A. KHO[1], D. HONG[1], B. KANG[1], M. PARK[1], S. LEE[1], H. YANG[1], C. LEE[1], S. WOO[1], S. PARK[1], D. KIM[1], H. SONG[2], H. CHOI[3], S. SUH[1];
[1]Hallym Univ., Hallym Univ., Chuncheon, Korea, Republic of; [2]Neurology, Kangdong Sacred Heart Hosp., Seoul, Korea, Republic of; [3]Neurology, Hallym Univ. Chuncheon Sacred Heart Hosp., Chuncheon, Korea, Republic of

Abstract: Epilepsy is a common progressive neurological disorder that, in addition to genetic factors, may be triggered by specific traumatic events that injure the brain, such as severe traumatic brain injury (TBI), infection or development of abnormal neuronal activity in the brain. Acid sphingomyelinase (ASMase) is an enzyme that hydrolyzes sphingomyelinase into ceramides, and the excessive ceramides produced at this time can cause viral and bacterial infection as well as a wide range of diseases such as cancer, cystic fibrosis, diabetes, Alzheimer's and depression. Ceramide acts as a pro apoptotic intracellular messenger and is also known to cause reactive oxygen species (ROS) production, increased inflammation and lysosomal damage. After seizure, excessive zinc release occurs in neurons and activates ASMase to promote ceramide formation, which plays a pivotal role in apoptotic signaling and cell death in this setting. Under pathological conditions, ASMase is excessively activated and leads to activation of apoptotic signaling pathways. Imipramine, an ASM inhibitor, can reduce the activity of ASMase, thereby reducing ceramide production and apoptosis. So, we hypothesized that seizure induces the generation of ceramide via ASMase activation and ceramide leads to DNA damage and finally neuronal apoptosis. To verify our hypothesis, we used pilocarpine-induced seizure animal model with rats. Imipramine (10 mg/kg, i.p.) was continuously injected once per day for 4 weeks after seizure. We performed histological and cognitive function analysis at 4 weeks after seizure. We found that post-seizure treatment with imipramine reduced markers of neuronal apoptosis and increased the number of newborn neurons in the dentate gyrus of hippocampus after seizure. In addition, we confirmed that imipramine treatment prevents seizure-induced cognitive impairment. Therefore, we suggest that imipramine may be a robust therapeutic approach to enhance the survival of newborn neurons and cognitive function after seizure.

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Poster

377. Mechanisms of Neurodegeneration and Neuroprotective Therapies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 377.21

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: GAOMA Therapeutics
PULSALYS, public grant
Bpifrance, i-lab Grant

Title: Gao-3-02: a promising lipidic drug-candidate in epilepsy, reducing seizures and improving cognition, with anti-inflammatory activity

Authors: *A. BELMEGUENAI[1,2], S. BODENNEC[2], J. BODENNEC[1,2], J. GUYON[2], L. BEZIN[1,2];
[1]Translational and Integrative Group in Epilepsy Research, Lyon Neurosci. Res. Center, Univ.

Lyon 1; CNRS UMR5292, Inserm U1028, Epilepsy Inst. IDEE, Bron, France; [2]GAOMA Therapeut., Bron, France

Abstract: Epilepsy is a neuronal disorder characterized by the occurrence of spontaneous recurrent seizures and a high prevalence of comorbidities, such as cognitive impairments. Despite the arsenal of marketed anti-seizure medications (ASMs), a high medical need persists for epilepsy treatment. The currently available ASMs only provide a symptomatic anti-seizure action, most of them presenting tolerability issues including cognitive alterations, while a significant part of patients still display pharmaco-resistance. Therefore, there is a real need to develop new drugs with innovative mechanistic approaches, addressing epilepsy beyond the control of seizures. We thus developed GAO-3-02, a derivative of the endocannabinoid-like N-docosahexanoylethanolamide (synaptamide), not only to control seizure but also to improve associated cognitive impairments, with a good tolerability. In this study, we investigated the anti-inflammatory effect of GAO-3-02 using in vivo assays and evaluated its anti-seizure effect in acute seizures and chronic epilepsy models. The present study also assessed the effects of GAO-3-02 treatment on brain plasticity in the rat lithium-pilocarpine (Pilo) model of temporal lobe epilepsy, by examining its ability to ameliorate behavioral and synaptic plasticity deficits. As a result, we found that GAO-3-02 reduces the transcripts levels of certain proinflammatory cytokines in the hippocampus of both LPS-induced mouse neuroinflammation and Pilo rat models. The results demonstrated also a dose-dependent protection of GAO-3-02 against PTZ-induced seizures and a significant reduction in the total number or severity of seizures in the Pilo or the fully amygdala-kindled rat models. In the Pilo rat model, GAO-3-02 significantly ameliorated spatial learning deficits and completely rescued hippocampal Long-Term Potentiation (LTP), contrary to other ASMs tested (sodium valproate, levetiracetam and cannabidiol). After a long-term exposure of healthy mice to GAO-3-02, no impairments of cognitive functions were observed in the open field, the elevated zero maze and the Morris water maze tests, indicating a favorable tolerability. Overall, GAO-3-02 displayed robust anti-seizure effects across validated seizure and epilepsy models and ameliorated spatial learning and synaptic plasticity, possibly via inhibition of inflammatory cytokine secretion. GAO-3-02 may therefore be a novel, promising therapeutic candidate for epilepsy and associated cognitive impairments.

Disclosures: **A. Belmeguenai:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); GAOMA Therapeutics. F. Consulting Fees (e.g., advisory boards); GAOMA Therapeutics. **S. Bodennec:** A. Employment/Salary (full or part-time):; GAOMA Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); GAOMA Therapeutics. **J. Bodennec:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); GAOMA Therapeutics. F. Consulting Fees (e.g., advisory boards); GAOMA Therapeutics. **J. Guyon:** A. Employment/Salary (full or part-time):; GAOMA Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); GAOMA Therapeutics. **L. Bezin:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); GAOMA Therapeutics. F. Consulting Fees (e.g., advisory boards); GAOMA Therapeutics.

Poster

377. Mechanisms of Neurodegeneration and Neuroprotective Therapies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 377.22

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: HU20C0206

Title: The effects of the L-type voltage gated calcium channel (LTCC) inhibitor, amlodipine, on hippocampal neuronal death after pilocarpine-induced seizure

Authors: *C. LEE[1], A. KHO[1], S. LEE[1], D. HONG[1], B. KANG[1], M. PARK[1], S. LEE[1], H. YANG[1], S. WOO[1], S. PARK[1], H. SONG[2], H. CHOI[3], S. SUH[1];
[1]Hallym university, Dept. of Physiology, Col. of Medicine, Hallym University, 3604, Chuncheon, Korea, Republic of; [2]Neurology, Kangdong Sacred Heart Hosp., Seoul, Korea, Republic of; [3]Neurology, Hallym Univ. Chuncheon Sacred Heart Hospital, Korea, Chuncheon, Korea, Republic of

Abstract: Epilepsy arises as a result of excessive or abnormally synchronous neuronal activity in the brain, which leads to the activation of L-type voltage gated calcium channels (LTCC), which are localized in neuronal membrane. When LTCC are open, calcium (Ca^{2+}) - in addition to other metal ions such as Zinc (Zn^{2+}) and magnesium (Mg^{2+}) - flow into the cytosol. Ca^{2+} entering through LTCC into the presynaptic terminal leads to Zn^{2+} and glutamate release from the presynaptic terminal to the postsynaptic terminal. Zn^{2+} released in such a fashion is translocated to the postsynaptic terminal via LTCC. When Zn^{2+} subsequently accumulates in neurons, it significantly increases the expression of NADPH oxidase subunits, which are then directly correlated with an increase in reactive oxygen species (ROS) generation, finally leading to neuronal death. Amlodipine (AML), an LTCC inhibitor, is most commonly known as a medication used to treat high blood pressure and coronary artery disease by binding directly to LTCC. We wondered whether AML could decrease the translocation and accumulation of Zn^{2+} in neuron by blocking LTCC and hypothesized that if it could, it may have a potentially protective effect on seizure-induced hippocampal neuronal death. To test our hypothesis, we established an rat epilepsy model using pilocarpine. AML (10mg/kg, p.o., once per day) was injected continuously for 7 days after epilepsy. We performed behavioral testing to evaluate cognitive function and histological measurements to determine to extent of pathological changes such as Zn^{2+} accumulation, oxidative stress, neuronal death, etc. The present study found that LTCC inhibition by AML decreased excessive Zn^{2+} accumulation, ROS production and ultimately hippocampal neuronal death after seizure. In conclusion, we suggests that amlodipine administration could have strong therapeutic potency after seizure. **Key Words**: Seizure, Amlodipine, L-type voltage gated calcium channel, Zinc, Neuronal death, Oxidative stress

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Poster

377. Mechanisms of Neurodegeneration and Neuroprotective Therapies

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Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Nora Jane Almany Foundation
ND NKH Research (Sarb)
Fighting for Fiona and Friends
NKH Crusaders
John Thomas NKH Foundation
Jacqueline Kirby NKH Fund
Lucas John Foundation

Title: Separation of neurological disease states in a preclinical mouse model of non-ketotic hyperglycinemia

Authors: *A. LOPEZ-RAMIREZ, K. HALDAR, C. BICKERTON, A. BALI, P. PADMANABHAN, M. ALAM;
Univ. of Notre Dame, Univ. of Notre Dame, Notre Dame, IN

Abstract: Glycine is catabolized to carbon dioxide and ammonia by the glycine cleavage system (GCS) pathway which is found at high concentrations within the brain and liver due to the constant demand of one-carbon units required in the folate and purine pathways. Defects in the GCS pathway leads to an accumulation of glycine in the plasma and central nervous system. This defect gives rise to a rare neuro-metabolic disease known as Non-Ketotic Hyperglycinemia (NKH), which leads to pathological deficits such as intractable seizures, developmental hindrance, and neural tube defect all of which are associated with an intrinsic genetic deficit in the brain and metabolic dysfunction in the liver. NKH symptoms can mimic those found in hepatic encephalopathy (HE) patients; for example, memory loss, gliosis, and N-methyl-D-aspartate (NMDA) dysregulation. Mutations within glycine decarboxylase (GLDC), the first protein required for glycine catabolism are responsible for 85% of NKH cases. Advancements in our lab have allowed for the first translation of the most prevalent human disease mutation GLDC, p.A389V, into a preclinical mouse model, p.A394V. Using young (1-month old) and mature (9-month old) mice, we compared the first comprehensive metabolome maps of the brain, liver, and plasma to understand the changes as a function of age and disease progression. All of these metabolomes indicate dramatic fluctuations in glycine metabolites, glycine conjugates, amino acid anabolism, and purine synthesis suggesting druggable pathways that can be targeted to treat NKH. Protein analysis suggests that heterozygous mice lost ~50% GLDC protein while

homozygous mutant mice demonstrated a 90% reduction, which reflect severity of changes seen in the metabolome. Additional histological analysis was performed on liver sections; wildtype mice demonstrated proper hepatocyte morphology while mutant mice showed increased steatosis. Cognitive defects in mice that survive early severe (and frequently fatal) cerebral defects were also investigated and revealed that mutants showed a reduction in cognition in the Morris Water Maze, suggesting long term liver-dysfunction alone may induce at least a subset of neurological sequelae. In summary, this prevalent *Gldc* mutation in mice allows for the separation of early neurogenic disease from subsequent systemic metabolic disease with neurological correlates in NKH. The insights gained may be generalizable across a wide spectrum of neurometabolic and neurodegenerative disorders.

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Poster

377. Mechanisms of Neurodegeneration and Neuroprotective Therapies

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Program #/Poster #: 377.24

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: ND NKH Research (Sarb)
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Fighting for Fiona and Friends
NKH Crusaders
John Thomas NKH Foundation
Jacqueline Kirby NKH Fund
Lucas John Foundation

Title: Efficacy of rAAV9 Gene Therapy in treating mice with Non-ketotic hyperglycinemia

Authors: *A. BALI, C. BICKERTON, S. CALHOUN, P. PADMANABHAN, A. L. RAMIREZ, S. ALAM, **K. HALDAR**;
Univ. of Notre Dame, Notre Dame, IN

Abstract: Non-ketotic hyperglycinemia (NKH) is a rare neurometabolic disorder caused by autosomal recessive defects in the genes encoding the glycine cleavage system (GCS), resulting in elevation of glycine in the blood and brain. Severe disease manifests within the first two weeks of life and causes epileptic seizures, hypotonia, and developmental delays. 85% of NKH cases stem from a genetic defect in Glycine decarboxylase (*Gldc)*, which encodes the GLDC or P-protein that catalyzes the first step of glycine cleavage. We used CRISPR-Cas9 technology to genetically engineer mice with the p.A394V mutation (mouse orthologue to a prevalent patient mutation p.A389V) to deliver the first mutation-based mouse model with significant cerebral and systemic disease *(Farris et al., 2021)*. Due to the early development of severe phenotypes, we

investigated gene therapy intervention for NKH. We designed a novel AAV9 viral vector recombinant with a copy of wild-type *Gldc* driven by constitutive promoter to examine consequences for systemic metabolic and severe neurological disease as well as death from NKH in an age dependent way. Mutant mice, post-weaning (P25 – P36) injected systemically with rAAV9 virus particles and tracked for 5 months post-injection showed significantly decreased plasma glycine levels compared to their control counterparts. To address severe neurogenic outcomes and survival, pre-weaned mice (P0 – P2) were injected with rAAV9 virus particle dose adjusted for mice age and weight. Our results show significantly increased in recovery of NKH mutants from cerebral disease as well as survival (measured at P60 adult mice). Our studies suggest that rAAV9 gene therapy has potential for treating both neurological and metabolic disruptions characteristic of NKH disease.

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Poster

377. Mechanisms of Neurodegeneration and Neuroprotective Therapies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 377.25

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: ForeBatten to JMW

Title: A small molecule inhibitor of Sortilin enhances lysosomal function and reduces brain pathology in diverse neurodegenerative lysosomal storage disorders

Authors: H. G. LEPPERT, J. ANDERSON, K. TIMM, C. SWANSON, M. A. PRATT, C. BOOTH, K. WHITE, J. J. BRUDVIG, *J. M. WEIMER;
Pediatrics and Rare Dis. Group, Sanford Res., Sioux Falls, SD

Abstract: Lysosomal storage disorders (LSDs) are a genetically and clinically diverse group of diseases characterized by lysosomal dysfunction. Batten disease (also referred to as neuronal ceroid lipofuscinosis) is a family of severe LSDs primarily impacting the central nervous system. Here we show that ART1001, a small molecule inhibitor of sortilin, improves lysosomal pathology in multiple LSD models. Live cell imaging and comparative transcriptomics revealed that transcription factor EB (TFEB), an upstream regulator of lysosomal biogenesis**,** was activated upon treatment with ART1001. Utilizing CLN2, CLN3, and CLN6- Batten disease mouse models, we show that treatment with ART1001 prevents the accumulation of lysosomal storage material and the development of neuroinflammation. Behavioral outcomes were also completely rescued in our CLN2 Batten disease model. These findings reveal sortilin inhibition as a novel and highly efficacious therapeutic modality for the treatment of lysosomal storage disorders.

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Poster

377. Mechanisms of Neurodegeneration and Neuroprotective Therapies

Location: SDCC Halls B-H

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Program #/Poster #: 377.26

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: SLC6A1 Connect 810616-0522-00

Title: Characterization of a novel in vitro modeling system for SLC6A1 neurodevelopmental disorder and its use for therapeutic testing

Authors: *F. ROUSSEL[1], C. N. DENNYS[2], A. HARTLAUB[1], S. SINHA RAY[1], A. SIERRA DELGADO[1], A. BRADBURY[1,3], K. MEYER[1,3];
[1]Res. Inst. Nationwide Childrens Hosp., Columbus, OH; [2]Alcyone Therapeut. Inc, Boston, MA; [3]Ohio State Univ., Columbus, OH

Abstract: Mutations in the SLC6A1 gene cause a neurodevelopmental disorder, characterized by absence, atonic and myoclonic seizures. Patients also present with developmental delay, EEG abnormalities, language impairment and intellectual disability. Currently, no treatment is available that could alter the disease course. Solute carrier family 6 member 1 (SLC6A1) encodes for the GABA transporter 1 (GAT-1) and is mainly expressed in the brain. The protein is responsible for the reuptake of GABA into presynaptic neurons and glia, clearing the extracellular space and modulating neurotransmission. Since little is known about the disease mechanism and the cell types involved, the development of in vitro models to study the disease in the context of patient cell lines and for testing of new therapeutics is urgently needed. We have previously developed a rapid reprogramming method to convert patient skin fibroblasts into induced Neuronal Progenitors Cells (iNPC) that can further be differentiated into induced Astrocytes (iAs). Here, we utilized this methodology in addition to a rapid chemical conversion method to produce neurons (iNs) from patient fibroblasts to investigate the impact of SLC6A1 mutations in both iNs and iAs. We evaluated the mitochondria and metabolic activities in the different cell types identified to be involved in the disease mechanism. Seahorse analysis indicates that cellular glycolysis and mitochondria activity did not differ significantly from healthy controls. In addition, we established an assay that allowed us to study an important role of astrocytes in the disease mechanism as they reduce the survival and alter morphology of co-cultured neurons. Thanks to this *in vitro* model system, we were able to evaluate different therapeutics that have been shown to be effective in other epileptic disorders. Further evaluation is needed to understand underlying disease mechanisms and specific roles of iAs and iNs in the disease physiopathology and to find better treatments for the patients.

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Poster

377. Mechanisms of Neurodegeneration and Neuroprotective Therapies

Location: SDCC Halls B-H

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Program #/Poster #: 377.27

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: PIPE-791, a potent brain-penetrant selective LPA1 antagonist, reduces neuroinflammation *in vivo*

Authors: *G. C. EDU, M. M. POON, A. O. LORENZANA, A. R. BROADHEAD, J. R. ROPPE, T. O. SCHRADER, L. J. VALDEZ, Y. XIONG, A. C. CHEN, D. S. LORRAIN; Pipeline Therapeut., San Diego, CA

Abstract: Lysophosphatidic acid (LPA) is a naturally occurring inflammatory lipid that is dysregulated in multiple sclerosis - an immune mediated demyelinating disease in the CNS. LPA activates the LPA1 receptor leading to aberrant cytokine and chemokine levels in the CNS, infiltration of peripheral immune cells (particularly autoreactive T cells), as well as microglial and astrocyte activation. Profiling of the inflammatory signaling in the mouse MOG-EAE model of demyelination demonstrated an upregulation of cytokines and chemokines in the CNS that was responsive to LPA1 antagonism. The inflammatory signaling factors induced in the EAE model are also increased after challenge with LPS (lipopolysaccharide), an endotoxin frequently used to induce widespread inflammation. LPA1 antagonists, such as AM152, are effective at reducing LPS-induced inflammation. However, since AM152 is peripherally-restricted, its ability to impact inflammation in the CNS is limited. To address this deficiency, Pipeline Therapeutics has identified PIPE-791, a potent, selective, and brain-penetrant LPA1 antagonist. To demonstrate the need for a brain-penetrant LPA1 antagonist, PIPE-791 was characterized in an LPS model of CNS inflammation. LPS rapidly increases cytokine and chemokine levels in the CNS and this upregulation is inhibited by PIPE-791, but not by the peripherally-restricted antagonist, AM152. In this model, PIPE-791 was effective at not only reducing cytokine and chemokine levels in the CNS, but also successful at reducing microglial activation in the retina. While these data highlight the role of LPA1 in neuroinflammation, they also underscore the need for a brain-penetrant antagonist, such as PIPE-791. PIPE-791 is a promising therapeutic for multiple sclerosis as it not only promotes remyelination, but also reduces neuroinflammation *in vivo*. As such, PIPE-791 shows promise for many CNS dysfunctions caused by neuroinflammation.

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Poster

377. Mechanisms of Neurodegeneration and Neuroprotective Therapies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 377.28

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Gsk343, an inhibitor of ezh2, reduces glioblastoma progression through inflammatory and apoptosis processes modulation

Authors: *G. CASILI, A. FILIPPONE, S. SCUDERI, R. BASILOTTA, D. MANNINO, A. CAPRA, M. CAMPOLO, S. CUZZOCREA, E. ESPOSITO, I. PATERNITI;
Dept ChiBioFarAm, Univ. of Messina, Messina, Italy

Abstract: Glioblastoma (GB) is a common tumor of the central nervous system (CNS). It is characterized by high proliferation, metastasis and invasiveness. The standard treatment for GB includes radiotherapy and chemotherapy; however, new therapies are needed. Recently, particular attention was given to the role of histone methyl-transferase enhancer of zeste homolog 2 (EZH2) in GB pathogenesis. Therefore, this study aimed to investigate the effect of GSK343, an EZH2 inhibitor, in an in vitro and in vivo xenograft model of GB. Our in vitro

results demonstrated that GSK343 treatment, at the concentrations of 1, 10 and 25 μM significantly reduced GB cell viability. Moreover, GSK343 treatment reduced NF-κB/IκBα pathway activation, IL-1β and TNFα expression. Additionally, in vitro GSK343 treatment increased pro-apoptotic protein expression as Bax and p53. The in vivo results, demonstrated that GSK343 treatment, at doses of 5 mg/kg and 10 mg/kg, was able to reduce subcutaneous tumor mass, tumor burden and tumor weight. Furthermore, GSK343 treatment, at doses of 5 mg/kg and 10 mg/kg, significantly modulated NF-κB/IκBα pathway activation and increased apoptosis process. Thus, based on the obtained results, GSK343 could be considered a therapeutic strategy to counteract GB progression, thanks its ability to modulate inflammatory and apoptotic processes.

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Poster

378. Neuroinflammation and Mechanisms of Neurodegeneration

Location: SDCC Halls B-H

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Program #/Poster #: 378.01

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: In vitro human CNS system exhibits neurodegenerative characteristics following disease relevant stimulation

Authors: *A. CHEONG, A. S. BLAZIER, J. D. PROTO;
Sanofi, Cambridge, MA

Abstract: Enhancement on our current model systems for human neurodegenerative disease is vital in gaining insights on the underlying pathological mechanisms and determining therapeutic efficacy. We have previously shown that an in vitro human iPSC triculture system composed of astrocytes, neurons and microglia is responsive to TLR4 stimulation as evidenced by the presence of inflammatory transcriptional signatures and release of pro-inflammatory cytokines. In this study, we extended our investigation to interrogate the effect of two neurodegenerative disease relevant stimuli: tau and α-synuclein preformed fibrils (PFFs). From RNA sequencing analysis, we observed both shared and stimulus-specific differential expressed genes after 24 hours. In depth pathway analysis revealed that both tau and α-synuclein stimuli induced EMT and disrupted metabolic processes which are known phenomena during neurodegeneration. Furthermore, inflammatory cytokine response related signaling was predicted to be altered based on the tau and α-synuclein PFFs transcriptomic signatures. In concordance with the transcriptomic profile, we observed an increased level of IL-18 following α-synuclein treatment which coincides with the predicted up-regulation of TGFβ signaling as well as previous finding in PD CSF. Based on these data, a reductionist, iPSC-based model system, may provide basic

insights in cellular responses to toxic protein aggregates and aid in the design of mechanism-based therapeutics and screening compound efficacy on CNS injury.

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Poster

378. Neuroinflammation and Mechanisms of Neurodegeneration

Location: SDCC Halls B-H

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Program #/Poster #: 378.02

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

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Title: Analgesic effects of tactile gamma entrainment on chemotherapy-induced peripheral neuropathy

Authors: *F. ABDURROB, T. KIM, H.-J. SUK, L.-H. TSAI;
Picower Inst. for Learning and Memory, MIT, Cambridge, MA

Abstract: Chemotherapy-induced peripheral neuropathy (CIPN) is one of the most prevalent long-lasting side-effects of cytotoxic cancer treatments. Damage to the dorsal root ganglion neurons can result in debilitating symptoms of allodynia hypersensitivity to pain and temperature in the periphery. Cisplatin, a commonly used chemotherapeutic agent, is known to induce neuroinflammation, DNA damage, and dysregulation of mechanoreceptor signaling in peripheral neurons. In this study, we investigate how tactile GENUS can modulate the neuropathic pain response in cisplatin treated mice. We observed increased mechanical pain threshold, as well as decreased CIPN-induced microglia and macrophage inflammation in the dorsal root ganglion, indicating potential analgesic effects of tactile sensory gamma entrainment.

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Poster

378. Neuroinflammation and Mechanisms of Neurodegeneration

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Program #/Poster #: 378.03

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

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NIH Grant R01EY029159
NIMH Grant R01MH119346

Title: Peripheral Nerve Trauma Causes Rapid Metabolic Reprogramming of Blood-Borne Immune Cells Entering the Injured Nerve

Authors: *M. ATHAIYA[1,2], X.-F. ZHAO[2], H. HAFNER[2], M. FINNERAN[1,2], C. JOHNSON[2], R. KAWAGUCHI[4], D. H. GESCHWIND[5], M. POPADICH[3], L. YANG[3], R. J. GIGER[1,2]; [1]Neurosci. Grad. Program, [2]Cell and Developmental Biol., [3]Neurosurg., Univ. of Michigan, Ann Arbor, MI; [4]Psychiatry and Neurol., [5]Dept. of Neurol., UCLA, Los Angeles, CA

Abstract: The most common forms of nervous system injury are traumatic lesions to peripheral nerves, and it has long been appreciated that the immune system plays a key role in peripheral nervous system (PNS) degeneration and regeneration. The temporal and spatial changes in the immune landscape of injured nerve tissue, the breath of cell-cell communication, and the underlying signaling networks remain incompletely characterized. To address this void, we conducted a longitudinal analysis of injured mouse PNS tissue using single cell RNA-sequencing (scRNAseq) of sciatic nerve at 0 day (naïve), 1-day, 3-days, and 7-days following crush injury (dpc). To assess how blood-borne immune cells change their transcriptomes as they become activated upon nerve entry, we carried out scRNAseq of peripheral blood mononuclear cells (PBMC) for comparison. Data integration revealed that the early immune response is pro-inflammatory and dominated by monocytes and macrophages (Mo/Mac) that are metabolically programmed for glycolytic energy production. The elevated expression of nearly all glycolytic enzymes, lactate dehydrogenase, and the lactate export channel MCT4 indicates that a Warburg-like effect is at play, coupling glycolytic energy production with a proinflammatory Mo/Mac phenotype. The presence of the corresponding protein products was validated by immunofluorescence staining of injured and naïve nerve tissue sections. The high glycolytic flux in the injured nerve stands in marked contrast to the low glycolytic activity observed in circulating Mo/Mac in naïve mice. This indicates that Mo/Mac undergo a rapid metabolic shift upon nerve entry following injury. The glycolytic burst in Mo/Mac is short-lived, however; at 3dpc expression of glycolytic enzymes begins to decline, and at 7dpc has reached levels similar to naïve nerve. As glycolytic activity declines, there is evidence for a metabolic shift toward oxidative phosphorylation (OXPHOS), and this coincides with the appearance of Mac with a resolving phenotype. Separation of the nerve injury site from the distal nerve revealed that mechanical nerve injury creates two separate immune microenvironments. To expand our work into the human PNS, we are currently focusing on specimens obtained from newborns and adult individuals that undergo brachial plexus reconstructive surgery. We established protocols for scRNAseq of naïve human nerves (sural nerves used for nerve grafting), proximal nerve segments, and distal nerve segments. Our studies provide insights into the cellular make up and single cell transcriptomes of healthy, injured, and diseased human PNS tissue.

Disclosures: **M. Athaiya:** None. **X. Zhao:** None. **H. Hafner:** None. **M. Finneran:** None. **C. Johnson:** None. **R. Kawaguchi:** None. **D.H. Geschwind:** None. **M. Popadich:** None. **L. Yang:** None. **R.J. Giger:** None.

Poster

378. Neuroinflammation and Mechanisms of Neurodegeneration

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 378.04

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NRF 2020R1A2C2010285
NRF I21SS7606036
HU22C0115

Title: Helicobacter pylori's cell-free supernatant ($_{HP}$CFS) induces neurodegenerative neuroinflammation in human mini-brain

Authors: *V. TRAN, H. CHO;
Sungkwunkwan Univ., Sungkwunkwan Univ., Suwon-si, Korea, Republic of

Abstract: Several epidemiological studies show the correlation between chronic *Helicobacter pylori* infection and neurological disorders. However, cellular interaction has not been validated yet, partly due to the lack of model systems that reflect the physiologically relevant innate immunity in the human brain. Here, we utilized the human brain platform reconstituting key aspects of neuroinflammation and neurodegeneration to study multicellular interaction under the *Helicobacter pylori*'s cell-free supernatant ($_{HP}$CFS) stimulation. Firstly, the $_{HP}$CFS were collected during their proliferation, followed by centrifuging and 0.2-um membrane filtering to remove bacterial pellets and collect toxic factors. We discovered that the $_{HP}$CFS-containing both VacA toxin/LPS induced inflammatory responses in microglia and astrocytes, producing neurotoxic factors (NO, H_2O_2) with 3-folds and pro-inflammatory cytokines (Il-8 and IL-18) with 4-folds compared to unstimulated microglia and astrocytes. We next observed that the $_{HP}$CFS elevated neuroinflammation (3-folds, CD86, microglia; 4.5-folds, GFAP, astrocytes) compared to non-stimulated cells. We also found that the combination of $_{HP}$CFS and soluble factors from stimulated astrocytes induced microglial autophagy (2-folds, LC3b), and migration (3-folds) compared to a single treatment. In addition, we explored that direct treatment with the $_{HP}$CFS exacerbates phosphoric tau (p-Tau) accumulation with 4-folds greater than control, respectively. Finally, neuroinflammation and neurodegeneration were partially reduced by inhibiting the LRP1 and TLR4 on the cell membrane for VacA and LPS inhibition, respectively. This study envisioned that *Helicobacter pylori* can be a potential infectious risk factor for neuroinflammation and neurodegeneration through the gut-brain axis.
Keywords: *Helicobacter pylori*, cell-free supernatant, cytotoxicity, brain inflammation, neurodegeneration.

Disclosures: **V. Tran:** None. **H. Cho:** None.

Poster

378. Neuroinflammation and Mechanisms of Neurodegeneration

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 378.05

Title: WITHDRAWN

Poster

378. Neuroinflammation and Mechanisms of Neurodegeneration

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 378.06

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Department of Veterans Affairs (Veterans Health Administration, Office of Research and Development, Biomedical Laboratory Research and Development (I21BX003514)
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The Assistant Secretary of Defense for Health Affairs through the Congressionally Directed Gulf War Illness Research Program (W81XWH-16-1-0626)

Title: A bacterial infection during deployment may have potentiated the effects of multi-chemical exposures on brain functioning and behavior

Authors: T. P. COMINSKI[1,2], *K. D. BECK[1,3], Y. HAN[4], V. DELIC[1,3], B. A. CITRON[1,3], J. R. RICHARDSON[4];
[1]Res., VA New Jersey Hlth. Care Syst., East Orange, NJ; [2]Veterans Bio-Medical Res. Inst., East Orange, NJ; [3]Rutgers - New Jersey Med. Sch., Newark, NJ; [4]Pharmaceut. Sci., Florida Intl. Univ., Miami, FL

Abstract: Bacterial infections during repeated exposure to permethrin and pyridostigmine bromide selectively cause neuroinflammation-induced changes in behavior and cerebellar functioning. Male and female C57Bl/6 mice were exposed to the type I pyrethroid insecticide permethrin (dermal) and the nerve-gas prophylactic pyridostigmine bromide (oral) over one month. Both have been demonstrated to cause neuroinflammation in rodents at high doses. Lipopolysaccharide (LPS) was used to model the immune response to an infection; LPS has also

been demonstrated to cause acute signs of neuroinflammation. The LPS was administered three times (i.p.) either during the first or last week of chemical exposure. Additional chemical-exposure alone and LPS-alone groups served as positive controls to assess the additional role of proinflammatory cytokine activity and combined chemical exposures. Behaviors were assessed 3 months and 6 months after the initial exposure. Behavioral analyses included tests of balance/coordination (rotor-rod), exploration/anxiety (open field), and memory (object recognition). Immunohistochemistry was utilized to assess group differences in astrocytes and astrocyte activation in the cerebellum. Motor functioning, as assessed by the rotor-rod test, was significantly impaired in the mice at 3 and 6 months, but only when LPS had been administered during the first week of chemical exposure. No other behaviors showed treatment-associated differences. The immunohistochemistry results of astrocyte prevalence and activation showed both early and late LPS treatments to combined chemical exposed mice exhibited more astrocytes and more activated astrocytes in the cerebellum. Although the activation of astrocytes in the cerebellum did not correlate with the differences in balance on the rotor-rod, it does demonstrate there are long-term changes in both behavior and at least cerebellar glial activation. There may be some additional brain areas that are differentially affected by either early or late LPS co-administration with the chemical exposures that explain the behavioral differences in balance.

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Poster

378. Neuroinflammation and Mechanisms of Neurodegeneration

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 378.07

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Macquarie University Fellowship

Title: Complement cascade and TDP43 pathology in MND and FTD

Authors: *F. M. BRIGHT, S. KNOTT, A.-N. CHO, N. MOREY, Y. D. KE, L. M. ITTNER; Macquarie Univ., Dementia Res. Centre, Macquarie Univ., Sydney, Australia

Abstract: Abnormal mislocalisation and aggregation of TDP43 is a pathological hallmark in the central nervous system (CNS) in 97% of motor neuron disease (MND) and ~50% of frontotemporal dementia patients (FTLD-TDP). Neuroinflammation is also a hallmark of neurodegeneration (ND), and a critical factor of the inflammatory response in ND is aberrant activation of the complement cascade (CC). Previously, we showed significantly altered expression of key CC components in serum of human FTD and MND patients. Consistent with these findings, RNA-sequencing of CNS tissue from a genetically modified (GM) TDP43 mouse model of MND/FTD showed significant dysregulation of the same components. To further

explore this, the expression of various complement components across all three CC pathways (classical, lectin, alternative) were individually knocked-down (KD) using shRNA-mediated adeno associated virus (AAV) injected into the brain and spinal cord at postnatal day 0, in four GM mouse models of human TDP43 pathology in comparison to three GM mouse models of other proteinopathies. Mice underwent extensive physiological testing and post-mortem analysis of neuropathology and gliosis within the brain, spinal cord, muscle and blood. Physiological testing showed significantly advanced pathological phenotypes including motor deficits, tremor, hind limb paralysis, gait abnormalities and premature death upon AAV mediated depletion of CC components, compared to those administered a control AAV. Importantly, pathological phenotypes were restricted only to models of mutant TDP-43 and not wild type TDP43 or other proteinopathies. To further explore the role of the CC in the context of mutant TDP-43, 3D human brain cortical and neuromuscular organoids derived from human embryonic stem cells (hESCs) expressing mutant and wild type TDP-43 respectively were treated with AAV's to KD the same panel of complement components. Consistent with mutant TDP43 mouse models, significant and detrimental phenotypes were observed in mutant TDP43 3D human brain organoids but not wild-type TDP-43. Collectively this work indicates a specific relationship between the CC and mutant TDP43 mediated ND in MND/FTD. To probe the mechanistic foundation of this relationship, including involvement of a specific CNS cell type or pathway, we employed a repertoire of techniques including ELISA, western blotting, RT-qPCR, MACS/FACS and spatial transcriptomics. This work provides evidence that harnessing specific elements of the CC within the CNS in the pathogenesis of TDP43 mediated ND will be advantageous in the development of disease specific therapeutics in MND and FTLD-TDP.

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Poster

378. Neuroinflammation and Mechanisms of Neurodegeneration

Location: SDCC Halls B-H

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Program #/Poster #: 378.08

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: MOST 110-2314-B-303 -018 -
TCRD111-042

Title: Severe Intracerebral Hemorrhage Permitting Hematoma Aspiration Rat Model

Authors: W.-F. HU[1], C.-H. LEE[3,4], H.-C. YU[5], H.-R. LIU[5], S. MOHAMMED THANGAMEERAN[2], H.-Y. HUANG[5], C.-Y. PANG[5,2], S.-T. TSAI[3,4,2], ***H.-K. LIEW**[5,1,3]; [1]PhD Program in Pharmacol. and Toxicology, [2]Inst. of Med. Sci., Tzu Chi Univ., Hualien, Taiwan; [3]Neuro-Medical Scientific Ctr., [4]Dept. of Neurosurg., [5]Dept. of Med. Res., Hualien Tzu Chi Hosp., Hualien, Taiwan

Abstract: Objective: Intracerebral hemorrhage (ICH) is the most devastating type of stroke without effective treatment. The progression of ICH causes primary brain injury, including hematoma formation, which compressed brain tissue, leading to brain edema, blood-brain barrier (BBB) disruption, and neurological deterioration. The secondary brain injury results from the degradation of products of blood clots and increased oxidative stress in the surrounding brain tissue resulting in the oxidation of DNA, proteins, and lipids. Reactive oxygen species (ROS) also over-activate microglia to trigger uncontrolled inflammatory responses and neuronal cell death. Management of ICH patients is mainly supportive treatments, allowing for the brain resorbing of the hematoma. Severe ICH is accompanied by a large hematoma causing compression (mass effect), increased intracranial pressure (IICP), midline shift, brain herniation, and ultimately death. Urgent surgery to remove a large hematoma can reduce the damage and save the patient's life. However, early surgical treatment failed to improve the patient's prognosis. No suitable animal model can currently mimic the Severe ICH permitting hematoma evacuation. **Materials & Methods:** We established a Severe ICH permitting hematoma aspiration rat model by intra-striatal microinjection (microinjection rate: 0.2 mL/min) of collagenase 0.6 U in 3 mL sterile saline. At 6 hours post-severe ICH induction, 0.15 mL of hematoma were aspirated. Oxidative stress and mitochondrial dysfunction were evaluated by protein carbonyl level, ATP levels, & ultrastructure of mitochondria, respectively. The 7T-MRI analyzes brain hematoma volume and midline shift. The modified Neurological Severity Scores and Forelimb Placement Test were used to evaluate the neurological severity. RT-qPCR is used to assess the mitochondrial biogenesis and antioxidant response elements expression. **Results:** Severe ICH causing rapid hematoma compression (approximately 200 mm3 hematoma volume 24 hours post-ICH), midline shift, neurological deficits, and a 30% mortality rate within one day. Aspiration of 0.15 mL of hematoma 6 hours after severe ICH significantly reduces hematoma volume, midline shift, neurological deficits, and mortality. Hematoma aspiration significantly restores mitochondrial dysfunction and ATP levels and relieves mitochondrial swelling. **Conclusion:** Our findings demonstrated that the newly established Severe ICH permitting hematoma aspiration rat model would closely mimic the clinical condition, which will aid in future research for the treatment of Severe ICH.

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Poster

378. Neuroinflammation and Mechanisms of Neurodegeneration

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 378.09

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: HK109-115

Title: The effect of sodium nitroprusside on lipopolysaccharide (LPS)-induced inflammatory response in rat cortical glial cells

Authors: *J.-Y. WANG[1], C.-L. CHEN[2], S.-Y. CHEN[1], C.-H. YANG[3], S.-N. YANG[3];
[1]Dept Nursing (Basic Med. Sci), Hungkuang Univ., Dept Nursing (Basic Med. Sci), Hungkuang Univ., Taichung, Taiwan; [2]Li-Shin Hosp., Taoyuan, Taiwan; [3]Departments of Pediatrics and Med. Research,, E-DA Hospital, Col. of Medicine, I-Shou Univ., Kaohsiung, Taiwan

Abstract: In central nervous system (CNS), nitric oxide (NO) plays an important role in neuroinflammation. Furthermore, neuroinflammation is closely related to various neurodegenerative diseases. The inducible nitric oxide synthase (iNOS) mainly expressed in glial cells is response to stress such as infection, hypoxia/ ischemia or neurodegenerative diseases. The growing evidence indicated that NO produced by activated glial cells was involved in neuronal injury. And the NO will modulate intracellular signals including activation of NFκB or induction of iNOS, that they will regulate the neuropathological pathways. Our previous data indicated the glial cells were activated in releasing NO by LPS treatment but the cell viability did not change significantly. The NO donor, sodium nitroprusside (SNP), may induce cell damage in high dose on glial cell cultures. Although NO-induced cytotoxicity has been extensively studied, but there is no consensus result. In this study, we want to investigate whether NO will enhance the LPS-induced inflammatory response in glial cells. Rat cortical mixed glial cell cultures will be subjected to (1) control; (2) LPS exposure for 1, 3 or 5 days with 100 ng/ml [final concentration in medium]; (3) Sodium Nitroprusside (SNP, NO donor) treated for 1, 3, 5 days with varying doses, including 1 μM, 10 μM, 100 μM, 1000 μM [final concentration in medium]; (4) SNP (100 μM) + LPS (100 ng/ml) treated for 1, 3, 5 days. Cell density and morphology will be observed by phase-contrast microscopy. Cell injury will be assessed by 3-(4, 5-Dimethylthianol-2-yl) 2, 5 diphenyltetrazolium bromide (MTT) reduction. The accumulation of nitrite in medium will be measured. The expression of NFκB, iNOS and TNF-α will be estimated by western blot to indicate inflammatory response. Our data indicate that the production of NO and the cell damage by SNP treatment revealed dose- and time- dependent response. SNP treatment only in high dose (1000 μM) significantly decreased the MTT reduction. The increasing nitrite accumulation in SNP with glial cells was higher than in SNP without glial cells at same concentration. However, the nitrite accumulation in SNP combination with LPS was not significant further increasing than in SNP or LPS alone. The expression ratio of TNF-α was the same in SNP combination with LPS and LPS alone. These results suggest that NO may induce cell injury at very high dose (more than 50 μM of nitrite accumulation) and this level of NO may not mediate further NO release or inflammatory response in glial cells.

Disclosures: J. Wang: None. **C. Chen:** None. **S. Chen:** None. **C. Yang:** None. **S. Yang:** None.

Poster

378. Neuroinflammation and Mechanisms of Neurodegeneration

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 378.10

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Ripk1 kinase activation drives neuroinflammation and contributes to disease pathophysiology in als.

Authors: *F. PONTARELLI, M. ZELIC, M. LAMORTE, A. BLAZIER, T. TRELEAVEN, E. MCGUIRK, Y. REN, J. DODGE, N. HAGAN, N. ATASSI, J. GANS, G. GAGLIA, T. HAMMOND, D. OFENGEIM;
Rare and Neurolog. Dis., Sanofi-Aventis Pharmaceuticals, Cambridge, MA

Abstract: Receptor interacting protein kinase 1 (RIPK1) is a key regulator of cellular signaling in multiple cell types. Here we show that RIPK1 kinase activity contributes to neuroinflammation response to LPS. We identified several RIPK1-dependent biomarkers in the brain following LPS stimulation. This suggests that this kinase mediates deleterious processes in neurodegenerative disease. Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that affects motor neurons in the spinal cord and brain. Based on pathological and genetic findings, ALS sits on a spectrum with frontotemporal dementia (FTD), a neurodegenerative disease that leads to the progressive degeneration of neurons in the temporal and frontal lobes of the brain. Recently, several lines of evidence have suggested that RIPK1 activation may be causally linked to the pathophysiology of ALS and FTD. Loss of function mutations in *OPTN* and *TBK1* can lead to familial ALS/FTD and their deficiency causes hyperactivation of RIPK1 kinase activity. Previous studies have suggested increased RIPK1 expression in mouse models and ALS patient samples. While these data suggest activation of RIPK1 in the central nervous system (CNS) may mediate ALS disease progression, we sought to definitively elucidate the role of RIPK1 kinase activity in the $SOD1^{G93A}$ mouse model and in ALS patient-derived post-mortem samples. Using a mouse active CNS-penetrant RIPK1 inhibitor, we show that blocking RIPK1 kinase activity delays the kinetics of symptom onset and motor impairment in the SOD1 model and assess RIPK1-dependent gene and pathway regulation by single cell RNA sequencing. We establish an iPSC-derived tri-culture system (motor neurons, microglia, astrocytes) to derive a human RIPK1 kinase-dependent gene signature. Furthermore, we demonstrate RIPK1 activity and expression are increased in human sporadic ALS spinal cord samples and interrogate gene changes correlating with RIPK1 expression by single nucleus RNA sequencing. Based on our data we suggest aberrant RIPK1 activation in various CNS cell types contributes to motor neuron loss in ALS.

Disclosures: **F. Pontarelli:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sanofi. **M. Zelic:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sanofi. **M. LaMorte:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sanofi. **A. Blazier:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sanofi. **T. Treleaven:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sanofi. **E. McGuirk:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sanofi. **Y. Ren:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sanofi. **J. Dodge:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sanofi. **N. Hagan:** E.

Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sanofi. **N. Atassi:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sanofi. **J. Gans:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sanofi. **G. Gaglia:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sanofi. **T. Hammond:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sanofi. **D. Ofengeim:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sanofi.

Poster

378. Neuroinflammation and Mechanisms of Neurodegeneration

Location: SDCC Halls B-H

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Program #/Poster #: 378.11

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NRF Grant 2020R1A2C2010285
NRF Grant I21SS7606036
MHWMS Grant HU22C0115

Title: Resistin and glucose stimulation promotes proinflammatory astro-microgliosis in human obesity hyperglycemia

Authors: *H. CHO;
Biophysics, Sungkwunkwan Univ., Suwon, Korea, Republic of

Abstract: Obesity is a major cause of hyperglycemia; the exact mechanism between obesity and degenerative brain diseases still needs further research. In obese people, this is important because there are high concentrations of adipokine resistin and glucose in the bloodstream, which may be the basis for astrocyte-derived neuroinflammation. Here, we applied the simultaneous stimulation of resistin (200 ng/mL) and high concentration of glucose (15 mM) to human astrocyte and microglia, respectively, to investigate the effects of proinflammatory astrogliosis and microgliosis. Resistin and glucose simultaneous stimulation increased expression of reactive astrocyte marker such as GFAP, iNOS, and NO expression by 2.13-fold, 2.67-fold, and 1.92-fold, respectively ($p < 0.001$), and inflammatory soluble factors such as IFN-γ, TNF-α, IL-1β, CXCL12, CCL1, and MIP-1 by 2.06-fold, 1.85-fold, 2.50-fold, 3.03-fold, 4.51-fold, and 4.26-fold, respectively ($p < 0.05$), compared to normal astrocyte. Resistin induces mild microglial activation with CD86 expression by 1.54-fold, compared to normal. However, resistance/glucose-stimulating astrocyte conditioned medium (ACM) recruited microglia by 2.61-fold ($p <0.001$) and increases CD86 expression by 2.13-fold ($p < 0.01$) and inflammatory soluble factors such as serpins, IL-16, IL-2, and TNF-a by 2.25-fold, 3.33-fold, 1.92-fold, and

4.77-fold, respectively ($p < 0.05$). Astro-microgliosis CM (AMCM) induced neuronal cytotoxicity by 2.18-fold ($p < 0.001$) confirmed with LDH assay. Our study suggests the obesity hyperglycemia could be a risk factor for neurodegeneration through inter-glial inflammation.
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Keywords: obesity, adipokine resistin, glucose, proinflammation, astrogliosis, microgliosis

Disclosures: H. Cho: None.

Poster

378. Neuroinflammation and Mechanisms of Neurodegeneration

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 378.12

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Race to Erase MS
Hilton Foundation

Title: The role of fatty acid metabolism in oligodendrocyte precursor cell response to inflammation

Authors: *J. J. LEE, M. D. KORNBERG;
Johns Hopkins Univ., Baltimore, MD

Abstract: Multiple sclerosis (MS) is an inflammatory disease with features of demyelination by self-reactive T cells and limited remyelination as oligodendrocytes that produce myelin are damaged. In MS lesions, the differentiation of oligodendrocyte progenitor cells (OPCs) to oligodendrocytes is blocked, resulting in a lack of mature oligodendrocytes to repair myelin. The mechanisms underlying this blockage of OPC differentiation are poorly understood. Therefore, it is critical to investigate the mechanisms of OPC differentiation to promote remyelination and develop regenerative treatments to complement current therapies that target the immune system and rely on anti-inflammatory effects. Interestingly, inflammatory cytokines have been shown to inhibit OPC differentiation and produce a distinct OPC phenotype featuring characteristics similar to activated immune cells. Because metabolic reprogramming plays critical roles in both immune activation and progenitor differentiation, we hypothesized that metabolic regulation represents a checkpoint between pro-inflammatory and remyelinating OPC phenotypes. In this study, we investigated the metabolic programming of OPCs in response to various factors to identify metabolic pathways and metabolites that regulate inflammatory vs. remyelinating OPC fates. OPCs were exposed to distinct stimuli promoting proliferative (PDGFaa), differentiating (T3), and inflammatory (IFN-γ) phenotypes. Untargeted metabolomics demonstrated downregulation of fatty acid levels in inflammatory OPCs when compared to differentiating

OPCs, implying a potential role of fatty acid metabolism in regulating OPC differentiation under inflammatory conditions. These results were corroborated by gene expression analysis, which demonstrated that transcriptional programs associated with fatty acid β-oxidation were upregulated in inflammatory OPCs when compared to differentiating OPCs. We are currently conducting studies to determine the key molecular events that regulate OPC fatty acid metabolism in response to inflammation, establish the physiologic relevance of these findings using in vivo models of inflammatory demyelination, and determine whether fatty acid metabolism can be targeted to enhance myelin repair.

Disclosures: **J.J. Lee:** None. **M.D. Kornberg:** None.

Poster

378. Neuroinflammation and Mechanisms of Neurodegeneration

Location: SDCC Halls B-H

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Program #/Poster #: 378.13

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: MOST 109-2314-B006-015-MY3
MOST 110-2811-B-006-522

Title: Expression of microRNA-204-5p in oligodendrocytes by interleukin-6 and in rat corpus callosum following exposure to cuprizone

Authors: *H.-T. HUANG, C.-Y. WANG, C.-H. HO, S.-F. TZENG;
Dept. of Life Sci., Natl. Cheng Kung Univ., Tainan, Taiwan

Abstract: Demyelination and dysregulated oligodendrocyte (OL) homeostasis in the white matter are common pathological features in the neurodegenerative diseases of the central nervous system, in which are mainly associated with gliosis and neuroinflammation. MicroRNA-204 (miR-204) has been considered as a positive regulator for OL differentiation in vitro. Although we have observed miR-204-5p was displayed in APC^+-OLs in rat corpus callosum, its expression in the damaged white matter is not yet examined. Here, we conducted a chemical-induced gliosis in the corpus callosum by feeding rats with cuprizone (CPZ)-containing diet. Exposure to CPZ-containing diet for three weeks caused an increase in the gene expression of interleukin-6 (IL-6), a pleiotropic cytokine that plays a detrimental role in the development of demyelination. In contrast, IL-6 expression was not elevated in the recovery group that was fed only with a two-week CPZ-containing diet and then by the regular diet for a week. Moreover, CPZ exposure for three weeks caused the loss of APC^+-OLs in the corpus callosum, whereas the restoration of APC^+-OLs were observed in the recovery group. This was in accompany with the upregulation of miR-204-5p, as well as myelin related genes (i.e. MBP, PLP, and Myrf). To determine whether IL-6 have the regulatory role in miR-204-5p expression in OLs, mature OLs were treated with IL-6, as well as TNF-α and IL-1β. Although the three cytokines were able to suppress miR-204-5p expression in OLs, only IL-6 decreased MBP gene expression. Taken

together, our findings suggest that IL-6 and miR-204-5p interplay could contribute to the occurrence or prevention of OL loss in a CPZ-treated animal model.

Disclosures: **H. Huang:** None. **C. Wang:** None. **C. Ho:** None. **S. Tzeng:** None.

Poster

378. Neuroinflammation and Mechanisms of Neurodegeneration

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Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

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National Eye Institute R01EY025687
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National Multiple Sclerosis Society FG-2108-38411

Title: Visual assessments predict neurodegeneration in experimental autoimmune encephalomyelitis and multiple sclerosis

Authors: *G. M. MEY[1], K. S. EVONUK[1], M. K. CHAPPELL[1], L. M. WOLFE[1], R. SINGH[3], J. C. BATOKI[3], M. YU[3], N. S. PEACHEY[3,4], B. ANAND-APTE[3], R. BERMEL[5], D. ONTANEDA[5], K. NAKAMURA[2], K. R. MAHAJAN[5,1], T. M. DESILVA[1];
[1]Neurosciences, [2]Biomed. Engin., Cleveland Clin. Lerner Res. Inst., Cleveland, OH; [3]Ophthalmic Res., Cleveland Clin. Cole Eye Inst., Cleveland, OH; [4]Louis Stokes Cleveland VA Med. Ctr., Cleveland, OH; [5]Neurolog. Inst., Mellen Ctr. for MS Treatment and Res., Cleveland, OH

Abstract: Multiple sclerosis (MS) is a chronic inflammatory, demyelinating, and neurodegenerative disease of the central nervous system (CNS) that ultimately leads to permanent neuronal loss and progressive disability. Immunomodulatory therapies reduce relapses and improve quality of life, but there are currently no effective treatments for neurodegeneration in MS. Thalamic atrophy is one of the strongest predictors of worsening clinical disability in MS, warranting investigation of its afferent and efferent projections to better understand neurodegenerative mechanisms. Therefore, we sought to evaluate pathological changes in the spino- and retino-thalamic pathways using the most common preclinical model of chronic autoimmune demyelination, experimental autoimmune encephalomyelitis (EAE). EAE was induced in adult male C57BL/6J mice using MOG_{35-55} peptide. Histopathological analyses were coupled with structural and functional visual tests including optical coherence tomography (OCT) to track retinal thickness and visual evoked potentials (VEP) to evaluate the neuronal response to visual stimuli. Acute and chronic disease assessments were performed at 15 days

post-EAE induction (dpi, peak of disease), 35 dpi (chronic disease), and 60 dpi (sustained chronic disease) and were compared with naïve (untreated) age-matched controls. Comparable $CD3^+$ T cell infiltration, demyelination, and axonal loss were observed in spinal cord white matter and optic nerve during the acute phase of disease. Concurrently, synaptic loss, but not neuronal cell body loss, was observed in the dorsal lateral geniculate nucleus of the thalamus. Retrograde loss of spinal cord neurons and retinal ganglion cells relative to their respective axons was not observed until the chronic phase of disease, consistent with reduced inner retinal thickness as detected by OCT. These data establish a spatio-temporal relationship between axonal injury, synaptic loss, and demyelination in the spino- and retino-thalamic pathways, suggesting that the EAE model can be a beneficial tool to investigate neurodegeneration and the effect of neuroprotective strategies in demyelinating disease. In patients with relapsing-remitting MS without a history of optic neuritis, OCT measures of inner retinal volume correlated with spino-thalamic (ventral posterior nucleus) and retino-thalamic (lateral geniculate nucleus) volume as well as neuroperformance measures. These data support the utility of visual assessments as a valuable and noninvasive predictor of neurodegeneration in MS.

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Poster

378. Neuroinflammation and Mechanisms of Neurodegeneration

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 378.15

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant F31NS115361

Title: Depression and Prefrontal Metabolism Explain Unique Variability in Processing Speed Ability in Multiple Sclerosis: A Calibrated fMRI study

Authors: *M. D. ZUPPICHINI[1], J. MA[2], D. SIVAKOLUNDU[4], K. WEST[2], D. OKUDA[5], B. P. RYPMA[3];
[1]Psychology, Univ. of Michigan, Ann Arbor, MI; [3]Behavioral & Brain Sci., [2]Univ. of Texas At Dallas, Dallas, TX; [4]Yale Sch. of Med., New Haven, CT; [5]Univ. of Texas Southwestern Med. Ctr., Dallas, TX

Abstract: Background: Cognitive processing speed deficits are common in multiple sclerosis (MS). Previous work has shown that cerebral metabolism in dorso-lateral prefrontal cortex (dlPFC) is known to be related to the slowed processing speed observed in MS. Depression is also common in MS and is associated with processing speed decline. In this study, we sought to assess the extent to which depression contributes to these metabolism-processing speed relationships.**Objective:** Assess whether depression and metabolism account for unique proportions of variability in MS-related processing speed decline.**Methods:** MS and healthy control (HC) participants who met inclusion criteria were scanned using a 3T MRI scanner with a dualecho calibrated fMRI (cfMRI) sequence which provided nearsimultaneous measures for both cerebral blood flow (CBF) and BOLD signal. During imaging, participants performed a block-design digit-symbol substitution task (DSST) that required the viewing of a digit-symbol pairing key and responding as to whether a probe digit-symbol pair matched the key as fast as they could using button boxes. A hypercapnia gas challenge involving periodic inhalation of room air (4 min) and 5% CO2 (6 min) permitted measures of cerebral metabolic rate of oxygen (CMRO2). Data were preprocessed and average percent signal change from baseline was calculated in each voxel providing BOLD and CBF time series. The anatomical region of interest (ROI) was defined as dlPFC after Freesurfer cortical parcellation. Regression analyses were performed controlling for ROI size to assess whether BOLD, CBF, or CMRO2 could explain variability in processing speed ability. Prior to imaging, participants were administered a cognitive assessment battery that included the Beck Depression Inventory (BDI).**Results:** An independent-samples *t*-test showed that the MS group had a significantly higher response time (RT) for the DSST ($t[42]=2.77$, $p=.008$) and higher BDI scores ($t[42]=3.02$, $p=.004$) compared to HCs. Within the MS group, regression analyses using RT for correct trials as the dependent factor were not significant for BOLD and CBF PSC but was significant for BDI ($R^2=.115$, $p=.045$) and CMRO2 ($R^2=.124$, $p=.024$). No regression analyses were significant within the HC group.**Conclusion:** Results suggest that depression and prefrontal metabolism account for unique proportions of variance in MS-related processing speed declines.

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Poster

378. Neuroinflammation and Mechanisms of Neurodegeneration

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 378.16

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: MS Society of Canada Grant 2302

Title: Machine learning prediction of myelin loss in multiple sclerosis disease progression

Authors: *P. JOHNSON[1], R. TAM[2], I. VAVASOUR[3], A. TRABOULSEE[1], S. KOLIND[1]; [1]Neurol., [2]Sch. of Biomechanical Engin., [3]Radiology, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Multiple sclerosis (MS) is a chronic autoimmune disease characterized by demyelinating white matter lesions and diffuse damage through normal-appearing white (NAWM) and grey matter caused by inflammation (lymphocyte, macrophages, and microglia). Currently it is difficult to predict disease progression for individual patients.Myelin water imaging (MWI) evaluates myelin content by measuring MRI signal from the water trapped between myelin bilayers. The amount of myelin water relative to total water signal is termed the myelin water fraction (MWF). The MWF standard deviation (SD) represents myelin heterogeneity which is expected to increase when there are more demyelinated fibres alongside intact fibers within a region. The myelin heterogeneity index (MHI) is defined as the MWF coefficient of variation (MWF SD/mean MWF). MHI captures myelin content and variability simultaneously to reflect MS-related abnormalities. We previously found that the mean MWF (R=-0.28), SD (R=0.3) and MHI (R=0.33) of non-lesional NAWM correlated with clinical disability score (Expanded Disability Status Score, EDSS). MS NAWM myelin was significantly affected (decreased MWF, increased SD and MHI) over 2 years consistent with disease progression.The aim of this study was to determine which baseline clinical and imaging features are predictive of disease progression as determined by myelin changes over two years. Baseline features include MWI values (mean MWF, SD, and MHI), MS subtype (relapsing remitting MS (rrMS) and progressive MS (pMS)), baseline EDSS, disease duration, and age, to predict change from baseline to year 2 MWF, SD and MHI values using a machine learning approach. We used multiple linear regression from the scikit-learn machine learning package in python. The MS subtypes were encoded into the algorithm using One Hot Encoding and the rest of the values were passed through unmodified as they were numerical. We used a 90/10% train/test split on 49 participants (24 rrMS, 25 pMS). The 5 uncollected EDSS values were imputed using the median EDSS value from the entire set. The best predicted change over 2 years was in MHI (R^2 of 0.22). Disease type was given little weight in each predictive model. In the MHI predictive model the most important factors were baseline mean MWF (coefficient of -2.3) and SD (coefficient of 3.6). Higher baseline myelin SD likely represents patients with a greater degree of myelin damage throughout their central nervous system. This is predictive of a larger change in global myelin content 2 years later. Myelin SD might provide an indication of basal rate of diffuse microglial activity that could be driving the rate of demyelination in MS.

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Poster

378. Neuroinflammation and Mechanisms of Neurodegeneration

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 378.17

Title: WITHDRAWN

Poster

378. Neuroinflammation and Mechanisms of Neurodegeneration

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 378.18

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NHI Grant 5P20GM103642
NHI Grant U54 MD007600

Title: Pnu- 120596 prevents diet induced obesity weight gain in an age dependent manner.

Authors: S. COTTO RÍOS[1], Á. F. RUÍZ GUERRERO[2], V. A. ROMÁN RIVERA[2], J. A. LASALDE-DOMINICCI[3], ***J. COLÓN-SÁEZ**[4];
[1]Chem. Dept., Univ. of Puerto Rico, Río Piedras, Puerto Rico; [2]Pharmaceut. Sci., Univ. of Puerto Rico, Río Piedras, PR; [3]Dept. of Biol., Univ. of Puerto Rico, SAN JUAN, Puerto Rico; [4]Univ. of Puerto Rico, San Juan, PR

Abstract: Obesity rates have nearly tripled in the past 45 years affecting globally close to 2 billion adults which are either overweight or obese1, cocurrently, the worldwide prevalence of dementia has also tripled in that same span2. One of the mechanisms that is thought to play a major role in obesity induced cognitive decline is the consumption of a High Fat Diet (HFD). HFD has been shown to activate neuroinflammation, which can result in neuronal injury3, and can lead to a decline in cognitive performance5,9-11. Neuroinflammation has been shown to be a common denominator of many neurodegenerative and neuropsychiatric disorders8. Diet induced neuroinflammation, is mediated by activation of the CNS resident immune cells, the microglia3-7. Recently an important role for the alfa-7-nicotinic acetylcholine receptor (α7-nAChR) has been found in the control of both systemic13,14 and CNS inflammation15-17. This receptor is a key component of the cholinergic anti-inflammatory response which is a neural system that controls both systemic and neuroinflammation. To study the therapeutic potential of the α7-nAChR for diet induced neuroinflammation we used the allosteric modulator PNU-120596. Surprisingly, we observed that the mice that received a daily intraperitoneal injection of PNU-120596 for a week loose weight during that week. When looking at total weight gain during the 12 weeks exposure to the different diet's mice receiving PNU-120596 that started the diet at 1

month of age had gain significantly less weight than control mice, while food consumption was unchanged. When we measured epidydimal fat pads as a percentage of total body weight we see a significant reduction in fat stored on these mice. Suggesting that activation of the cholinergic system is able to prevent the additional gain in weight and fat accumulation associated with a high fat diet during an early age.

Disclosures: S. Cotto Ríos: None. **Á.F. Ruíz Guerrero:** None. **V.A. Román Rivera:** None. **J.A. Lasalde-Dominicci:** None. **J. Colón-Sáez:** None.

Poster

378. Neuroinflammation and Mechanisms of Neurodegeneration

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 378.19

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Kuwait University

Title: The influence of interleukin-1β on ABCB1 transporter function in blood-brain barrier endothelial cells

Authors: *A. ALRABEEA[1,2], J. PENNY[1];
[1]Dept. of Pharm. and Optometry, Univ. of Manchester, Greater Manchester, United Kingdom;
[2]Dept. of Pharmacol. and Toxicology, Kuwait Univ., Kuwait City, Kuwait

Abstract: *Background:* The blood-brain barrier (BBB) helps maintain central nervous system (CNS) homeostasis and neurological function. Adenosine triphosphate-binding cassette (ABC) efflux transporters expressed in the BBB reduce penetration of xenobiotics, including therapeutic drugs, into the CNS. Release of inflammatory cytokines within the CNS, or periphery, may alter BBB integrity which could contribute to progression of neurodegenerative diseases. Pro-inflammatory cytokines including interleukin-1 β (IL-1β), interleukin-6 (IL-6) and tumour necrosis factorα (TNFα) are released in conditions including sepsis, epilepsy, Alzheimer's disease and Parkinson's disease. The signalling pathways involved in regulating cytokine-induced changes in ABC transporter activity are highly complex and not well understood. Understanding the activity of ABC transporters during inflammation is crucial for better therapeutic interventions. *Aim:* to understand the influence of IL-1β on the activity and expression of the ABCB1 transporter, also known as P-glycoprotein (P-gp), in blood-brain barrier primary endothelial cells and the role of the c-Jun N-terminal Kinase (JNK) pathway in regulating ABCB1 activity. *Methods:* Porcine brain endothelial cells (PBECs) were isolated and purified. The Neutral red assay was used to assess cell viability. PBECs were treated with either IL-1β, at 0.5, 2.5, 5 and 10 ng.ml-1 alone, or combined with IL-6, TNFα and VEGF (where each individual cytokine in the combination was used at the concentration specified), for 24 h (n≥4), or treated with the JNK pathway inhibitor SU3327, 10 μM for 24h. ABCB1 activity was determined by measuring intracellular accumulation of calcein.

ABCB1 expression was measured using Western blotting.*Results:* Exposure of PBECs to IL-1β, alone, or in combination, IL-6, TNFα and VEGF at 0.5, 2.5, 5 and 10 ng.ml-1, for 24 h ($n \geq 4$) or JNK pathway inhibitor SU3327, 10 µM for 24h, had no significant effect on cell viability. Treatment of PBECs with IL-1β alone, or in combination ($n \geq 4$) significantly, $p < 0.0001$, increased ABCB1 efflux activity in a concentration dependent manner, and increased ABCB1 protein expression. The cytokine-mediated enhancement of ABCB1 activity was reduced, but not significantly, by 13 % when PBECS were co-treated with IL-1β and SU3327 for 24h. *Conclusion:* IL-1β, which is produced during central and peripheral disease conditions, influences ABCB1 transporter activity, with inhibition of the JNK signalling pathway contributing somewhat to changes in ABCB1 activity. These findings are important as they may allow for designing better therapeutic strategies in treating CNS diseases.

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Poster

378. Neuroinflammation and Mechanisms of Neurodegeneration

Location: SDCC Halls B-H

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Program #/Poster #: 378.20

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: DFG / Germany's Excellence Strategy—EXC 2067/1-390729940
DFG research grants Ga354/16-1

Title: Polyclonally expanded CD8 T cells orchestrate CNS inflammation and brain atrophy in type I interferon-mediated neuroinflammation of RNaseT2 deficient mice

Authors: *M. KETTWIG[1], K. TERNKA[1], A. ALIA[3], S. NESSLER[2], J. GÄRTNER[1]; [1]Dept. of Pediatrics, Div. of Pediatric Neurol., [2]Inst. of Neuropathology, Univ. Med. Ctr. Göttingen, Göttingen, Germany; [3]Inst. for Med. Physics and Biophysics, Univ. of Leipzig, Leipzig, Germany

Abstract: Introduction: We have recently established Rnaset2-/- mice as a model system to improve our pathophysiological understanding of infantile-onset RNaseT2 deficient leukoencephalopathies and type I interferonopathies. Rnaset2-/- mice were viable and healthy at birth, but type I interferon-stimulated genes were upregulated in all organs examined. RNaseT2 deficient animals developed an IFNAR1-dependent neuroinflammation at 20 weeks of age, predominantly composed of CD8 T cells and inflammatory monocytes. The neuroinflammatory phenotype was accompanied by a homeostatic dysfunction of glial cells and neurons in single nuclei transcriptome analyses and by an hippocampal-accentuated brain atrophy on magnetic resonance imaging. *Kettwig et al. 2021* - www.doi.org/10.1038/s41467-021-26880-x
Aims/ Questions: To assess the contribution of CD8 T cells to neuroinflammation and neurodegeneration in RNaseT2 deficient mice. To evaluate if CD8 T cells have to be antigen-specific to sustain neuroinflammation.

Methods: Crossbreeding of Rnaset2-/- mice to CD8-deficient animals; ex vivo MRI, flow cytometry, histology.
Results: Rnaset2-/- CD8a-/- mice displayed normal T2-relaxation times on MRI, suggesting a significantly reduced neuroinflammatory response in these animals compared to CD8 competent Rnaset2-/- mice. In line, flow cytometric analyses of CNS leukocytes demonstrated that Rnaset2-/- CD8a-/- mice had no CD8 T cells and significantly reduced numbers of CD4 T cells and inflammatory monocytes infiltrating into the CNS. Interestingly morphometric analyses of the CNS by MRI showed also less hippocampal atrophy in Rnaset2-/- CD8a-/- compared to Rnaset2-/- controls. Among CNS CD8 T cells, we so far found no evidence for clonally expanded TCR V beta families using a flow cytometric approach.
Conclusion: In summary, our data suggest that CD8 T cells significantly contribute to neuroinflammation and neurodegeneration in Rnaset2-/- animals. On a broader scope, RNaseT2 deficient mice will provide an excellent model system to gain mechanistic insight how CD8 T cells mediate neurodegeneration.

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Poster

378. Neuroinflammation and Mechanisms of Neurodegeneration

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 378.21

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Ameliorative effect of 6-bromo indirubin-3-oxime (6BIO) dual inhibitor of GSK3B and CDK5 via targeting Wnt signalling pathway in experimental model of Alzheimer's disease

Authors: *M. PRAJAPAT[1], A. PRAKASH[2], B. MEDHI[3];
[1]PGIMER, PGIMER: Post Grad. Inst. of Med. Educ. and Res., Chandigarh, India; [2]Univ. Inst. of Pharmaceut. Sci. (UIPS), Univ. Inst. of Pharmaceut. Sci. (UIPS), Chandigarh, India; [3]PGIMER, Chandigarh, India

Abstract: Wnt signalling have role in neuroprotection mechanism against amyloid beta (Aβ) induce toxicity. Dysfunction of Wnt signalling associated with activation of GSK3B, CDK5, Phospho-YAP protein, which are involved in hyper phosphorylation of Tau protein and inhibits the entry of beta catenin into the nucleus directly or indirectly via regulation of hippo signalling. thus, it led to neuronal apoptosis and implicated in Alzheimer disease. In this study, we identified 6-bromo indirubin-3-oxime(6BIO) inhibit GSK3B and CDK5, and this is the first study that explores the effect of 6BIO on Wnt/hippo signalling pathway through Aβ42 induced experimental model of AD. Docking and Dynamic simulation (DS) of 6BIO compound with GSK3B and CDK5 protein crystal structure (PDB: 1uv5 and 1UNH) was performed using Schrodinger software at 100ns. Aβ (25uM) induced AD model were develop on differentiated SHSY5Y cells. Further, cells treated with 6BIO (solvent DMSO) in 5,10,15uM concentration for

24hr. the effect of drug was analysed by MTT assay, molecular markers expressions by RT-PCR and immunofluorescence. In in vivo study, Aβ42(10ul) was injected intracerebroventricular to induce AD in male Wister rat(n=6). 6BIO was given daily in 3 different doses (5.95ug/kg, 11.9ug/kg, 23.8ug/kg intra-peritoneally) for 7 days after 14 days of Aβ42 administration in rat(n=18). the treatment was assessed by neuro-behavioural (Morris water maze, Elevated plus maze) and correlated with biochemical and molecular markers (oxidative stress, ELISA, RT-PCR and morphological alteration by H&E staining). All data were analysed by one way ANOVA test. We found that 6BIO had favourable pharmacokinetics, blood brain barrier permeability. GSK3B and CDK5 docking score is −10.451 and -9.865, RMSD 1.8Å and 2.0Å, RMSF 1Å and 0.4Å, respectively in DM score. In in-vitro, AD induced cells treated with 6BIO (15uM) shown significant neuroprotective effect, reduced cytotoxicity, decreased expression of Aβ, DKK1, CDK5, caspase-3 while significantly rising expression of beta-catenin, Wnt3A, GSK3B(ser9), compared to Aβ model. In animal model, Treatment with 6BIO at the dose (23.8ug/kg) significantly improved memory, raised expression of Wnt3a, beta-catenin, YAP protein while decreasing oxidative stress, Aβ, GSK3B(P216), CDK5, p-Tau, DKK1 along with improvement in morphological changes were observed. Protective effect of 6BIO was seen by dual inhibitory effect on GSK3B and CDK5 activity. It is also regulating hippo signalling by which beta-catenin and YEP protein enter into nucleus which further transcript cell survival genes. It may prove 6BIO to be a useful therapeutic compound for AD.

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Poster

378. Neuroinflammation and Mechanisms of Neurodegeneration

Location: SDCC Halls B-H

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Program #/Poster #: 378.22

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: PAPIIT-DGAPA IN216821
COMECyT FICDTEM-2021-066

Title: Vanadium pentoxide (v_2o_5) inhalation effect on memory and cytoskeleton alterations in brain structures related to alzheimer disease.

Authors: *C. DORADO-MARTÍNEZ, E. MONTIEL-FLORES, Sr., J. L. ORDONEZ LIBRADO, A. GUTIERREZ VALDEZ, J. ESPINOSA-VILLANUEVA, L. REYNOSO-ERAZO, V. RODRÍGUEZ-LARA, M. AVILA-COSTA;
UNAM, CDMX, Mexico

Abstract: Alzheimer disease (AD) is the most common neurodegenerative pathology worldwide, it has been reported that approximately 15 million people suffer from this disease, the incidence annually increases 0.5% in 65 year old people and 8% in 85 year olds; although it was described more than a 100 years ago and there is a lot of research being done about this

pathology, it has been difficult to find an animal model that replicates all AD characteristics and neurodegenerative processes. Previous experiments in our laboratory have shown that chronic exposure to V_2O_5 in rats causes morphological and behavioral changes similar to those seen in AD. To this end 40 male Wistar rats were randomly divided into two control (n= 5) and two experimental groups (n = 10) with an initial weight of 180-200 gr. The experimental groups were exposed to V_2O_5 0.02M one hour, three times a week, for six months, after 6-month exposure one experimental group was leaved in a 6-month recovery phase. To measure behavioral changes, the three groups were trained in the T-maze test that assesses spatial memory and an open field test for 10 mins. All groups were evaluated once a month for six or twelve months. To measure histological alterations, after 6 or 12 months, frontal and entorrinal cortex, CA1, subiculum and amygdala, underwent Congo red or argentic Bielschovsky impregnation and were analyzed. Memory results in the T-maze show memory impairment since the group had been exposed for three months to V_2O_5. During the open field test, differences were observed in the locomotion pattern of the experimental group. Motor activity decreased (less lines crossed) while freezing increased. Freezing behavior in control rats is nearly absent, this immobility behavior appears in rats exposed to V_2O_5 from the first month. In the recovery group there were no memory nor locomotion normalization in the spatial memory and open field tests. Congo red and Bielschovsky impregnation showed that affected neurons have the same morphological characteristics as the neurons of AD patients, (i.e. the affected cells are shaped like a "flame"), and structures similar to fibrillary tangles are observed.

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Poster

378. Neuroinflammation and Mechanisms of Neurodegeneration

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 378.24

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: 2R01NS091617-06/GRANT13161291
The University of Virginia Program in Fundamental Neuroscience
The University of Virginia Raven Fellowship

Title: Neurodegeneration is regulated by phagocyte interactions with axonal spheroids

Authors: *S. HUNTER-CHANG[1], Y. YONG[2], T. VEGIRAJU[3], R. VERMA[3], S. C. KUCENAS[3], C. DEPPMANN[4];
[1]Univ. of Virginia Neurosci. Program, Charlottesville, VA; [3]Biol., [4]Biology, Neurosci. and Biomed. Engin., [2]Univ. of Virginia, Charlottesville, VA

Abstract: Axon degeneration is a key process leading to neuronal death in many neurodegenerative contexts and can determine health outcomes independently of neuronal survival. Phases of axon degeneration include latency**,** a quiescent stage after the degenerative trigger in which axons remain structurally and functionally intact, and catastrophic degeneration**,** a sudden switch into rapid, irreversible disintegration. However, much remains unknown about the processes underlying this binary transition. Curiously, bubble-like "axonal spheroids" have long been observed on axons exiting the latent phase, which we recently described rupturing and releasing degeneration-inducing axonal contents. Additionally, we observed that spheroids expose phosphatidylserine, which induces engulfment by phagocytes. Thus, it is tempting to speculate that spheroids could regulate pro-degenerative factor release into extra-axonal space via interactions with phagocytic cells. We examine this potential role *in vivo* using laser axotomy in mixed-sex zebrafish, *Danio rerio*, and *in vitro* using mixed-sex mouse neuronal culture enucleation in microfluidic devices. Our preliminary data identifies spheroid rupture (N=5 fish, N=3 cultures) or interaction with phagocytes (N=1 culture, N=5 fish) after injury. *In vitro,* we find that axons degenerate more slowly in the presence of macrophages ($p<0.0001$ by two-way ANOVA, N=5). Ongoing work seeks to determine if this protection is spheroid rupture-dependent and to verify these effects *in vivo*. This work highlights axonal spheroids as regulators of degeneration and downstream processes, providing new avenues for basic and therapeutic research.

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Poster

378. Neuroinflammation and Mechanisms of Neurodegeneration

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Program #/Poster #: 378.25

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Uncovering the Role of Progranulin in Ameliorating Lysosomal Storage Disease

Authors: *A. MALAGODI[1], I. VILLALPANDO[2], L. MANGINI[2], M. LOPEZ[3];
[1]BioMarin Pharmaceuticals, San Rafael, CA; [2]BioMarin Pharmaceut., San Rafael, CA;
[3]BioMarin Pharmaceut., Novato, CA

Abstract: This study investigates the role of granulin (*GRN*) in the disease progression of lysosomal storage disorders (LSD). Brain expression profiles of LSD mice models, such as Sandhoff, show *GRN* expression increase during pathogenesis. To explore whether loss of *GRN* worsens neuroinflammation, our lab generated and characterized a mouse deficient in both *GRN* and *HEXB* genes. Unexpectedly, the double knockout ($HEXB^{-/-}/GRN^{-/-}$) mice lived a median of 192 days compared to127 for Sandhoff ($HEXB^{-/-}$ / $HEXB^{+/+}$) mice (p-value = 0.0001). This survival benefit was reflected by lower levels of blood neurofilament light chain (NF-L). RNA-seq was performed on mice hindbrains at multiple timepoints for the following genotypes,

HEXB$^{-/-}$*/GRN*$^{+/+}$, *HEXB*$^{+/+}$*/GRN*$^{-/-}$, *HEXB*$^{-/-}$*/GRN*$^{-/-}$, and *GRN*$^{+/+}$*/HEXB*$^{+/+}$. *HEXB*$^{-/-}$*/GRN*$^{-/-}$mice showed equivalent or elevated levels of neuroinflammation markers, including *CD68, GPNMB, GFAP*, and *HPSE*, compared to *HEXB*$^{-/-}$*/GRN*$^{+/+}$mice. High glial activity was confirmed through immunohistochemistry staining on brain slices. Since PGRN and GRNs are known components of the lysosome, studies were performed to see if there was any beneficial alteration of the lysosomal defect that would protect from neurodegeneration. We preformed mass spectrometry on brain samples at day 65 for primary accumulates and found significant reduction (p-value of < 0.05) of lipid catabolism intermediates bis(monoacylglycero)phosphate (BMP) and GA2, albeit with a counter effect of increased GM2, a primary storage lipid. To better assess lysosomal health, lysosomal-associated membrane protein 1 (*LAMP1)* location and content were analyzed via microscopy of fibroblasts from each genotype. Results showed a significant decrease of *LAMP1* intensity in the *HEXB*$^{-/-}$ */GRN*$^{-/-}$ compared to *HEXB*$^{-/-}$ */GRN*$^{+/+}$ cells. These results indicate that there is an altered lysosomal pathway, contributing to the Sandhoff disease mitigation in the *HEXB*$^{-/-}$*/GRN*$^{-/-}$model. Further work aims to characterize lysosomal status of both glia and neurons in vivo and ex vivo to determine their contribution to the disease pathology and whether active inflammation can be decoupled from neuronal injury in this scenario.

Disclosures: **A. Malagodi:** A. Employment/Salary (full or part-time):; Part time. **I. Villalpando:** A. Employment/Salary (full or part-time):; Full Time employee. **L. Mangini:** A. Employment/Salary (full or part-time):; Full time employee. **M. Lopez:** A. Employment/Salary (full or part-time):; Full time.

Poster

378. Neuroinflammation and Mechanisms of Neurodegeneration

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 378.26

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Departmental funding

Title: Cerebrospinal fluid exosome profiling: A new research direction into the pathophysiology of pediatric hydrocephalus

Authors: *M. GARCIA BONILLA[1], A. ISAACS[4], D. M. MORALES[1], O. OSORIO[2], J. ALEXANDER-BRETT[2], K. SHUMILOV[3], M. CELORRIO NAVARRO[3], S. FRIESS[3], J. MCALLISTER[1], D. D. LIMBRICK[1];
[1]Neurosurg., [2]Pulmonary and critical care medicine, [3]Pediatrics, Washington Univ. in St Louis, St Louis, MO; [4]Neurosurg., Univ. of Calgary, Calgary, AB, Canada

Abstract: Introduction: Intraventricular hemorrhage (IVH) can lead to post-hemorrhagic hydrocephalus (PHH) with multifactorial pathophysiology that includes ventriculomegaly, alterations of white matter and the stem cell niche, and inflammation. Exosomes, extracellular vesicles involved in intercellular signaling, can be detected in the cerebrospinal fluid (CSF).

There is an emerging interest in exosome biology due to its potential to improve diagnosis, prognosis, and treatment. Our hypotheses are (1) CSF exosomes contain proteins related to neurodevelopmental and inflammation processes that provide insight into the pathophysiology of PHH; (2) CSF-based exosomal signaling and related CSF T-cell activation mediate the pathogenesis and neurological disability of PHH. **Methods:** Exosomes from CSF of neonates with PHH (lumbar, n=4; ventricular, n=5), IVH without PHH (lumbar, n=9), congenital hydrocephalus (ventricular, n=9), and controls/no known neurological injury (lumbar, n=9) were isolated and analyzed by electron microscopy, western blot and mass spectrometry (MS)-based high-throughput proteomics. Analysis of T cell activation was conducted in post-mortem brain samples and exposing them to exosomes for 3 hours *in vitro*. Levels of pro-inflammatory cytokines were studied by flow cytometry, ELISA, and immunofluorescence. **Results:** Lumbar CSF in PHH contained a significant increase in exosome proteins involved in the immune response, including neutrophils, antigen presentation molecules, and Fc receptor pathways compared to control patients. Ventricular PHH samples exhibited a significant increase in neutrophil activation/degranulation and oxygen transport proteins compared to congenital hydrocephalus patients. Lumbar PHH samples also showed a significant reduction in cell-cell adhesion proteins compared to IVH. No significant differences between control and IVH patients were detected. In contrast, congenital hydrocephalus, compared to control, revealed an increase in proteins related to stem cell differentiation. Exosomes activated the production of IL1beta, IL6, and TNFalpha in T cells in vitro. T cell recruitment and same cytokine production was detected in the choroid plexus of post-mortem PHH samples. **Conclusion:** Exosomes can play a role in the pathogenesis of acquired hydrocephalus, yield insight into IVH/PHH developmental sequelae, and examine the intersection of neuro-inflammation and repair.

Disclosures: M. Garcia Bonilla: None. **A. Isaacs:** None. **D.M. Morales:** None. **O. Osorio:** None. **J. Alexander-Brett:** None. **K. Shumilov:** None. **M. Celorrio Navarro:** None. **S. Friess:** None. **J. McAllister:** None. **D.D. Limbrick:** None.

Poster

378. Neuroinflammation and Mechanisms of Neurodegeneration

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 378.27

Title: WITHDRAWN

Poster

379. Mechanisms of Abnormal Movement in Stroke

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 379.01

Topic: C.09.Stroke

Title: Necessary plastic processes to account for the Brunnstrom stages of recovery post-stroke in isometric arm tasks

Authors: *K. LEE[1], V. BARRADAS PATINO[2], N. SCHWEIGHOFER[1];
[1]USC, Los Angeles, CA; [2]Inst. of Innovative Res., Tokyo Inst. of Technol., Yokohama-Shi, Japan

Abstract: Despite a wide variety in lesion types, recovery of arm movements post-stroke follows a stereotypical time course characterized by the Brunnstrom stages of recovery, although not all patients go through all stages. For the simple case of isometric arm tasks in multiple directions, we consider a reduced version of the Brunnstrom stages: 1) Flaccidity, that is, no movement due to weakness, 2) appearance of weak movements constrained to few directions due to strong abnormal synergies, 3) return to regular movements and decrease in abnormal synergies, and 4) complete recovery. Here, we develop computational neural models for isometric arm tasks to study the minimum necessary plastic processes that account for these stages. The base model contains a motor cortex neural model with a cortico-spinal tract, spinal motor neurons, and a data-driven arm musculoskeletal system with ten muscles (Barradas et al., 2019). We incrementally add plastic processes to build complex models and compare the models' capabilities to reproduce the recovery stages. We monitor three variables: overall force amplitude as a measure of weakness, number of abnormal synergies determined by non-negative matrix factorization, and generation of fine motor control via the dot product of desired forces and actual forces.
In the first model (Model 1), we simulated plasticity in the motor cortex via reinforcement learning, similar to the model of Reinkensmeyer et al. (2012), and partially lesioned the motor cortex to simulate a stroke. This model can solve stage 1 but not stage 2. In Model 2, we added a reticulospinal neural model, also under reinforcement learning, with a reticulospinal tract that projects to synergistic muscle. Model 2 can account for the increase in abnormal synergies but not the recovery of strength (stage 3). Since the remaining weakness is due to low activity in the motor neurons related to the loss of cortical inputs, we simulated Model 3 with motor neuron plasticity in the form of homeoplasticity (Triesch 2005), as has been previously reported both following spinal lesions and post-stroke. Model 3 can account for all stages except stage 4 of complete recovery. Therefore, we developed Model 4 with the premotor cortex as a "reserve" area (Reinkensmeyer et al., 2012), which accounted for all stages. We systematically simulated the four plastic processes in all possible combinations and concluded that these processes are necessary to account for the four stages of recovery. In future work, our study can help to pinpoint and enhance plasticity in different brain regions that are essential to improve recovery post-stroke.

Disclosures: K. Lee: None. **V. Barradas Patino:** None. **N. Schweighofer:** None.

Poster

379. Mechanisms of Abnormal Movement in Stroke

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 379.02

Topic: C.09.Stroke

Support: T32 HD101395-01A1

Title: Quantifying the Effect of Trunk Postural Control Deficits on Arm Reaching in Hemiparetic Stroke

Authors: *K. SUVADA[1], J. DEOL[2], J. P. DEWALD[1], A. ACOSTA[1];
[1]Physical Therapy and Human Movement Sci., Northwestern Univ., Chicago, IL; [2]Univ. of Alberta, Edmonton, AB, Canada

Abstract: The trunk provides a stable base of support and facilitates successful interaction of the limbs with the environment. Post hemiparetic stroke, damage to descending corticospinal pathways impacts motor control and activities of daily living. In the arm, impairments include weakness, hyperactive stretch reflexes, and involuntary coupling of shoulder abduction with elbow, wrist, and finger flexion, or flexion synergy. In the trunk, studies found excessive trunk movements, larger sway area during sitting, weakness, and altered coordination compared to controls. Yet, little is known about the interaction of the flexion synergy and the trunk during trunk-arm coordination. This study provides evidence of the impact of stroke on trunk-arm coordination while reaching, possibly due to increased use of the reticulospinal system wherein the arm and trunk are utilizing the same pathways. Previous studies suggest that the flexion synergy is a result of increased reliance on reticulospinal pathways, also responsible for maintenance of posture. I designed a novel experimental paradigm, to understand the consequences of controlling the trunk in the context of flexion synergy, consisting of a robotic device to create virtual environments, a motion capture system measuring trunk and arm kinematics, surface EMGs to measure trunk and arm musculature activity, and pressure mats to quantify trunk center of pressure.Four control and nine stroke subjects participated in the study after providing informed consent. Participants were seated with their arm coupled to a robotic device, allowing motion on a horizontal plane while either supported on a virtual table or generating 25% or 50% of their maximum shoulder abduction torque (SABT). Participants were instructed to reach as fast and as far as possible. When the trunk was unrestrained, participants were instructed to maintain their trunk posture. For those post stroke, reaching distance (difference between shoulder and hand where LL is limb length) was reduced for trunk unrestrained while reaching with the paretic limb: Table (84.95 +/- 4.87 vs 83.26 +/- 4.68 % LL), 25% SABT (77.33 +/- 5.26 vs 73.43 +/- 5.24 % LL), and 50% SABT (76.30 +/- 5.63 vs 73.35 +/- 5.37 % LL). For the non-paretic limb, individuals had reduced reaching distance in trunk unrestrained, Table (95.24 +/- .98 vs 93.55 +/- 1.09 %LL), 25% SABT (96.00 +/- 1.31 vs 94.05 +/- 1.42), 50% SABT (95.27 +/- 1.20 vs 93.54 +/- 1.47 % LL). For controls, reaching distance was reduced for trunk unrestrained when reaching with the dominant limb: Table (96.42 +/- 3.24 vs 90.98 +/- 2.34 %LL), 25% SABT (97.12 +/- 2.77 vs 93.28 +/- 2.28 %LL), 50% SABT (97.56 +/- 2.93 vs 91.89 +/- 2.71 %LL).

Disclosures: K. Suvada: None. **J. Deol:** None. **J.P. Dewald:** None. **A. Acosta:** None.

Poster

379. Mechanisms of Abnormal Movement in Stroke

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 379.03

Topic: C.09.Stroke

Support: NIH Grant P30GM103400
University of New Mexico BBHI 2017-1006
National Science Foundation award IIA-1301346

Title: Endogenous Zinc Protoporphyrin FormationCritically Contributes to Hemorrhagic Stroke-induced Brain Damage

Authors: *R. PAN, S. YU, H. ZHANG, G. TIMMINS, J. M. WEAVER, Jr., Y. YANG, X. ZHOU, K. LIU;
Dept. of Pharmaceut. Sciences, Univ. of New Mexico Hlth. Sci. Ctr., Albuquerque, NM

Abstract: Hemorrhagic stroke is a leading cause of death. The causes of intracerebral hemorrhage (ICH)-induced brain damage are thought to include lysis of red blood cells, hemin release and iron overload. These mechanisms, however, have not proven very amenable to therapeutic intervention, and so other mechanistic targets are being sought. Here we report that accumulation of endogenously formed zinc protoporphyrin (ZnPP) also critically contributes to ICH-induced brain damage. ICH caused a significant accumulation of ZnPP in brain tissue surrounding hematoma, as evidenced by fluorescence microscopy of ZnPP, and further confirmed by fluorescence spectroscopy and supercritical fluid chromatography-mass spectrometry. ZnPP formation was dependent upon both ICH-induced hypoxia and an increase in free zinc accumulation. Notably, inhibiting ferrochelatase, which catalyzes insertion of zinc into protoporphyrin, greatly decreased ICH-induced endogenous ZnPP generation. Moreover, a significant decrease in brain damage was observed upon ferrochelatase inhibition, suggesting that endogenous ZnPP contributes to the damage in ICH. Our findings reveal a novel mechanism of ICH-induced brain damage through ferrochelatase-mediated formation of ZnPP in ICH tissue. Since ferrochelatase can be readily inhibited by small molecules, such as protein kinase inhibitors, this may provide a promising new and druggable target for ICH therapy.

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Poster

379. Mechanisms of Abnormal Movement in Stroke

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 379.04

Topic: C.09.Stroke

Title: The affected side in the phase of stroke has a higher motor unit mean firing rate than the less affected side

Authors: *M. YOSHIDA[1], T. ITO[2], T. KOKUBUN[3];
[1]Grad. school of Saitama Prefectural Univ., Saitama, Japan; [2]Grad. school of Saitama Prefectural Univ., Koshigaya, Japan; [3]Grad. Sch. of Hlth. and Social Services, Grad. Sch. of Saitama Prefectural Univ., Koshigaya-shi, Japan

Abstract: Stroke causes a variety of physical dysfunctions. In particular, impairment of voluntary muscle contraction is one of the most common sequelae. Voluntary muscle contraction is controlled by the smallest units called motor units. Previous studies have revealed the recruitment characteristics of motor units in chronic stroke patients. However, even though the subacute phase is the most suitable for recovering physical functions after stroke, the recruitment characteristics during this phase remain unresolved. Since the recruitment characteristics of motor units reflect abnormalities in central nervous system muscle activity, elucidating the characteristics of the subacute phase is an important guideline for rehabilitation. The purpose of this study was to clarify the motor unit mobilization characteristics in patients in the subacute phase of stroke.The subjects were six hemiplegics in the subacute phase of stroke. Wireless surface electromyography and load cells were synchronized. The target muscles were the biceps brachii on the less affected and affected side. The subjects performed the Maximum Voluntary contraction (MVC) task and the Hold task, in which they held a constant force in an isometric contraction of the elbow flexion. The coefficient of variation (CV) of maximum voluntary isometric contraction force of elbow flexion in MVC and elbow flexion force in hold task were calculated, respectively. The composite action potentials acquired from the surface electromyograph were decomposed into motor unit action potentials. Then the mean firing rate of the less affected and the affected sides were calculated. Statistical analysis was performed using the Shapiro-Wilk test to confirm normality, followed by a paired t-test.The maximum voluntary isometric contraction force was significantly greater on the less affected side than on the affected side ($p=0.00$). The CV was significantly greater on the affected side than on the less affected one ($p=0.02$). The mean firing frequency was significantly greater on the affected side than on the less affected side ($p=0.02$). In the present study, recruitment characteristics of motor units in patients with subacute stroke were different from those in previous studies. The higher mean firing rate on the affected side suggests that this was an alternative means of exerting muscle strength in response to a decrease in the lesion-side subcortical descending pathway in the subacute phase. We found that the recruitment characteristics of post-stroke motor units differed according to the stage of the disease. In the future, it could be used as a biomarker for post-stroke rehabilitation.

Disclosures: **M. Yoshida:** None. **T. Ito:** None. **T. Kokubun:** None.

Poster

379. Mechanisms of Abnormal Movement in Stroke

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 379.05

Topic: C.09.Stroke

Support: Doctoral Dissertation Fellowship (DDF)

Title: Robot-aided assessments of ankle proprioception in chronic stroke survivors

Authors: *Q. HUANG[1], N. ELANGOVAN[1], M. ZHANG[2], J. KONCZAK[1];
[1]Human Sensorimotor Control Lab., Univ. of Minnesota, Minneapolis, MN; [2]Shenzhen Key Lab. of Smart Healthcare Engin., Southern Univ. of Sci. and Technol., Shenzhen, China

Abstract: BACKGROUND AND PURPOSE: About 56% of people with stroke present with compromised lower limb proprioception leading to increased falls and poor walking ability. Thus, accurate and objective assessment of proprioceptive deficits is needed in clinical settings. This study seeks to understand the nature and the extent of lower limb (i.e., ankle) proprioceptive impairment in stroke survivors by evaluating ankle joint proprioceptive acuity using a robotic device.**METHODS:** Eight adults with stroke (7 ischemic, 1 hemorrhagic; mean ± SD age 57.3 ± 5.9 years; 2 females) were recruited. Age and gender-matched healthy participants served as controls. All participants completed ankle position and motion discrimination assessments using an ankle robotic device. A psychophysical 2-alternative forced choice paradigm was applied, which requires participants to make verbal judgments discriminating specific stimulus pairs - a pair ankle position or motion. During testing, the ankle robotic device passively moved the ankle joint to specific plantar flexion position stimulus pairs or moved at specific constant velocity pairs. Stimulus difference size was determined by an adaptive Bayesian algorithm based on participant responses. Just-noticeable-difference (JND) thresholds - a measure of bias, and intervals of uncertainty (IU) - a measure of precision, were obtained as measures of ankle position sense and motion sense acuity.**RESULTS:** Stroke participants showed a highly elevated mean motion sense JND threshold (+82%, $p < 0.05$, $d = -1.38$), as well as IU or variability ($p < 0.05$ $d = -1.30$) than healthy controls. Elevated position sense IU ($p < 0.05$, $d=-1.30$) was observed in stroke participants. 7/8 stroke participants showed an average of 73% elevated mean position sense JND thresholds than healthy controls. Unsurprisingly, position sense thresholds were highly correlated with motion sense thresholds in healthy controls ($r = 0.73$, $p < 0.05$). However, this relationship was not observed in stroke participants indicating that different levels of impairment in the submodalities of proprioception. In addition, position sense JND (bias) and IU (precision) were correlated only among the stroke group ($r=0.87$, $p<0.01$).**CONCLUSION:** Our results demonstrate the nature and the extent of ankle proprioceptive impairment in stroke survivors. It also shows the need to evaluate both measures of perceptual bias and precision to comprehensively understand proprioceptive dysfunction following stroke. Furthermore, joint position and motion sense are compromised differently in neurological population. These insights inform the design of future stroke rehabilitation therapies.

Disclosures: Q. Huang: None. **N. Elangovan:** None. **M. Zhang:** None. **J. Konczak:** None.

Poster

379. Mechanisms of Abnormal Movement in Stroke

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Program #/Poster #: 379.06

Topic: C.09.Stroke

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NIH/NINDS (U01-NS108916)
McDonnell Center for Systems Neuroscience
Fondazione Neurone

Title: The interaction between cortical oscillation and muscle synergies in patients with hemiparesis

Authors: *G. TAN[1,2,3], Y. SHENG[4], J. LIU[5], J. WANG[6], H. LIU[4], P. BRUNNER[2,1,3];
[1]Dept. of Biomed. Engin., [2]Dept. of Neurosurg., Washington Univ. in St. Louis, Saint Louis, MO; [3]Natl. Ctr. for Adaptive Neurotechnologies, Albany, NY; [4]Sch. of Mechanical Engin. and Automation, Harbin Inst. of Technol., Shenzhen, China; [5]Zhejiang Lab., Hangzhou, China; [6]Dept. of Rehabil. Med., Shanghai Jiao Tong Univ., Shanghai, China

Abstract: More than 60% of chronic stroke patients are affected by hemiparesis. Motor recovery in these patients is mainly attributed to neuronal network reorganization. Recent studies suggest that the cortex controls muscle synergies rather than individual muscles. The interaction between the cortex and synergies could serve as an explicative target for rehabilitation therapies. Our study aims to quantify cortical oscillation-synergy interaction (OSI) based on EEG and EMG and understand its relationship with motor outcome. In our study, 9 chronic stroke patients with hemiparesis performed an isokinetic motor task using their affected upper limb once per week for 15 minutes. As a control, 5 healthy volunteers performed the same task once. Participants held the control stick of a robot and consecutively performed push, horizontal abduction, pull, and horizontal adduction. Muscle activation was calculated using a 2^{nd}-order linear model from the EMG signals. A synergy includes the involvement of muscles and their collective activation, which were identified using non-negative matrix factorization. K-means clustering was used to label synergy compositions in controls. Synergy compositions in patients were labeled based on their cosine similarity with compositions found in controls. The center frequency and bandwidth of the oscillation were identified from EEG signals, and power was extracted from their spectrogram. OSI was quantified as the t-statistics of the linear regression between synergy activation and oscillation power. Surrogates of t-statistics generated by shuffling oscillation power were used to normalize OSI. The explained variance of the regression models were calculated to affirm OSI. We found 5 relevant muscle synergies in healthy controls. Of those, 2 related to extension, 2 related to flexion, and 1 activated in 4 phases. The averaged explained variance of the regression model in both groups was more than 1 standard error higher than the surrogate explained variance. The largest difference between the two groups was OSI between the ipsilateral frontal area and an extension-related synergy, which is significantly higher in patients. Motor recovery, as measured by Fugl-Meyer assessment was positively correlated with OSI (R^2=0.33, $p<0.01$). In summary, contralateral damage resulted in higher OSI in the ipsilateral frontal area in hemiparesis patients which continued to increase as they recovered. This could lead to more rapid assessments of motor recovery through measuring OSI throughout

the rehabilitation sessions, which could facilitate the optimization of rehabilitation strategies in people affected by hemiparesis post stroke.

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Poster

379. Mechanisms of Abnormal Movement in Stroke

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Program #/Poster #: 379.07

Topic: C.09.Stroke

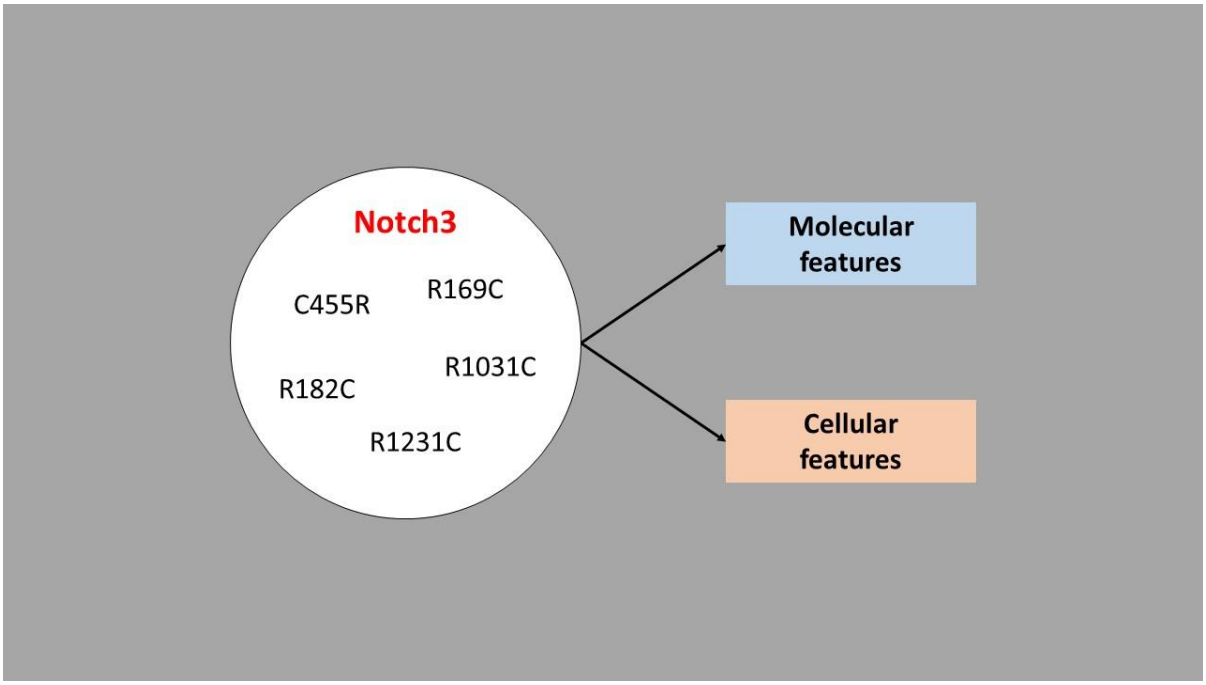
Support: NIH/NINDS/NIA Grant U01NS119560
NIH/NINDS Grant RF1 NS110048

Title: Molecular and cellular characterization of Notch3 mutations causing cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy

Authors: *T. H. DOAN[1,2], R. C. MAZZARINO[1,2], C. MARINO[1,2], S. J. AREVALO-ALQUICHIRE[1,2], J. F. ARBOLEDA-VELASQUEZ[1,2];
[1]Schepens Eye Res. Inst., Massachusetts Eye and Ear Infirmary, Boston, MA; [2]Harvard Med. Sch., Boston, MA

Abstract: Cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) causes recurrent ischemic events. The NOTCH3 gene, a member of the NOTCH family, is associated with CADASIL by missense mutations or small deletions in extracellular domain of this transmembrane protein. The aim of this study is to characterize different mutations and identify which features may play a role in pathology of CADASIL. Notch3 receptors with the C455R, R169C, R182C, R1031C, and R1231C CADASIL mutations under a TET-inducible promoter were stably expressed in human embryonic kidney (HEK) 293 cell line. The expression of Notch3 was confirmed using western blotting. Different features of the NOTCH3 mutations were examined, including the translocation of Notch3 protein, the interaction with activating ligands and the expression of downstream targets. To investigate the cell-surface charge, the zeta potential was measured using laser doppler electrophoresis technique. We found that after induction of NOTCH3 gene by Doxycycline, the ratio between the full-length unprocessed protein ($N3^{fl}$) and the processed form (Notch3 ectodomain, $N3^{ECD}$) was similar between the wild-type and C455R, R182C, R169C, R1231C Notch3 receptors. In contrast this ratio was higher for mutation R1031C ($N3^{fl}/N3^{ECD}$ ratio of WT = 1.34, C455R = 1.63, R182C = 1.21, R169C = 1.20, R1231C = 1.58, and R1031C = 2.92). Induction of NOTCH3 caused the neutralization of the HEK293 zeta potential. The zeta potential changed from negative to positive [$F(11, 24) = 4.255$, $p = 0.0015$, one-way ANOVA]. Comparing between mutations, the positive zeta potential value were also observed through all cell lines, and the significant change was found between C445R and R1031C mutations [$F(7, 16)$

= 3.322, p = 0.0221, one-way ANOVA]. This study is the first step to characterize and group more than 140 NOTCH3 mutations based on their characteristics that may be involved in the progression of vascular impairment. This also provides the advantageous for development of novel therapies that target individual genotype of CADASIL patients.



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Poster

379. Mechanisms of Abnormal Movement in Stroke

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 379.08

Topic: C.09.Stroke

Support: NICHD-R01HD096071

Title: Measuring upper limb kinetics and kinematics in an inpatient stroke rehabilitation facility

Authors: *G. C. BELLINGER, M. D. ELLIS;
Physical Therapy and Human Movement Sci., Northwestern Univ., Chicago, IL

Abstract: Individuals with stroke experience a myriad of motor impairments. Two distinct but often concurrent impairments are paresis and loss of independent joint control (i.e., flexion synergy). Weakness is profound soon after stroke and as movement re-emerges it is synergistic.

Flexion synergy is a muscle coactivation pattern and manifests as the involuntary coupling of shoulder abduction (i.e., lifting the arm) with flexion across all distal joints. There is growing interest in evaluating impairment level and motor performance with greater precision and rigor. This study uses sensor-based measures to quantify strength, independent joint control, and reaching performance during early subacute recovery. Patients recovering from stroke are recruited within an inpatient rehabilitation facility. The ongoing study involves a baseline evaluation and weekly inpatient evaluations until patients are discharged with the goal of differentiating the recovery trajectories of paresis and flexion synergy. Shoulder abduction and elbow extension kinetics are measured with a force and torque sensor while kinematic measures are collected using a custom spring/pulley-based device instrumented with encoders. This device allows manipulation of the vertical force applied to the limb, thereby controlling for different levels of strength while assessing flexion synergy and reaching performance. The first ten participants (6 men, 4 women) were 56.4 ± 16.7 years of age and their first evaluation occurred 34.1 ± 22.4 days after stroke. Collectively, participants' Fugl-Meyer score at baseline was 18.7 ± 14.4 and the average number of evaluations was 3 ± 1. At baseline, shoulder abduction strength was 18.31 ± 16.76 Nm (0.84 - 49.96 Nm) and elbow extension strength was 17.11 ± 18.37 Nm (0.00 - 49.20 Nm). Flexion synergy expression eliminated reaching ability at 79.02 ± 32.46% of shoulder abduction strength. Flexion synergy emergence was only captured for one participant which is expected since the protocol involves reaching to end range. Participants were able to reach 65.88 ± 33.63% of distance to the target while fully supported but only 32.14 ± 24.94% while lifting against 50% of shoulder abduction strength. Only three participants were able to reach against gravity at baseline. These preliminary data demonstrate the feasibility of assessing motor impairments longitudinally in the inpatient rehabilitation facility. While functional measures such as reaching distance against gravity may not be possible in early stroke recovery for individuals with profound hemiparesis, other precision measures are available to provide valuable insight into the development of motor impairment.

Disclosures: G.C. Bellinger: None. **M.D. Ellis:** None.

Poster

379. Mechanisms of Abnormal Movement in Stroke

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Program #/Poster #: 379.09

Topic: C.09.Stroke

Support: NSF Grant 1934650
University of Delaware Research Foundation Seed Grant

Title: Understanding the relationship between limb position sense and oculomotor proprioceptive estimations in individuals with stroke

Authors: *D. T. TULIMIERI[1], D. S. AUSTIN[1], J. ESKANDER[1], T. SINGH[3], J. A. SEMRAU[2];

[1]Biomechanics and Movement Sci., [2]Kinesiology and Applied Physiol., Univ. of Delaware, Newark, DE; [3]Kinesiology, Pennsylvania State Univ., University Park, PA

Abstract: Proprioception refers to our sense of body position in space and is comprised of static (i.e., position sense) and dynamic (i.e., kinesthesia) sub-components. Recent work from our group has demonstrated that proprioceptive impairments are common in individuals with stroke (~50%). Many of these individuals cannot use vision of the limb to improve performance on proprioceptive tasks (~20%) despite not having impairments in vision or visual attention. This result is perplexing, as vision of the limb is thought to typically improve movement and is a common rehabilitation approach for upper limb function after stroke. The presence of these deficits and lack of visual impairments in stroke survivors is suggestive of impairments in multisensory integration rather than unimodal sensory impairments. Here, we aim to examine how limb-based proprioceptive signals inform skeletalmotor- and oculomotor-based movements during a proprioceptive matching task. We hypothesized that individuals with stroke would have greater position sense deficits when using the eye as an end effector compared to age-matched controls due to impaired proprioceptive processing from the limb. Twenty older adults (OA) and 10 individuals with unilateral stroke were tested on a mirror-matching task to assess eye and limb output as proxies of limb position sense using the Kinarm robotic exoskeleton with integrated eye tracking. Participants were instructed to mirror-match the end position of a robot-generated movement of one limb with an eye movement (eye-matching) or limb movement of the opposite arm (arm-matching). For stroke participants, the robot moved the more-affected arm. To quantify proprioceptive accuracy, we examined end point error (EPE) of arm and eye movements to understand positional error, as well as the total number of times participant eye-movements crossed the mid-line of the workspace to understand a potential strategy of referencing the more affected arm. We found that older adults had less limb EPE (OA: 3.4 ± 1.4 cm, Stroke: 6.0 ± 3.1 cm; $p = 0.001$) and eye EPE (OA: 7.2 ± 2.8 cm, Stroke: 9.9 ± 3.3 cm; $p = 0.016$), compared to stroke participants during hand and eye blocks, respectively. Stroke participants also made an increased number of workspace crossings compared to controls (OA: 5.1 ± 3.1, Stroke: 14.8 ± 22.6; $p = 0.053$). Our results suggest that individuals with stroke, in the absence of visual impairment, have difficulty transforming proprioceptive information from the stroke-affected limb to guide oculomotor behavior. This has important implications for understanding the efficacy of how the oculomotor system can be used to guide upper limb movement in stroke survivors.

Disclosures: D.T. Tulimieri: None. **D.S. Austin:** None. **J. Eskander:** None. **T. Singh:** None. **J.A. Semrau:** None.

Poster

379. Mechanisms of Abnormal Movement in Stroke

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 379.10

Topic: C.09.Stroke

Support: NIH Grant GR1054771

Title: Control of interaction torques during single-joint arm movements in stroke survivors

Authors: *Y. DARMON[1], G. E. LOEB[2], V. BARRADAS PATINO[4], C. J. WINSTEIN[3], E. ROSARIO[5], N. SCHWEIGHOFER[1];
[1]USC, Los angeles, CA; [2]Biomed Engin., [3]Div. Biokinesiol/Physical Ther, USC, Los Angeles, CA; [4]Inst. of Innovative Res., Tokyo Inst. of Technol., Yokohama-Shi, Japan; [5]Res. Inst., Casa Colina Hosp. and Centers For Healthcare, Pomona, CA

Abstract: Sensory-motor strokes abruptly impair motor control of goal-directed movements. Generating fast, smooth and straight trajectories for multi-joint planar reaches requires adequate compensation for the interaction torques that arise at both proximal and distal joints. It has been proposed that stroke survivors exhibit deficits in the control of these interaction torques (Beer et al., 2000). Here, we study to what extent stroke survivors activate shoulder muscles to control for anticipated interaction torques during fast movements restricted to the elbow. Neurotypical individuals learn to compensate for these torques by activating anticipatory shoulder muscles proportionally to the interaction torques (Gribble and Ostry, 1999). We hypothesize that, compared to the less-affected arm, the more-affected arm of stroke survivors will exhibit a diminished scaling between the generated interaction torques at the shoulder and the Electromyographic (EMG) activity of the posterior deltoid during fast single-joint elbow extension. We recruited 18 (10 females), moderately to mildly impaired (UE-FM 47 ± 2.1), supra-tentorial chronic stroke survivors (6 by right hemisphere damage), and tested their more- and less-affected arms. We studied the relationships between the interaction torques at the shoulder (computed from arm morphometry, shoulder and elbow angle, angular velocity, and angular acceleration) and the Posterior Deltoid (PD) EMG activity. PD EMG preceded movement onset for both the more (-60 ± 11.8 ms) and the less affected sides (-52 ± 16ms), but the amplitude was more weakly correlated with interaction torque on the more-affected side (mean $R^2 = 0.21 \pm 0.03$) than the less-affected side (mean $R^2 = 0.5 \pm 0.05$). Our results indicate that stroke survivors maintain the temporal strategy needed to counteract the interaction torques in our sample but show a diminished ability to scale the anticipatory muscle activities properly. In future work, we will use structural imaging (DTI) to seek the neural correlate of this impairment. Because feedforward motor commands are thought to be learned via the cerebellum, damage in the cerebro-cerebellar pathways may preclude stroke survivors from updating their feedforward controllers.

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Poster

379. Mechanisms of Abnormal Movement in Stroke

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Topic: C.09.Stroke

Support: Japan Society for Promotion of Science, KAKENHI (grant number 20H01785)

Title: Optimization processes of movement trajectory to gravitational perturbation in stroke patients

Authors: *L. ANDO[1], M. YOSHIOKA[2], S. DAIMON[3], Y. ITAGUCHI[4];
[1]Dept. of Informatics, Shizuoka Univ., Hamamatsu-shi, Japan; [2]Dept. of Rehabil., JA Toride medical center, Toride, Japan; [3]Dept. of Rehabil., CLARK Hosp., Sapporo, Japan; [4]Fac. of letters, Keio Univ., Tokyo, Japan

Abstract: In a daily life, we often perform arm movements with a weight; for example, we grasp a doorknob with a shopping bag hanging from our arm. A previous study investigated the adaptation processes to the arm-weight increase on the kinematics of reaching and reach-to-grasp movements and suggested that healthy participants optimize their movements by raising the trajectory height. Stroke patients, however, would have a difficulty in the optimization of their movements. Although our bodily movements are usually performed in a three-dimensional space against gravity, many clinical studies have paid attention to movement trajectories in a horizontal plane. One study showed that patients with a parietal lobe damage did not modulate the trajectory height in reaching movements when the speed of the movement changed. To further clarify the height modulation against gravitational influences on a daily movement in stroke patients, we investigated the kinematics of reaching movements with or without a weight. In the experiment, three stroke patients with a mild hemiparalysis performed reaching movements on a desk under two weight conditions: 0 g and 200 g conditions. The patients performed two blocks that were consisted of twenty trials for each condition. Side-view images of the movements were recorded, and the positions of participants' index finger in the sagittal plane were estimated using DeepLabCut. The height and velocity of the index finger were analyzed. We mainly obtained two results; first, maximum heights of the index finger in the patient with most severe hemiparalysis were comparable in both weight conditions between affected and intact hands, whereas the maximum heights in the patients with less severe symptoms were higher in the second block than in the first block only in 200 g condition. This result suggests that the severe patient could not optimize his heights of movements for the increased arm weight or muscle fatigue. Second, particularly with the affected hand, the number of tangential velocity peaks increased in the deceleration (descending) phase in both weight conditions. Interestingly, the velocity in the gravitational direction, rather in the heading direction, contributed to the increase of the number of peaks. This result suggests that the stroke symptoms are more apparent in the disturbances of the trajectory in gravitational direction. The analysis of the kinematics in the gravitational direction as well as the tangential direction is not difficult in clinical settings but may help to further understand motor symptoms in stroke patients.

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Poster

379. Mechanisms of Abnormal Movement in Stroke

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Program #/Poster #: 379.12

Title: WITHDRAWN

Poster

379. Mechanisms of Abnormal Movement in Stroke

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 379.13

Topic: C.09.Stroke

Support: NIH R01NS093057

Title: Investigating Neuronal Activation in the Contralesional Cortex After Stroke

Authors: *T. CHIANG, M. ITO, S. HARVEY, M. Y. CHENG, G. K. STEINBERG; Stanford Univ., Stanford, CA

Abstract: Background: Functional neuroimaging studies have reported increased activation in the contralesional cortex in both experimental and clinical settings of stroke. However, the patterns of activation and its neuronal subtype in the contralesional cortex is unclear. In this study, we characterized the time course of contralesional cortical activation and its neuronal subtypes during post-stroke recovery using neuronal activity and various cell type markers. Methods: Adult male C57Bl6 mice (11-13 weeks) were subjected to transient middle cerebral artery occlusion (MCAO). Stroke lesion was verified at post-stroke day (PD) 3 by T2-weighted MRI and/or histology. Mice with infarcts in both cortical and striatum were included in the study. Brains were collected at PD3, 7, 15, and 28 and were processed for immunostaining using activity-dependent marker Early growth response 1 (Egr1). Egr1 positive cells in the primary motor cortex (M1), pre-motor cortex (M2), and somatosensory cortex (SS) in the contralesional hemisphere were quantified. Egr1 was also co-stained with NeuN (mature neurons), CaMKII and SATB2 (excitatory neurons), Parvalbumin (GABAergic neurons) and GFAP (astrocytes) to identify its cell type specificity. Results: Egr1 expression in the contralesional cortex was predominantly colocalized with NeuN, indicating that Egr1 is primarily expressed in mature neurons. Following stroke, contralesional cortical activation was detected on PD1-3 and peaked at PD7-15. Contralesional cortical activation persisted but began to decrease in some regions at PD21-28. Region-wise in-depth analysis revealed that these changes occurred in contralesional M1, M2 and SS. Cell type analysis showed that Egr-1 expressing cells were mostly colocalized with excitatory neuronal marker SATB2, but not inhibitory neuronal marker PV, indicating that majority of activated neurons in the contralesional cortex are excitatory. Conclusions: Our results demonstrated increased Egr-1 activation in excitatory neurons of contralesional cortex following stroke, particularly in somatosensory and primary motor cortex, suggesting a potential important role of these cortical regions in stroke recovery.

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Poster

379. Mechanisms of Abnormal Movement in Stroke

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Program #/Poster #: 379.14

Topic: C.08. Ischemia

Support: NIH NINDS R01 NS 113921

Title: Clinical and telemetric electroencephalographic seizure monitoring in a neonatal piglet model of hypoxic-ischemic encephalopathy and therapeutic hypothermia

Authors: *C. JAVDAN, C. PRIMIANI, C. E. O'BRIEN, M. W. CHEN, E. KULIKOWICZ, S. ADAMS, B. LESTER, N. RIVERA-DIAZ, V. OLBERDING, M. NIEDZWIECKI, E. RITZL, C. HABELA, R. C. KOEHLER, J. K. LEE, L. J. MARTIN;
Johns Hopkins Med. Inst., Baltimore, MD

Abstract: Neonatal hypoxic-ischemic encephalopathy (HIE) is a risk factor for seizure disorders. No clinically relevant large animal model exists for modeling perinatal brain damage, therapeutic hypothermia, progression of electrical seizure activity and associated motor phenotypes, and single-cell neuropathology in combination. A gyrencephalic neocortex is necessary to best model human neonatal neuropathology and epileptogenesis. We sought to develop such a model. Neonatal piglets received hypoxia-ischemia (HI)+normothermia (n=9), HI+overnight hypothermia (n=6), sham+normothermia (n=6), or sham+overnight hypothermia (n=6). Piglets were sedated, intubated, anesthetized, subjected to 45 min hypoxia (FiO_2 0.1), followed by 5 min room air, and then 8 min asphyxia. Piglet resuscitation was achieved with 50% oxygen, chest compressions, and epinephrine. Some piglets, before the HI protocol, were instrumented with sterile epidural 4-lead electrode telemetry arrays. Secure electrode placement was done by craniotomy and micromanipulation of contact screw insertion precisely onto the dura mater. The transmitter was placed subdermally. Piglets had continuous electroencephalography (EEG) during recovery and overnight manual video recording. All piglets were unmedicated. Piglets that developed intractable seizures were euthanized. Piglet survival was 2-7 days. Piglets were perfused with 4% paraformaldehyde for electrode placement verification and neuropathology. Clinical seizures usually emerged 24 hours after extubation. They often appeared during sleep, consisting of sudden arousal, repetitive rooting, and orofacial twitching and then clonic movement. Clonic movements spread from the head region to the shoulder and forelegs appearing as more generalized tonic clonic seizures. Some piglets ultimately developed fictive running movements using forelegs and hindlegs and advanced to apparent status epilepticus. Continuous EEG confirmed the presence of seizures in some piglets as seen by rhythmic spike-wave complexes appearing in specific areas of neocortex and then generalizing to other areas of neocortex. Seizures were seen in HI piglets with and without hypothermia. The neuropathology

in neocortex was topographically organized and ranged from selective neuronal injury, laminar necrosis, to pan-laminar necrosis. Subcortical areas were also damaged. We thus have developed a unique gyrencephalic large animal model that comprehensively integrates clinically relevant neonatal HIE, a therapeutic standard of care, continuous EEG, survival, a neurologic phenotype, and cellular neuropathology.

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Poster

379. Mechanisms of Abnormal Movement in Stroke

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Program #/Poster #: 379.15

Topic: C.09.Stroke

Support: NIH – DP5-OD029571

Title: Utilizing EMG for stroke diagnostics: identifying metrics that indicate degree of hemiparesis

Authors: *D. LOPEZ[1], C. THOMSON[2], J. A. GEORGE[3];
[1]Interdepartmental Neurosci. Program, [2]Biomed. Engin., [3]Mechanical Engin., Univ. of Utah, Salt Lake City, UT

Abstract: The long-term goal of this research is to provide stroke patients with more reliable and accurate diagnostics for tracking recovery from stroke-induced upper-limb spastic hemiparesis. Destruction of cortical upper motor neurons resulting from ischemic stroke can diminish the ability to relax muscles and can result in upper-limb spastic hemiparesis. Upper-limb spastic hemiparesis limits hand function and negatively impacts quality of life for roughly 80% of stroke survivors. Treatment plans for spastic hemiparesis, which often consist of various physical therapy regimens, are informed by the Modified Ashworth Scale (MAS) evaluation of spasticity. The MAS consists of an attempt to passively move a joint through its range of motion and evaluating resistance and muscle tone throughout on a five-point categorical scale. Due to the MAS's qualitative nature, its inter-rater reliability is only moderate ($\kappa = 0.54$).
Here we explore the use surface electromyography (EMG) to provide a continuous and quantitative measure of upper-limb spasticity. We recruited one male and one female stroke patient to perform a series of voluntary contractions of hand grasping and then hand opening while EMG was recorded from the extrinsic hand muscles in their forearm with a 32-electrode sleeve. We hypothesized that the time it took the patients to begin and terminate hand grasping and hand opening could serve as a measure of spasticity, with longer times indicating more severe spasticity. We measured the time constants for each electrode for the average hand grasp

and hand opening as the time it took from the start of the movement to reach 63.21% of peak EMG activity and the time it took from peak EMG activity to fall below 36.79% of peak value. Preliminary results suggest that the time it takes to terminate hand opening may be able to provide a continuous and quantifiable measure of spasticity. Preliminary results from the three participants show that the time constants of EMG decay after hand opening were significantly greater in paretic hands compared to healthy hands (1.02 ± 0.07 s vs 0.71 ± 0.02 s; $p < 0.05$, unpaired t-test). The time constant of EMG decay showed a positive correlation with MAS score ($r^2 = 0.74$). These results lend further credibility to the notion that stroke results in the failure of inhibitory neurons to reduce motor-unit activation after contraction.
We anticipate this metric will be a starting point in creating a comprehensive set of quantitative indicators of motor recovery after stroke. More quantitative measures of motor function throughout recovery may ultimately lead to individualized treatment plans that enhance upper-limb recovery after stroke.

Disclosures: D. Lopez: None. **C. Thomson:** None. **J.A. George:** None.

Poster

379. Mechanisms of Abnormal Movement in Stroke

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 379.16

Topic: C.09.Stroke

Title: Using a computerized tablet device to understand spatial precision and movement time of the upper limb in individuals with stroke.

Authors: D. AUSTIN[1], J. ESKANDER[2], M. DIXON[2], D. TULIMIERI[2], J. CASHABACK[2], *J. SEMRAU[2];
[1]Biomechanics and Movement Sci., [2]Univ. of Delaware, Newark, DE

Abstract: Recent recommendations suggest that the utilization of technology can improve sensorimotor assessment after stroke. Our lab has previously used robotic tasks to precisely quantify sensorimotor function of the upper limb after stroke. However, the availability, training, and cost of robotic systems make it difficult for this technology to be universally available. Computerized tablets (e.g., iPad) have been used for sensorimotor rehabilitation protocols, but have seen limited implementation for post-stroke assessment in the upper limb. The objective of this pilot study was to design and implement a tablet-based maze task to examine upper limb sensorimotor behavior in individuals who have had a stroke. We expected that as maze complexity increased, participants with stroke would have a greater number of spatial errors, longer movement times, and slower speeds compared to age-matched controls. Participants (7 individuals with stroke, 17 controls) used an iPad with digitizing stylus to navigate 8 pseudorandomly generated paths (levels). Participants completed five trials per level, with each level becoming more complex by increasing the number of 90° turns (Level 8 = 8 turns). Participants were instructed to navigate the level as quickly and accurately as possible while

avoiding the path's boundaries. If contact was made with a boundary, the boundary lit up to notify participants of their error. All participants performed the task with both hands, which was counterbalanced within groups. Sensorimotor behavior was quantified using the following parameters: total number of boundary hits, peak speed, and movement time of each level between and within groups. Preliminary analyses revealed that, on average, individuals who have had a stroke had significantly greater boundary hits ($p<0.001$) and movement time ($p=0.002$) for all levels, while peak speed was significantly lower for four of the eight paths ($p=0.01$). The within-group analysis for both groups did not reveal differences in the number of spatial errors, movement time, or speed as a function of task level. Further analysis of total path length and other spatial parameters may reveal unique strategies that different participants utilized to maximize success. Overall, metrics related to movement time, peak speed, and the number of errors demonstrate spatial and temporal impairments of upper limb function in individuals who have had a stroke during a complex sensorimotor task. This work has the potential to contribute to specific rehabilitation interventions related to complex navigation during upper limb movements.

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Poster

379. Mechanisms of Abnormal Movement in Stroke

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Program #/Poster #: 379.17

Topic: C.09.Stroke

Support: NRF Grant 2021R1A2C1012113

Title: Preservation of cerebellar afferent pathway may be related to good hand function in patients with stroke

Authors: *S. LIM[1], B. SHIN[1], T.-W. KIM[2], H.-Y. PARK[1], Y. YOO[1], M.-J. YOON[1], B. HONG[1], H. RIM[1], J. JUNG[1], S. PAEK[1];
[1]Rehabil. Med., Col. of Medicine, The Catholic Univ. of Korea, Seoul, Korea, Republic of;
[2]Rehabil. Med., Natl. Traffic Injury Rehabil. Hosp., Gyeongki-do, Korea, Republic of

Abstract: Many chronic stroke patients suffer from worsened hand function, and functional recovery of the hand does not occur well after 6 months of stroke. Therefore, predicting final hand function after stroke through acute phase imaging would be an important issue in counselling with the patients or their family. Thus, we investigated the remaining white matter integrity in corticospinal tract (CST) and cortico-ponto-cerebellar tract (CPCT) at acute stage of stroke and chronic hand function after stroke, and present the cut-off value of fiber number (FN) and fractional anisotropy (FA) of CST and CPCT at acute stage for predicting final hand function after recovery period. This retrospective case-control study included 18 stroke patients

who were classified into two groups: poor hand function with stroke (n = 11) and good hand function with stroke (n = 7). DTI was done within 2 months ± 15 days after onset, and the Jebson's Hand Function test was conducted 6-12 months after onset. The investigation of white matter were focused on the values of FN and FA for CST and CPCT, were measured separately(Figure 1). The normalized (affected/non-affected) FA values in the CPCT in the good hand function group were higher than those in the poor hand function group. The normalized cut-off value that distinguished the good hand function group from the poor hand function group was 0.8889 for FA in the CPCT. The integrity of the CPCT in the acute stage was associated with hand function in the chronic stage after a stroke. Ultimately, the integrity of the CPCT in the early stage after onset can be used to predict chronic hand function. Based on these results, cerebellar afferent fiber measurements may be a useful addition to predict hand function and plan specific rehabilitation strategies in stroke patients.

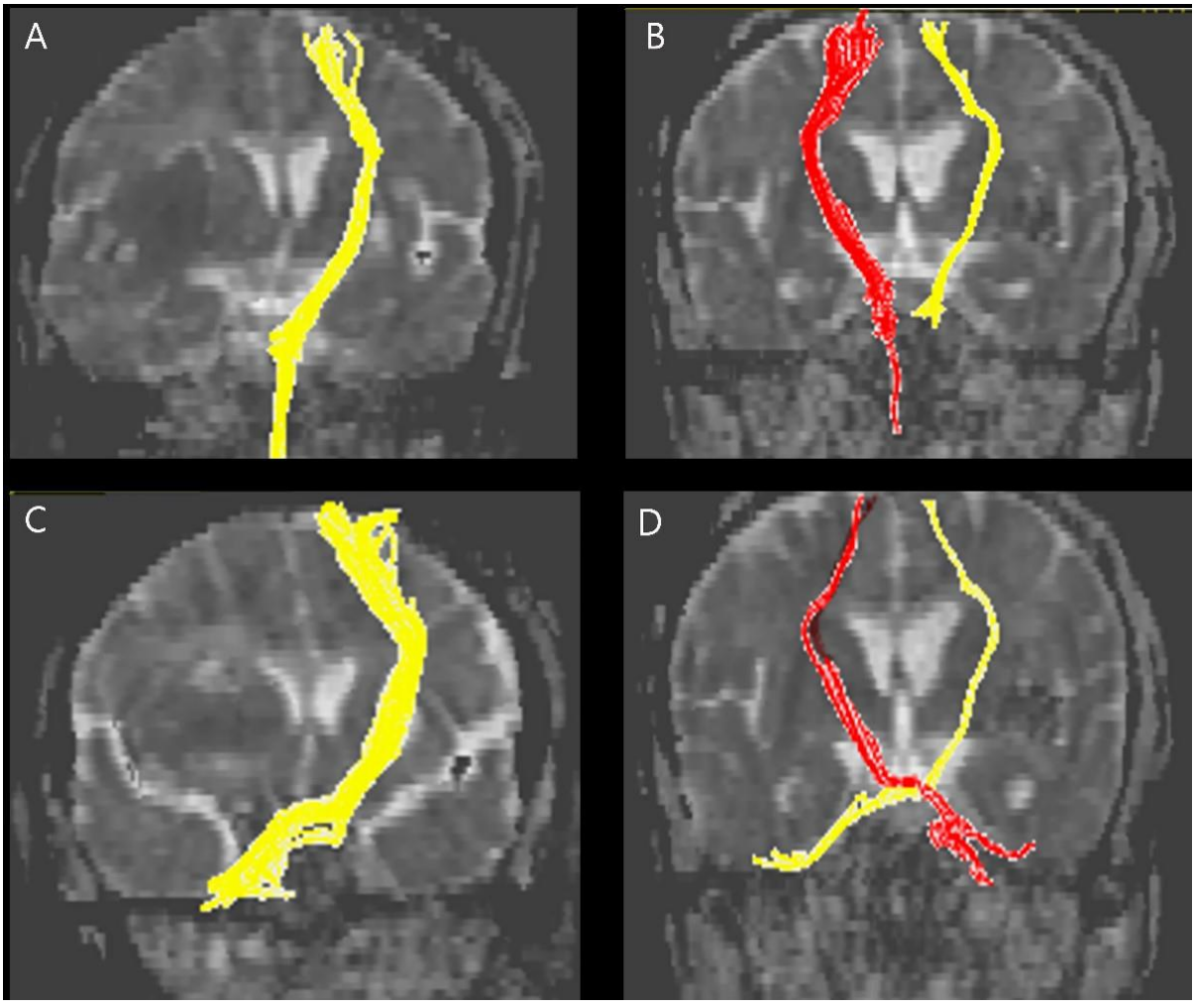

Figure 1. Representative diffusion tensor tractography images of the CST in typical subjects from the (A) poor hand function group and the (B) good hand function group, CPCT in typical subjects from the (C) poor hand function group and the (D) good hand function group.

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Poster

379. Mechanisms of Abnormal Movement in Stroke

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Topic: C.09.Stroke

Support: NIH/NICHD grant R01HD58301
NIDILRR grant RERC 90RE5021

Title: Eeg based resting state connectivity predicts motor recovery in the subacute phase post stroke

Authors: S. H. SALEH[1], J. PATEL[2], I. PATTISON[3], M. GLASSEN[1], Q. QIU[2], G. FLUET[2], E. KAPLAN[1], E. TUNIK[5], K. NOLAN[1], A. MERIANS[2], ***S. ADAMOVICH**[4,2];
[1]Kessler Fndn., West Orange, NJ; [2]Rehabil. and Movement Sci., Rutgers Univ., Newark, NJ; [3]Biomed. Engin., [4]New Jersey Inst. of Technol., Newark, NJ; [5]Dept. of Physical Therapy, Movement, and Rehabil. Sci., Northeastern Univ., Boston, MA

Abstract: This ongoing study evaluates resting state connectivity (RSC) measured via EEG as a neural biomarker of responsiveness to treatment in subacute stroke. Biomarkers can help clinicians understand the potential for recovery and thus help them establish therapy goals and determine treatment plans. Current predictive models include various measures of corticospinal integrity obtained via transcranial magnetic stimulation (TMS) and/or MRI. However, our ongoing clinical trial in one of the leading rehabilitation hospitals in the US demonstrated that many stroke survivors are either ineligible or refuse to participate in these types of testing during the first month post stroke. Therefore, we seek to develop a predictive model based on baseline EEG connectivity measures. We evaluated the change in resting state connectivity (RSC) in motor areas, as well as motor recovery of the affected upper limb, in the subacute phase post-stroke. Fifteen participants who had sustained a subcortical stroke were included in this study. The group made significant gains in upper limb impairment as measured by the Upper Extremity Fugl-Meyer Assessment (UEFMA) from baseline to four months post-stroke (mean (SD) of 24.78 (5.4)). During this time, we observed a significant increase in RSC in the beta band from contralesional M1 to ipsilesional M1. We propose that this change in RSC may have contributed to the motor recovery seen in this group.

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Poster

379. Mechanisms of Abnormal Movement in Stroke

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Topic: C.09.Stroke

Support: OCAST HR21-164
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Title: Cortical Somatosensory Reorganization in Hemiparetic Stroke

Authors: W. SIKORA[1], J. WILLIAMSON[1], N. PARMAR[2], L. V. LEPAK[3], C. CHEEMA[3], J. P. DEWALD[5], D. WU[4], E. V. SIDOROV[4], ***Y. YANG**[1];
[1]Univ. of Oklahoma, Norman, OK; [2]Univ. of Oklahoma, Tulsa, OK; [3]Univ. of Oklahoma Hlth. Sci. Ctr., Tulsa, OK; [4]Univ. of Oklahoma Hlth. Sci. Ctr., Oklahoma City, OK; [5]Physical Therapy and Human Movement Sci., Northwestern Univ., Chicago, IL

Abstract: Background. Stroke is the leading cause of serious, long-term disability. Previous studies have demonstrated that post-stroke motor impairments are likely due to maladaptive recruitment of the contralesional motor system in the brain. However, it is unknown how the somatosensory system may reorganize to align with the change of the motor system to provide sensory feedback to the movement control of the paretic arm. Methods. This study assessed cortical somatosensory processing in chronic hemiparetic stroke participants (n=9) and compared it with age-matched controls (n=9) using high-density electroencephalograph (EEG) recordings during electrical tactile finger stimulation. The somatosensory evoked potentials (SEPs) will be extracted from the EEG signals with the components P50 and N100 measured. The laterality index will be calculated to determine the hemispheric dominance of the ERP. Results. It was found that the latencies of P50 and N100 were significantly delayed in stroke participants, in comparison to controls ($p < 0.05$). In stroke, these two components are prolonged in response to the stimulation at the paretic hand as compared to the non-paretic hand ($p < 0.05$). Bilateral cortical responses were detected in stroke participants, while only contralateral cortical responses are shown in control participants, resulting in a significant difference in the laterality index. Conclusion. This research extends the current understanding of the neural circuitry change in the brain post-stroke with new findings in a change of the cortical somatosensory system. Somatosensory reorganization after stroke might involve the increased recruitment of ipsilateral cortical regions during the processing of the somatosensory signals from the paretic hand. The somatosensory reorganization might delay the latency of somatosensory processing in the stroke brain. Significance. As such, this research provided new knowledge to understand motor deficits induced by somatosensory organization in the injured brain. It will pave the way to provide a sensitive biomarker based on EEG to enrich future hypothesis-driven therapeutic rehabilitation strategies from a sensory or sensory-motor perspective, thus improving stroke recovery.

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Poster

379. Mechanisms of Abnormal Movement in Stroke

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Program #/Poster #: 379.20

Title: WITHDRAWN

Poster

379. Mechanisms of Abnormal Movement in Stroke

Location: SDCC Halls B-H

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Topic: C.09.Stroke

Support: NIH Grant HD096071
Department of Physical Therapy and Human Movement Sciences

Title: Stability of Kinetic and Kinematic Measures of Stroke-Related Paresis, Flexion Synergy, and Reaching in a Mechatronic Evaluation System

Authors: F. S. E. ABIUSI, A. M. CROOKSTON, N. B. HOFFMAN, R. E. IBRAHIM, E. LINNE, S. MATTA, N. MEHTA, C. R. NISSEN, M. SANKARAN, A. ACOSTA, ***M. D. ELLIS**;
Northwestern Univ., Chicago, IL

Abstract: A new mechatronic evaluation system along with a Matlab-based software app was used in two consecutive sessions to measure abduction and elbow extension strength, shoulder/elbow flexion synergy impairment, the impact of flexion synergy on reaching performance and reaching function in individuals with chronic stroke. Stability of each repeated measurement was evaluated along with the construct validity of measuring the impact of flexion synergy on reaching performance in order to support application of the mechatronic evaluation system in rehabilitation research. Thirteen individuals (7 males, 6 females) age 55.8 ± 16.9 years with chronic stroke (6.4 ± 8.7 years) took part in the study. Abduction and elbow extension strength were measured isometrically with a 2-DOF force and torque sensor. A mechatronic device measured reaching distance at shoulder height under three conditions (virtual horizontal table, at 50% abduction strength, against gravity) to a standardized target near end range of motion. The boundaries of expression of flexion synergy impairment were measured as the

highest abduction load achieved at the end and beginning of reaching range of motion (emergence and takeover thresholds respectively). There was no difference ($p > 0.05$) between repeated measures (session 1 vs. session 2) for absolute abduction strength (24.9 ± 13.8Nm, 25.8 ± 13.1Nm), absolute elbow extension strength (16.0 ± 16.2Nm, 17.5 ± 14.8Nm), normalized and absolute flexion synergy emergence threshold (53.1 ± 26.3, 48.8 ± 30.8; 45.1 ± 34.2Nm, 40.8 ± 27.1Nm), and takeover threshold (88.3 ± 22.9, 91.4 ± 18.8; 64.8 ± 29.8Nm, 72.8 ± 33.7Nm), normalized reaching against gravity (0.63 ± 0.32, 0.64 ± 0.36), and normalized reaching at 50% abduction strength (0.63 ± 0.26, 0.62 ± 0.30). There was a difference ($p < 0.05$) between normalized reaching distance on the table (0.87 ± 0.23, 0.86 ± 0.23) and normalized reaching distance at 50% of abduction strength (0.63 ± 0.26, 0.62 ± 0.30). The findings of this interim analysis suggest that the precision measurements were stable supporting test-retest reliability. Furthermore, the results suggest that measuring reaching distance at 50% of abduction strength was detecting the impact of flexion synergy on reaching performance supporting construct validity. These data support continued evaluation of psychometric properties in a large and generalizable sample. Precision measures are critical to finding subtle but meaningful responses to novel interventions that are presently being developed in rehabilitation research. Precision measurements will augment clinical decision making and encourage targeted impairment-based interventions.

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Poster

380. Understanding Traumatic Brain Injury (TBI)

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 380.01

Topic: C.10. Brain Injury and Trauma

Title: Biomarkers of neurotransmitter metabolism and bioenergetic pathways are chronically altered in individuals with mild traumatic brain injury and are related to the presentation of specific symptoms

Authors: *A. N. GROSSBERG[1], L. KOZA[1], C. PRUSMACK[2], D. A. LINSEMAN[1];
[1]Univ. of Denver, Denver, CO; [2]Resilience Code, Denver, CO

Abstract: According to recent estimates, ~2-million individuals sustain a traumatic brain injury in the US annually. Around 80% of those injuries are classified as mild (mild traumatic brain injury or mTBI). Most individuals with mTBI recover within days-weeks; however, a small subset of patients develop protracted symptoms that may last for months-years. These non-specific, multidimensional symptoms often mimic those associated with other disorders, vary widely in severity and presentation, and may have distinct pathophysiological underpinnings. Consequently, diagnosis and treatment can be frustrating, time-consuming, and costly for

patients, caretakers, and providers. Thus, the development of specific biomarker profiles is urgently needed to expand the diagnostic repertoire of clinicians and to help scientists better understand distinct clinical presentations in patients with mTBI. Previous research has reported that common post-injury symptoms, including depression, anxiety, fatigue, “brain fog”, and cognitive impairment may be associated with alterations in energy metabolism and neurotransmitter networks. The aim of this retrospective study was to investigate whether biomarkers of neurotransmitter metabolism (precursors and metabolites) and bioenergetic pathways are chronically altered in individuals with a history of mTBI compared to controls and to determine whether these biomarkers are related to specific symptom clusters. Data from 201 outpatients, treated at a Colorado-based clinic, were collected and analyzed. Patient data included biomarkers as well as compiled scores on the PROMIS-29, an instrument that assesses Health-Related Quality of Life (HRQoL) across seven domains, and scores on a symptom checklist, the Medical Symptom Questionnaire (MSQ). Individuals with a history of mTBI had significantly increased affective/psychological, somatic, and cognitive/neurological symptoms compared to controls. Interestingly, we also found that individuals with mTBI were more likely to display an increased frequency and severity of depressive symptomology compared to those without a history of brain trauma and moreover, distinct biomarker profiles (e.g., decreased serotonin or norepinephrine metabolites or Kreb’s cycle intermediates) were specifically related to symptom presentation. Overall, this work significantly contributes to our understanding of chronic alterations in energy metabolism and neurotransmitter dysfunction as contributing factors in the development of disruptive affective, somatic, and cognitive symptoms that linger past the acute phase of injury in certain groups of mTBI patients.

Disclosures: **A.N. Grossberg:** None. **L. Koza:** None. **C. Prusmack:** None. **D.A. Linseman:** None.

Poster

380. Understanding Traumatic Brain Injury (TBI)

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 380.02

Topic: C.10. Brain Injury and Trauma

Support: NHMRC APP1127007
NHMRC APP1104692

Title: Brain age in chronic traumatic brain injury

Authors: *G. SPITZ[1], A. HICKS[1], C. ROBERTS[1], C. C. ROWE[2], J. PONSFORD[1];
[1]Monash Univ., Melbourne, Australia; [2]Austin Hlth., Heidelberg, Australia

Abstract: Traumatic brain injury (TBI) is associated with greater ‘brain age’ that may be caused by atrophy in grey and white matter. Here, we investigated ‘brain age’ in a chronic TBI (over 10 years) sample. We examined whether ‘brain age’ increases with years post injury, and whether it

is associated with injury severity, cognition and functional outcome. We recruited 102 participants with moderate to severe TBI aged between 40 and 85 years. TBI participants were assessed on average 22 years post-injury. Seventy-seven healthy controls were also recruited. Participants' 'brain age' was determined using T1-weighted MRI images. TBI participants were estimated to have greater 'brain age' compared to healthy controls. 'Brain age' gap was unrelated to time since injury or long-term functional outcome on the Glasgow Outcome Scale-Extended. Greater brain age was associated with greater injury severity measured by post traumatic amnesia duration and Glasgow Coma Scale. 'Brain age' was significantly and inversely associated with verbal memory, but unrelated to visual memory/ability and cognitive flexibility and processing speed. A longitudinal study is required to determine whether TBI leads to a 'one-off' change in 'brain age' or progressive ageing of the brain over time.

Disclosures: G. Spitz: None. **A. Hicks:** None. **C. Roberts:** None. **C.C. Rowe:** None. **J. Ponsford:** None.

Poster

380. Understanding Traumatic Brain Injury (TBI)

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 380.03

Topic: C.10. Brain Injury and Trauma

Title: Heart rate variability is decreased in adults with mild traumatic brain injury

Authors: *E. BAT-ERDENE[1], E. TUMURBAATAR[1], G. TUMUR-OCHIR[2], M. DASHTSEREN[3], O. BYAMBASUKH[4], C. ERDENEBAATAR[1], A. BADARCH[5], T. JADAMBA[1], T. OKA[6], B. LKHAGVASUREN[6];
[1]Brain and Mind Res. Institute, Mongolian Acad. of Sci., Ulaanbaatar, Mongolia; [2]Mental Hlth., [3]Family Med., [4]Endocrinol., [5]Mongolian Natl. Univ. of Med. Sci., Ulaanbaatar, Mongolia; [6]Psychosomatic Med., Intl. Univ. of Hlth. and Welfare, Otawara, Japan

Abstract: Introduction: A mild traumatic brain injury (MTBI) may cause autonomic dysfunctions that contribute to increased mortality. However, the effects of MTBI on the autonomic functions with regard to mental health in the general population are less studied. We aimed to investigate the autonomic dysfunctions and psychological symptoms of those who reported a lifetime MTBI in the general population.
Methods: This cross-sectional study was conducted in the adult population of Ulaanbaatar in 2020. The dysfunction of the autonomic nervous system was evaluated by a noninvasive analysis of the heart rate variability (HRV). Anxiety, depression, sleep quality, and quality of life were examined using the Hospital Anxiety and Depression Scale (HADS), the Pittsburgh Sleep Quality Index (PSQI), and the brief version of World Health Organization Quality of Life (WHOQOL-BREF), respectively.
Results: A total of 177 participants (74% women) with a mean age of 39.85±9.82 years were enrolled. Among them, 16 participants reported a history of MTBI (prevalence: 9%). Body

temperature, heart rate, anxiety score, pNN50, and RMSSD were different between the participants with and without MTBI (P=0.028, P=0.039, P=0.04, P=0.021, and P=0.043, respectively). Overall, pNN50 was correlated with the WHOQOL-BREF domains (psychological: r=0.194, social: r=0.156, and environmental: r=0.204), and inversely correlated with age, BMI, waist circumference, and HADS total score (r=-0.215, r=-0.272, r=-0.218, and r=-0.159, respectively). The LF/HF was correlated with the neck and waist circumferences (r=0.229 and r=0.185) and inversely correlated with depression (r=-0.167). In participants with MTBI, the pNN50 was correlated with the systolic blood pressure (r=0.629), and inversely correlated with age and heart rate (r=-0.716, r=-0.582). In contrast, LF/HF was correlated with the neck circumference (r=0.650) and inversely correlated with depression (r=-0.567). Logistic regression analysis suggests that decreases in pNN50 and WHOQOL-BREF, and increases in body temperature and anxiety were the contributing predictors of MTBI.
Conclusion: Individuals with MTBI had decreased HRV and increased anxiety compared with the general population with no lifetime history of MTBI. Furthermore, these results suggest that MTBI negatively affects the autonomic functions, mental health, and quality of life.

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Poster

380. Understanding Traumatic Brain Injury (TBI)

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 380.04

Topic: C.10. Brain Injury and Trauma

Support: NINDS/NIA Grant U54NS115266

Title: Cortical Blood Vessel Microvascular Proliferation in Chronic Traumatic Encephalopathy and Alzheimer's Disease

Authors: G. A. ROSEN[1], D. KIRSCH[2], S. HOROWITZ[1], J. D. CHERRY[3], R. NICKS[4], H. KELLEY[4], R. MATHIAS[4], K. A. CORMIER[5], C. A. KUBILUS[4], J. MEZ[4], Y. TRIPODIS[4], M. ALOSCO[4], T. D. STEIN[6], V. E. ALVAREZ[1], A. C. MCKEE[4], *B. R. HUBER[7];
[1]VA Boston Healthcare Syst., Boston, MA; [2]Boston Univ., Boston, MA; [3]Pathology and Lab. Med., Boston Univ. Sch. of Med., Boston, MA; [4]Neurol., [5]Boston Univ. Alzheimer's Dis. Res. Ctr. and CTE Ctr., Boston, MA; [6]Boston VA Med. Ctr., Boston, MA; [7]VA Boston Healthcare, VA Boston Healthcare, Boston, MA

Abstract: Chronic traumatic encephalopathy (CTE) is a neurodegenerative tauopathy linked to repetitive head impact (RHI) exposure. In CTE, hyperphosphorylated tau (p-tau) pathology is primarily located at the cortical sulcal depths, where shear stress and deformation of the brain during impacts are greatest. Alzheimer's Disease (AD) is a more common tauopathy with a

laminar distribution of p-tau pathology distinct from CTE that affects both the gyrus and sulcus of the cortical grey matter. In CTE and AD, p-tau pathology is associated with neuroinflammation. Head impacts contribute to neuroinflammation at cortical sulcal depths where impact forces are concentrated. Tissue injury and neuroinflammation are associated with dysregulated angiogenesis. We hypothesize that evidence for dysregulated angiogenesis will be located primarily in the sulcus in CTE and in both the gyrus and sulcus in AD. To investigate vascular proliferation in these diseases, we used a modified version of the CLARITY method optimized for human brain tissue to optically clear and examine 200µm-thick sections of postmortem human dorsolateral frontal cortex (DLF). This region was chosen as it is affected early in CTE disease course and progressively accumulates pathology, and is also frequently affected in Braak stages V and VI in AD. Study participants from the VA-BU-CLF (UNITE) Brain Bank and VA National PTSD Brain Bank included those with CTE (high severity n=10, mean age=76 years , low severity n=10 mean age=66 years), comorbid CTE and AD (AD-CTE, n=5, mean age =71), AD with RHI exposure (n=4, mean age =76), as well as controls without AD or CTE that were RHI-exposed (n=8, mean age=58) and non-RHI controls (n=7, mean age=65). Passively cleared tissue was stained with fluorescently labeled tomato lectin allowing high-resolution observation of three-dimensional microvasculature morphology. We quantified vessel branch density and fraction volume, measurements that would be increased as a result of angiogenesis. The high CTE group branch density was significantly increased compared to non-CTE groups and fraction volume was increased compared to RHI-exposed controls at the DLF sulcal depths. The AD-CTE group showed increased branch density in both the gyrus and sulcus compared to pure CTE groups and controls. In CTE, AD, AD-CTE, and RHI-exposed controls, vessel fraction volume in the DLF sulcus correlated positively with both p-tau and CD68 staining density, an inflammatory microglial marker. Together, these findings suggest AD is associated with both sulcus and gyrus gray matter angiogenesis, while in CTE, chronic neuroinflammation may further increase vascular changes restricted to the sulcus.

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Poster

380. Understanding Traumatic Brain Injury (TBI)

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 380.05

Title: WITHDRAWN

Poster

380. Understanding Traumatic Brain Injury (TBI)

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 380.06

Topic: C.10. Brain Injury and Trauma

Support: The Dana Foundation
NIH R01NS123374
NIH R01NS123445

Title: Soccer heading and the microstructure profile of gray-white matter interface

Authors: *J. SONG, M. LIPTON, R. FLEYSHER;
Albert Einstein Col. of Med., Bronx, NY

Abstract: Repetitive subconcussive head impacts (RSHI) from soccer heading are an integral part of the sport, but high levels of heading are associated with adverse cognitive performance. Characterizing mechanisms underlying these adverse effects is a prerequisite to improving public health policies and developing protective and restorative interventions. Noninvasive imaging findings have demonstrated adverse associations of brain microstructure with high RSHI exposure. However, these studies have largely employed voxel-wise analyses. We applied a novel approach to assess hard-to-investigate gray-white matter interface by calculating slope of fractional anisotropy (FA) to analyze diffusion tensor imaging (DTI) collected from n=283 adult amateur soccer players (18-50; 38% women) enrolled in the Einstein Soccer Study. The slope of FA across the cortical gray-white interface (GWI) in each brain region was estimated as the slope of the 7^{th} degree polynomial fitted to the average FA value along the shortest Euclidean distance to the GWI (defined by FreeSurfer segmentation), which is always orthogonal to the GWI. The slope of FA was averaged separately across 6 different gray matter lobe regions: cingulate, frontal, occipital, orbitofrontal, parietal and temporal. Lower slope of FA was significantly associated with greater RSHI exposure in the orbitofrontal region (p=.0036), parietal region (p= 0.0212) and frontal region (p=0.0199). This supports our hypothesis that the microstructure profile of the GWI is adversely affected by RSHI exposure. The pattern of findings is consistent with microstructural pathology due to RSHI-induced shear force injury arising from the differential density of gray and white matter and preferentially affecting anterior and posterior brain regions likely to be subject to rapid acceleration-deceleration during heading.

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Poster

380. Understanding Traumatic Brain Injury (TBI)

Location: SDCC Halls B-H

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Program #/Poster #: 380.07

Topic: C.10. Brain Injury and Trauma

Support: NINDS U54 NS100064
NIH R01NS111744

Title: Comparative analysis of manual lesion segmentation methods in patients with traumatic brain injury

Authors: *A. BENNETT[1], C. ALBA[1], R. GARNER[1], M. D. MORRIS[2], M. LA ROCCA[3,1], G. BARISANO[1], R. CUA[4], J. LOON[1], P. CARBONE[1], P. VESPA[2], A. W. TOGA[1], D. DUNCAN[1]; [1]USC Stevens Neuroimaging and Informatics Inst., Los Angeles, CA; [2]David Geffen Sch. of Medicine, Univ. of California, Los Angeles, Los Angeles, CA; [3]Dept. Interateneo di Fisica "M. Merlin", Univ. degli studi di Bari "A. Moro", Bari, Italy; [4]Dept. of Radiology, USC Keck Sch. of Med., Los Angeles, CA

Abstract: Post traumatic epilepsy (PTE) is a debilitating disorder characterized by recurrent seizures more than one week after traumatic brain injury (TBI). Due to the heterogeneous nature of TBI, it is difficult to utilize automated methods in TBI patients. Therefore, manual segmentations provide ground truths for TBI analysis. Here, we report a methodology for a three-step manual segmentation process for the Epilepsy BioInformatics Study for Antiepileptogenic Therapy (EpiBioS4Rx) [1]. We compare the Dice similarity coefficients (DSC) of lesion segmentation masks after each review stage to quantify inter-rater variability. Finally, we investigate DSC differences for seizure and non-seizure groups.
The dataset consists of 129 subjects (100 male; 29 female, age=42.82 +/- 20.34, Glasgow Coma Scale= 7.98 +/- 3.99). Manual traces of parenchymal hemorrhagic lesions were completed on T2-Weighted-Fluid-Attenuated Inversion Recovery (T2-FLAIR) using ITK-SNAP [2]. Initial segmentations completed by student researchers underwent two reviews: first review by a team of staff researchers and medical doctors, and second review by a medical doctor with neuroradiology expertise.
DSC measures the overlap between two samples, and is often used for image segmentation comparison [3]. We compared DSC values for the following steps: 1) initial segmentation to first review, 2) first review to second review, and 3) initial segmentation to second review. We analyzed lesion core, edema, and total pathological lesion as separate regions of interest (ROI). All groups were tested using one-way ANOVA, and Tukey's HSD test was used for post hoc analysis. DSC values were significantly lower for the initial segmentation to first review than the first review to the second review for all ROIs ($p<0.05$). This establishes inter-rater reliability between the first and second reviewers. Further, DSC values for the initial segmentation to the second review were significantly different between lesion core and total lesion ($p<0.05$).
Seizure outcomes after two-year follow up were available for 70 patients. There was no significant difference between DSC values for the seizure group (n=21) and non-seizure group (n=49). ROIs at each step in the review process were compared using the Mann Whitney U test. These analyses provide insight into the robustness of manual segmentation processes used for PTE analysis. Our results suggest that the lesion core is a complex ROI to segment. The ground truths obtained from our manual segmentation process may help fine-tune automated methods to account for subtle complexities in TBI-induced lesions.

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Poster

380. Understanding Traumatic Brain Injury (TBI)

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 380.08

Topic: C.10. Brain Injury and Trauma

Title: Electroencephalogram classification of traumatic brain injury from nonspecific population using neural networks

Authors: *M. CAIOLA, M.-J. YE;
FDA, Silver Spring, MD

Abstract: Traumatic brain injury (TBI) presents a significant challenge affecting an estimated 2.5 million people annually. Current clinical scores can classify TBI by severity but not with enough sensitivity to detect mild TBI or monitor progression. Therefore, ongoing efforts seek alternative clinical assessment tools for TBI. EEG has advantages of being non-invasive, easy-to-use, portable, and cost effective. However, when applied to TBI research, EEG yields mixed results with some studies showing significant differences in EEG-based power spectra data between mild TBI and normal groups, while others report no such distinction. By comparing EEGs recorded between normal and TBI patients, our previous work using machine learning showed we could receive high accuracy classification between normal, TBI, and stroke, however it required the computation of >1300 precomputed features. Deep learning techniques give us the opportunity to surpass this accuracy without the restriction of a priori features, ultimately allowing us to find significant features we did not previously consider. The Temple University EEG Corpus consists of over 30,000 clinical EEG recordings and associated textual clinician report, however there is no concrete subpopulation of TBI patients. Using these clinical reports, we determined a small subset of subjects with recent TBIs and no other obvious comorbidities. This data was then used to train a Natural Language Processing model and classify the remaining subjects. We then trained a deep neural net consisting of short-time Fourier transforms and convolutional layers to classify a random subset (N=375) of this “ground truth” data with 50 subjects withheld for independent validation. We further augmented our training data by allowing a random 90 second piece of the signal to be trained each epoch, allowing for new variation and less over-fitting. Our deep-learning approach was able to surpass our previous machine learning approach in accuracy without any user supplied features, receiving an accuracy of 0.83 in unseen data compared to our SVM accuracy of 0.73. We investigated how changes to the signal can influence this accuracy, by occluding various windows in spatiotemporal space. We found that our model was most affected by changes to overall alpha (8-12 Hz) power. By occluding individual channels, we found changes in delta power (1-4 Hz) in channels F7-T3 and C4-T4 and changes in alpha power in channel C3-CZ can also affect model performance. Our study suggests that EEG deep learning can be a more powerful tool for TBI classification and can provide significant features to separate different neurological conditions without a priori assumptions.

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Poster

380. Understanding Traumatic Brain Injury (TBI)

Location: SDCC Halls B-H

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Program #/Poster #: 380.09

Topic: C.10. Brain Injury and Trauma

Support: #W81XWH-13-1-0095

Title: Amygdala Structural Abnormality as a Predictor of Post-Traumatic Stress Disorder Severity in Veterans with Mild Traumatic Brain Injury

Authors: *C. WANG[1], S. I. GIMBEL[2], M. L. ETTENHOFER[3];
[1]Psychiatry, UCSD, La Jolla, CA; [2]Traumatic Brain Injury Ctr. of Excellence, Def. Hlth. Agency, Naval Med. Ctr. San Diego, San Diego, CA; [3]Psychiatry, Univ. of California, San Diego Sch. of Med., SAN DIEGO, CA

Abstract: High rates of comorbidity of mild traumatic brain injury (mTBI) and post-traumatic stress disorder (PTSD) in the military population have been consistently reported. The comorbidity may involve a unique developmental trajectory and the neural mechanism remains understudied. It has been established that stress-induced abnormal neuroplasticity within the corticolimbic pathway may be associated with the neurobiology of PTSD. Emotional learning and memory are coordinated by the integration of sensory information within the basolateral amygdala and its projection to other brain areas. Subcortical structures of the limbic system involved in emotional processing, such as the amygdala, are especially vulnerable to biomechanical forces often causing mTBI. Preclinical studies suggest that traumatic injury may induce structural neuroplasticity within the amygdala, which may increase neural communication and alter information processing. Specifically, persistent dendritic remodeling (e.g. increase spine density, dendritic hypertrophy) of neurons in the amygdala post-mTBI is associated with anxiety-like behaviors in animal models. Previous clinical studies using structural neuroimaging to examine the amygdala as a potential biomarker for emotional deficits in patients with mTBI have produced conflicting results. The current study aims to explore amygdala volume as a predictor of PTSD severity in patients with chronic mTBI. We hypothesize that PTSD symptom severity post-mTBI is mediated by the enlargement of the amygdala due to increased neural communication altering information processing. Structural magnetic resonance imaging volumetric measurement and PTSD checklist for DSM-5 (PCL-5) were collected from 44 Active Duty Service Members (n=21 control, n=23 mTBI). Preliminary analyses showed that mTBI participants had significantly more severe PTSD symptoms than healthy control participants. Bilateral amygdala volume showed a trend of enlargement in patients with comorbid mTBI and more severe PTSD symptoms ($d = 0.306 \sim 0.329$), but this finding was not statistically significant. The current finding differs from some previous work with a larger sample size and effect size, which found significant amygdala enlargement in the cormorbid group. This suggests

further investigation with a larger sample size is needed to understand the relationship between amygdala structural alteration and PTSD in patients with mTBI.

Disclosures: C. Wang: None. **S.I. Gimbel:** A. Employment/Salary (full or part-time):; General Dynamics Information Technology. **M.L. Ettenhofer:** A. Employment/Salary (full or part-time):; Naval Medical Center San Diego, Traumatic Brain Injury Center of Excellence, Defense Health Agency.

Poster

380. Understanding Traumatic Brain Injury (TBI)

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

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Topic: C.10. Brain Injury and Trauma

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Title: Chronic postconcussive symptoms in mild TBI are associated with acute thalamic hyperconnectivity: prognostic and treatment implications

Authors: *R. E. WOODROW[1,2], S. WINZECK[4,1], A. I. LUPPI[1,2], I. R. KELLEHER-UNGER[1], V. F. J. NEWCOMBE[1], J. P. COLES[1], G. CENTER-TBI MRI SUB-STUDY PTS AND INVESTIGATORS[5], D. K. MENON[1,3], E. A. STAMATAKIS[1];
[1]Univ. Div. of Anesthesia, Dept. of Med., [2]Dept. of Clin. Neurosciences, [3]Wolfson Brain Imaging Ctr., Univ. of Cambridge, Cambridge, United Kingdom; [4]BioMedIA Group, Dept. of Computing, Imperial Col., London, United Kingdom; [5]Antwerp Univ. Hosp., Antwerp, Belgium

Abstract: Mild traumatic brain injury (mTBI) lacks prognostic tools and clinical support, as many individuals experience enduring postconcussive symptoms without sufficient prediction or rehabilitation. We aimed to characterise imaging-derived thalamic alterations in acute mTBI and their prognostic potential for chronic postconcussive symptoms, using multimodal neuroimaging to identify future treatment targets. Previous research shows particular thalamic vulnerability to injury, implicated in long-term outcome following severe TBI with preliminary postconcussive relationships. However, this is under-investigated in a large mild cohort with long-term outcomes. The Thalamus and its functional relationships to the rest of the brain were investigated using acute structural MRI and resting-state functional MRI from 108 mTBI participants and 76 healthy controls from European multicentre project CENTER-TBI. The mTBI cohort showed no

structural damage or history of previous concussion or neuropsychiatric condition, thus presenting the 'mildest' form of mTBI. We assessed thalamic change in terms of acute volume and functional connectivity, and its relationship to postconcussive symptoms and GOSE at 6 months post-injury. Such changes were further associated with neurochemical targets by correlation with healthy PET imaging. In this large cohort, 47% of mTBI participants show incomplete symptomatic or functional recovery at 6 months post-injury. We find vast functional, but not structural, changes of thalamic hyperconnectivity in mTBI across local and global measures, with nuclei-specific vulnerabilities in the left and right ventral anterior and right ventrolateral dorsal nuclei. These further differentiate participants with chronic postconcussive symptoms, not explained by general functional outcome, with greatest relationships to cognitive and emotional symptoms associated with noradrenergic and dopaminergic targets respectively. In a sub-cohort followed longitudinally at 6 and 12 months post-injury, thalamic connectivity showed time-dependent and outcome-dependent relationships. We therefore propose acute thalamic functional hyperconnectivity has prognostic potential for enduring postconcussive symptoms following mTBI, whereby functional imaging provides earlier markers of outcome than routine structural imaging. Given high rates of incomplete recovery even after mild TBI, thalamic function and multimodal imaging could guide vital drug development for chronic postconcussive symptoms.

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Poster

381. Human DRGs and iPSC Sensory Neurons

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 381.01

Topic: D.02. Somatosensation – Pain

Support: NS111929

Title: The epigenetic landscape of human dorsal root ganglia

Authors: *Ú. FRANCO-ENZÁSTIGA, P. RAY, J. MWIRIGI, S. V. NEERUKONDA, T. J. PRICE;
Sch. of Brain and Behavioral Sci., UTD, Richardson, TX

Abstract: Chromatin architecture influences transcriptional activation by fine-tuning the access of transcription factors and epigenetic remodelers to DNA. Although the transcription factors that are expressed in human dorsal root ganglia (hDRG) are known, the epigenetic landscape of human DRG cells, particularly putatively accessible transcription factor binding sites are largely unexplored. We performed the Assay for Transposase-Accessible Chromatin with sequencing (ATAC-seq) as an unbiased approach to capture the epigenetic landscape in hDRG from female and male healthy organ donors. To do this, we transposed a total of 100,000 nuclei per hDRG. Following DNA purification and PCR amplification of tagmented DNA using Illumina's Nextera-based adapters, we acquired paired-end sequencing reads on a Nextera500 sequencer. We identified ~100,000 ATAC-seq peaks per sample. We mapped these peaks to genomic regions, and found a higher proportion in intronic, and intergenic regions compared to promoter regions. We also identified various important pain-related genes that are accessible at steady-state in hDRG including *SCN9A*, *TRPV1/3*, *NTRK3* and *ISL1* suggesting that an important proportion of the ATAC-seq signal is neuronal. We further analyzed DNA consensus sites and detected SP4, a transcription factor that regulates pain-related genes in open chromatin regions. Additionally, we found peak differences in the promoter regions of various genes that we have previously shown to be enriched in a sexually dimorphic manner. Of these, *ISL2*, was accessible in the male data set, whereas *NGF* and *IL12A* were accessible in the female data set. This outcome suggests that some sexual dimorphisms are regulated at the level of chromatin accessibility. Collectively, this work provides novel insight into the gene regulatory mechanisms underlying neuronal and non-neuronal gene expression patterns in hDRG and enables future genome interventions to target pain in a sex-specific fashion.

Disclosures: Ú. Franco-Enzástiga: None. **P. Ray:** None. **J. Mwirigi:** None. **S.V. Neerukonda:** None. **T.J. Price:** None.

Poster

381. Human DRGs and iPSC Sensory Neurons

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 381.02

Topic: D.02. Somatosensation – Pain

Support: NS111929

Title: Mechanisms of axonal integrity and implications for peripheral neuropathies

Authors: *D. TAVARES FERREIRA[1], S. SHIERS[1], S. NEERUKONDA[1], I. SANKARANARAYANAN[1], P. RAY[1], A. CRAIN[1], M. KOTAMARTI[1], J. MWIRIGI[1], G. THOMAS[2], D. WUKICH[2], T. J. PRICE[1];
[1]Dept. of Neurosci., Univ. of Texas At Dallas, Richardson, TX; [2]Dept. of Orthopaedic Surgery, UT Southwestern Med. Ctr., Dallas, TX

Abstract: Sensory neurons are highly polarized cells with axons extending from the cell body for up to a meter or longer in most of the larger vertebrates. Thanks to the advancement of technologies such as electron and confocal microscopy and the development of high-throughput RNA-sequencing, recent studies clearly demonstrate the presence of translational machinery and mRNAs in peripheral axons. These studies also show that local translation of mRNAs in distal axons is important for axon growth, maintenance and regeneration and facilitates communication with the cell body. However, the majority of these studies have been conducted in rodents and the question of which mRNAs are localized to axons of human neurons has not been addressed. To address this in humans, we used human peripheral nerves collected from amputation surgeries combined with bulk-RNA and single-nuclei sequencing, in situ hybridization and Visium spatial transcriptomics. Most of our samples are from diabetic patients and show signs of neuropathy, such as axonal loss.In our human sural nerve datasets, we detect sensory genes with known function in pain such as *NTRK1* that are not expressed in non-neuronal cells present in sural nerves. This suggests that certain mRNAs may be transported to the peripheral sensory axon. Our data also shows several differences in gene expression across nerves with different axonal loss. We observe a decrease in Schwann cell and myelin genes in sural nerves with severe axonal loss. We also detect a difference in the expression of classic neuronal genes: *NMB* and *PRPH* are increased in nerves with severe axonal loss while *NTRK2, NTRK3, SCN9A* and *PIEZO2* are decreased.Next, we conducted an analysis of RNA-binding proteins (RBPs). We identified RBPs, such as the fragile X mental retardation protein (FMRP) and heterogeneous nuclear ribonucleoproteins (hnRNPs), in the sural nerve. These RBPs are predicted to bind to motifs present in mRNAs such as *SCN9A, NTRK1* and *TRPV1*.Ribosomal protein RPL10A, which is associated with actively translating ribosomes, is also detected in human sural nerves. In conclusion, we identified a subset of mRNAs that likely localize to the axons of human DRG neurons. We also identified RBPs and translational machinery in the axons of human sural nerves. Our preliminary data suggests that changes in mRNAs and RBPs could be associated with axonal loss severity. Maintaining the integrity of long axons is crucial and this work provides new, fundamental insight into mechanisms underlying peripheral neuropathies.

Disclosures: **D. Tavares Ferreira:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Doloromics. **S. Shiers:** None. **S. Neerukonda:** None. **I. Sankaranarayanan:** None. **P. Ray:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Doloromics. **A. Crain:** None. **M. Kotamarti:** None. **J. Mwirigi:** None. **G. Thomas:** None. **D. Wukich:** None. **T.J. Price:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Doloromics.

Poster

381. Human DRGs and iPSC Sensory Neurons

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 381.03

Topic: D.02. Somatosensation – Pain

Support: NS065926
NS111929

Title: Oncostatin M induces nociceptive signaling and satellite glial activation in human dorsal root ganglia

Authors: *J. MWIRIGI, S. SHIERS, P. RAY, D. TAVARES FERREIRA, I. SANKARANARAYANAN, Ú. FRANCO-ENZÁSTIGA, K. NATARAJAN, A. SHRIVASTAVA, S. BANDARU, T. J. PRICE;
Sch. of Brain and Behavioral Sci., Univ. of Texas At Dallas, Richardson, TX

Abstract: Oncostatin M (OSM) is one of the least studied cytokines in the interleukin-6 family especially considering that its expression correlates with hallmarks of chronic itch, rheumatoid arthritis, irritable bowel syndrome, and more recently neuropathic pain. This gap in knowledge is attributed to numerous species differences in the protein structure of OSM, and its receptor usage both of which affect physiological function. Here we uncover some of these discrepancies across mouse, rat, and human models, further underpinning the importance of studying OSM in human context. We characterized the receptors expression profile of OSMR in human dorsal root ganglia (hDRG) from healthy organ donors and confirmed its presence in small-diameter neurons and surrounding glial-like cells via RNAScope *in situ* hybridization. To investigate OSM-mediated signaling in hDRG, we treated acutely sliced explants with 10ng/ml OSM for 30min and immunoassayed with markers of translation regulation via the **M**itogen activated protein ki**N**ase interacting **K**inase (MNK) pathway and its downstream target **e**ukaryotic translation **I**nitiation **F**actor 4E (eIF4E). We noted significant increases in the p-eIF4E intensity signal in small-diameter neurons and glial-like cells suggesting that OSM activates MNK-eIF4E signaling in these cell types. Our findings cumulatively suggest that blocking OSM signaling in hDRG may attenuate nociceptive hyperexcitability and presents a viable therapeutic target for the treatment of pain.

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Poster

381. Human DRGs and iPSC Sensory Neurons

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Program #/Poster #: 381.04

Topic: D.02. Somatosensation – Pain

Support: NIH Grant R01NS111929

Title: Regulation of electrophysiological properties of human dorsal root ganglion neurons by apoA-I binding protein (AIBP)

Authors: *K. E. MCDONOUGH[1], Y. LI[1], M. L. UHELSKI[1], C. E. TATSUI[2], R. Y. NORTH[2], J. P. CATA[3], G. CORRALES[3], T. L. YAKSH[4], Y. I. MILLER[5], P. M. DOUGHERTY[1]; [1]Anesthesia and Pain Med., [2]Neurosurg., [3]Anesthesiol. & Perioperative Med., Univ. of Texas MD Anderson Cancer Ctr., Houston, TX; [4]Anesthesiol., [5]Med., The Univ. of California San Diego, La Jolla, CA

Abstract: The level of cell membrane cholesterol is critical to cell viability, growth, and proliferation, and is regulated by the rate of its biosynthesis and the internalization and degradation of lipoproteins from the plasma membrane. One key area of interest in this process centers on the role of cholesterol-rich membrane domains termed lipid rafts. The amount, size and function of lipid rafts can change rapidly in response to cell metabolic conditions, and the malfunction of lipid rafts plays a key role in the pathogenesis of hematopoietic, neurological, inflammatory, and infectious diseases, as well as that of cancer. Neuronal lipid rafts serve as a platform for the organization of many excitatory ion channels and receptors, and their disruption in neurons alters cellular excitability. A subtype of lipid rafts in which toll-like receptor 4 (TLR4) is localized has a specific and potent role in the regulation of neuroinflammation and pain. It was recently shown that Apolipoprotein A-I binding protein (AIBP) binds to TLR4 to augment cholesterol efflux from cell membranes, resulting in the disruption of lipid rafts in TLR4-expressing cells, and this effect appears to attenuate the generation of inflammatory and neuropathic pain. Human dorsal root ganglion (DRG) neurons that express nociceptor markers also express TLR4 and exhibit these lipid rafts, so the current study sought to examine whether AIBP could potentially alter the excitability of these cells. Action potential properties and excitability of cultured small-diameter human DRG neurons were analyzed before and after acute application of AIBP (0.1 μg/ml) to the extracellular bath solution using whole cell voltage- and current-clamp. The localization of AIBP was also examined. The results showed that AIBP treatment rapidly inhibited neuronal excitation and action potential (AP) generation in human DRG neurons. After AIBP, DRG neurons had lower AP amplitude and overshoot and greater AP rise time. Resting membrane potential was more depolarized and current thresholds lower after AIBP treatment. In spontaneously active DRG neurons, AIBP suppressed AP firing. Immunohistochemistry studies showed that AIBP is localized on human DRG neurons and satellite cells as well as infiltrating macrophages in patient DRGs that innervated dermatomes with radiculopathy. Based on the results of these studies and given that TLR4 expression is largely confined to nociceptors, AIBP may have potential as a novel pain therapeutic.

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Poster

381. Human DRGs and iPSC Sensory Neurons

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Topic: D.02. Somatosensation – Pain

Support: NIH Clinical Center Intramural Research Program
NINDS Intramural Research Program
NIH NCCIH Bench to Bedside Grant
NIH Office of Behavioral and Social Science Research Funding

Title: Peripheral Expression of Pain Insensitivity Genes in Human Dorsal Root Ganglion Neurons and Satellite cells

Authors: *M. R. SAPIO[1], A. P. MANALO[2], D. MARIC[3], E. S. STAEDTLER[1], A. J. MANNES[2], M. J. IADAROLA[2];
[1]NIH Clin. Ctr., Bethesda, MD; [2]Perioperative Med., Natl. Inst. of Health, Clin. Ctr., Bethesda, MD; [3]NINDS/NIH, Bethesda, MD

Abstract: Genetic pain insensitivity syndromes are rare diseases resulting in the inability to register pain or painful sensations. The most common and well-characterized underlying mechanisms of such syndromes are abnormalities in the peripheral nociceptive transduction apparatus. This can encompass lack of development of primary afferent nociceptors in the DRG, death of DRG neurons, loss of function in sensory transducing channels, or degradation of sensory axons. Loss of axonal function is further subcategorized into axonal degradation or demyelination, both of which lead to loss/impairment of signaling by the primary afferent nociceptive neurons. Using in situ hybridization to examine gene expression in the human DRG, we characterized the expression patterns of most known human pain insensitivity genes including their co-expression with known nociceptive markers, and expression patterns in neurons, Schwann cells and satellite glia. These pain insensitivity genes queried included PR/SET Domain 12 (*PRDM12*), With-No-Lysine Kinase 1 (*WNK1*), the Sphingosine Palmitoyltransferase Long Chain Base Subunits 1 and 2 (*SPTLC1* and *SPTLC2*), Atlastin 1 and 3 (*ATL1* and *ATL3*) and sodium channels NaV1.7 (*SCN9A*), NaV1.8 (*SCN10A*), and NaV1.9 *SCN11A*). Additionally, we examined genes such as the mu-opioid receptor (*OPRM1*) and Transient Receptor Potential Vanilloid 1 (*TRPV1*), which are expressed in nociceptive neurons that are also responsive to morphine analgesic agents. The co-expression profile of the canonical pain insensitivity gene Neurotrophic Receptor Tyrosine Kinase 1 (*NTRK1*), which when mutated can produce cell death of all nociceptive neurons and severe pain insensitivity, was also examined. All of the pain insensitivity genes were coexpressed with noci-responsive ion channels by in situ hybridization, and were robustly detected in human DRG using RNA-Seq. The co-expression analysis with noci-responsive ion channels showed a high degee of overlap, but for some genes co-expression also was observed with molecular markers of non-nociceptive neurons and satellite cells. This latter observation is consistent with the range of clinical symptoms described for the affected individuals and involvement of non-painful neurons and support cells in the pathophysiology of neuropathy.

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Poster

381. Human DRGs and iPSC Sensory Neurons

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 381.06

Topic: D.02. Somatosensation – Pain

Support: NIH CC IRP
NINDS IRP
Bench to Bedside NCCIH
OBSSR

Title: Molecular phenotyping of human DRG somatosensory neurons involved in nociception and analgesia

Authors: *M. IADAROLA[1], **E. S. STAEDTLER**[1], M. R. SAPIO[1], D. MARIC[2], A. GHETTI[3], A. J. MANNES[1];
[1]Dept. of Perioperative Med., NIH, Bethesda, MD; [2]NINDS/NIH, Bethesda, MD; [3]AnaBios, San Diego, CA

Abstract: Nociceptive input to the spinal cord is transmitted by primary afferent neurons in the dorsal root ganglia (DRG). Critically involved in this process are neurons expressing the heat- and inflammation-sensitive ion channel TRPV1. Clinical studies have shown that targeting these neurons peripherally with the ultrapotent TRPV1 agonist resiniferatoxin (RTX) results in a long-lasting functional blockage or even a chemoaxotomy and provides potent pain relief. At the same time, a subset of DRG neurons can mediate analgesia through expression of the μ-opioid receptor (encoded by *OPRM1*), resulting in attenuation of nociceptive transmission. The relationship between these two neuronal populations and their coexpression patterns with other transcripts involved in nociception and analgesia has not yet been evaluated in the human. In this study, we use high resolution multiplex fluorescence *in situ* hybridization to visualize molecular coexpression patterns of seven algesic (*TRPV1, TRPA1, TRPM8, TAC1, P2RX3, SCN10A, SCN11A*), four analgesic (*OPRM1, OPRD1, OPRK1, OPRL1*), and other markers of somatosensation (*PIEZO2, SPP1*). *TRPV1*-expressing neurons represent a large proportion of DRG neurons with a range of expression level (mean 83.5±3.3% - pooled data, four tissue donors, 2 F, 2 M, mean 22.5±3.1 years). When only neurons with moderate - high *TRPV1* expression are considered, they represent 71.3% of DRG neurons, which aligns with recent publications. The most common coexpression with *TRPV1* are DRG-specific voltage-gated Na^+ channels involved in nociception (*SCN11A* in 96.4%, and *SCN10A* in 94.6% of $TRPV1^+$ neurons), as well as P2X3 (coded by *P2RX3*), an ATP-gated ion channel expressed in 91.8% of $TRPV1^+$ neurons. The transcripts for opioid receptors show different expression profiles in DRG neurons. *OPRM1* is expressed in 58.3±1.5% of neurons, *OPRD1* in 51.1%, while *OPRK1* is

scarcely transcribed, being found only in 1.5% of neurons, but widely expressed in satellite glial cells. 65.8% of *TRPV1*$^+$ neurons coexpress *OPRM1*, and 57.8% *OPRD1*. Three main different opioid-receptor expressing *TRPV1*$^+$ populations could be identified: selective expression of either *OPRM1* or *OPRD1*, or coexpression of both opioid receptors. The *TRPV1*$^+$*/OPRM1*$^+$ neurons were the dominant population showing the highest levels of opioid receptor transcript. *TRPV1*$^+$*/OPRM1*$^+$ neurons show a high degree of coexpression of other algesic markers, thus emphasizing the central role of the μ-opioid receptor in the DRG-spinal nociceptive circuit. The present molecular characterization defines a population of DRG neurons that mediate both nociception and anti-nociception and are clinically relevant to human pain.

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Poster

381. Human DRGs and iPSC Sensory Neurons

Location: SDCC Halls B-H

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Program #/Poster #: 381.07

Topic: D.02. Somatosensation – Pain

Support: NCATS Grant 1UG3TR003149
NINDS Grant R01NS100788
NINDS Grant R01NS114018

Title: Global analysis of mRNA expression in human sensory neurons reveals eIF5A as a conserved target for inflammatory pain

Authors: ***P. R. SMITH**[1], R. CHASE[2], J. DE LA PENA[1], J. LAWSON[3], T.-F. LOU[2], A. D. STANOWICK[2], B. BLACK[4], Z. T. CAMPBELL[1];
[1]Dept. of Anesthesiol., Univ. of Wisconsin - Madison, Madison, WI; [2]Biol. Sci., Univ. of Texas at Dallas, Richardson, TX; [3]Dept. of Biomed. Engin., Univ. of Massachusetts Lowell, Lowell, MA; [4]Dept. of Biomed. Engin., Univ. of Massachusetts Lowell, Lowel, MA

Abstract: Nociceptors are a type of sensory neuron that are integral to most forms of pain. Targeted disruption of nociceptor sensitization affords unique opportunities to prevent pain. An emerging model for nociceptors are sensory neurons derived from human stem cells. Here, we subjected five groups to high-throughput sequencing: human induced pluripotent stem cells (hiPSCs) prior to differentiation, mature hiPSC-derived sensory neurons, mature co-cultures containing hiPSC-derived astrocytes and sensory neurons, mouse dorsal root ganglion (DRG) tissues, and mouse DRG cultures. Co-cultureof nociceptors and astrocytes promotes expression of transcripts enriched in DRG tissues. Comparisons of the hiPSC models to tissue samples reveal that many key transcripts linked to pain are present. Markers indicative of a range of neuronal subtypes present in the DRG were detected in mature hiPSCs. Intriguingly, translation factors were maintained at consistently high expression levels across species and culture

systems. As a proof of concept for the utility of this resource, we validated expression of eukaryotic initiation factor 5A (eIF5A) in DRG tissues and hiPSC samples. eIF5A is subject to a unique posttranslational hypusine modification required for its activity. Inhibition of hypusine biosynthesis prevented hyperalgesic priming by inflammatory mediators in vivo and diminished hiPSC activity in vitro. Collectively, our results illuminate the transcriptomes of hiPSC sensory neuronmodels. We provide a demonstration for this resource through our investigation of eIF5A. Our findings reveal hypusine as a potential target for inflammation associated pain in males.

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Poster

381. Human DRGs and iPSC Sensory Neurons

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 381.08

Topic: D.02. Somatosensation – Pain

Support: University of Texas at Dallas Center for Advanced Pain Studies SCI SEED award

Title: Electrophysiological properties of PIEZO1 mechanosensitive channels in human iPSC-derived dorsal root ganglia nociceptors

Authors: *R. GRANJA-VAZQUEZ[1], P. HAGHIGHI[1], J. PANCRAZIO[1], T. J. PRICE[2], V. TRUONG[3], P. WALSH[3];
[1]The Univ. of Texas at Dallas, Richardson, TX; [2]Sch. of Behavioral and Brain Sci., Univ. of Texas At Dallas Neurosci. Undergraduate Program, Richardson, TX; [3]Anatomi Corp, Minneapolis, MN

Abstract: To perceive and interact with our world the human body utilizes highly specialized sensors which are capable of transforming physical cues into electro-chemical signals which we then interpret as sight, sound, taste, smell, touch and proprioception. In contrast to the other specialized senses, touch is not organ specific as evidenced by the discovery of the mechanosensitive PIEZO receptors and its implication in both normal and pathological conditions in many organ-systems. In the context of touch and pain, there is evidence to suggest that there is an important role for PIEZO2 in the conversion of input into painful stimuli. The role for PIEZO1, however, has not been fully described. Here, we utilize a multi-well, microelectrode array (MEA) platform to interrogate human-induced pluripotent stem cell (hiPSC) nociceptors for the functional properties of PIEZO1. Nociceptors (RealDRG, Anatomic, Inc) were seeded on pre-coated electrode grids and matured during 3 weeks with media exchanged every other day at 50% volume with SensoMM. Electrophysiology recordings were acquired at day in vitro 21 and 23 with a Maestro Classic (Axion Biosystems, Atlanta, GA) at 12.5kHz sampling rate in a 5% CO2 environment. The signal was band pass filtered (single pole Butterworth, 300-5000Hz) and individual action potentials were identified with an adaptive

threshold of ± 5.5σRMS per electrode in AxIS software. Each recording session consisted of a 30 minute period of spontaneous activity documented at 37 °C followed by a 42 °C temperature ramp via the integrated heating plate. After one session, one group of wells (n=3, 48 electrodes) received a combination of cytokines mimicking the conditions of neuropathic pain, while a second group of wells (n=5, 80 electrodes) received vehicle and then, a second 30 minute recording with its subsequent 42 °C temperature ramp was documented. In a third recording, all wells received a 30µM concentration of the PIEZO1 agonist, Yoda1. We find that both groups responded to the agonist with a positive modulation of the AVG MFR (1.52 Hz, ±0.35 SEM, Cytokine+Yoda1 and 0.62 Hz, ±0.15 for Yoda1) with the cytokine group demonstrating an enhanced response (p=0.018, t-test) over the Yoda1-only group. We also confirm a positive modulation during temperature ramps (AVG MFR 1.6Hz, ±0.2 SEM) when compared to 37°C, suggestive of the functional expression of TRPV1 channels expected in nociceptors. Interestingly, we describe different populations of responsive electrodes which either responded during both thermal and chemical stimuli (n=31), or only responded to temperature (n=13) or Yoda1 (n=2) suggestive of the heterogeneous profile of these hiPSC nociceptors.

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Poster

381. Human DRGs and iPSC Sensory Neurons

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Program #/Poster #: 381.09

Topic: D.02. Somatosensation – Pain

Support: NIH Grant 5R01NS111929-02

Title: Regulation of electrophysiological properties in human dorsal root ganglion neurons by Tomivosertib (eFT508)

Authors: Y. LI[1], ***M. UHELSKI**[1], C. TATSUI[1], R. NORTH[1], G. CORRALES[1], J. CATA[1], K. MCDONOUGH[1], T. J. PRICE[2], P. DOUGHERTY[1];
[1]MD Anderson, Houston, TX; [2]Sch. of Behavioral and Brain Sci., Univ. of Texas At Dallas Neurosci. Undergraduate Program, Richardson, TX

Abstract: Tomivosertib (eFT508) is a potent, highly selective inhibitor of mitogen-activated protein kinase (MAPK) interacting protein kinase (MNK) 1 and 2. Translational changes induced by MNK signaling are involved in nociceptor sensitization, and inhibition of MNK signaling by

pharmacological or genetic manipulation attenuates hyperexcitability in rodent sensory neurons. The effects of eFT508 on the excitability of human dorsal root ganglion (DRG) neurons has not been elucidated, nor the mechanisms by which these effects occur. We acutely treated cultured human DRG neurons with eFT508 (25 nM, 30min) and analyzed action potentials properties and membrane excitability before and after treatment using whole-cell patch clamp electrophysiology. We also examined whether eFT508 could attenuate hyperexcitability induced by application of oncostatin M (10 ng/mL). We found that eFT508 significantly decreased the frequency of spontaneous action potential firing in human DRG neurons previously associated with spontaneous pain. Bath application of 25 nM eFT508 also reduced net current densities in DRG neurons from female, but not male patients. In neurons where the current threshold was <100 pA, resting membrane potential was significantly depolarized after a 30-minute treatment with eFT508, but this did not occur in neurons where the current threshold was >100 pA. Application of oncostatin M (10 ng/mL) induced spontaneous action potentials, an effect which was blocked by co-treatment with eFT508 (25 nM). Based on analysis of action potential characteristics, incubation of human DRG neurons with 25 nM eFT508 resulted in inhibition of fast tetrodotoxin-sensitive sodium currents, high threshold calcium channels, and activation of potassium currents. We postulate that eFT508 inhibits the excitability of human DRG neurons not at the ion channel translation level, but by direct modulation of membrane potential via inhibition of sodium and calcium voltage-gated channels and by activation of potassium voltage-gated channels. Treatment with eFT508 also prevented, but did not reverse, paclitaxel-induced inhibition of neurite outgrowth and degeneration. Our results are in line with eFT508 findings from rodent sensory neurons and suggest this compound may prove to be a useful pain therapeutic.

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Poster

381. Human DRGs and iPSC Sensory Neurons

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 381.10

Topic: D.02. Somatosensation – Pain

Support: NIH Grant 5T32GM132006-02
NIH Grant NS109936 R01

Title: Comparative analysis of Gs coupled receptor signaling cascades in mouse and human sensory neuron models

Authors: *Z. AL-ABBASI[1,2], R. GEGUCHADZE[1], B. J. HARRISON[1], D. C. MOLLIVER[1]; [1]Biomed. Sci., Univ. of New England, Biddeford, ME; [2]Grad. Sch. of Biomed. Sci. and Engin., Univ. of Maine, Orono, ME

Abstract: Evaluation of target translatability from rodent to human is a major challenge to advancing novel pain therapeutics into the clinic. Significant efforts have been made to characterize human dorsal root ganglion (DRG) sensory neurons and to use human tissues for basic research where feasible. We report here an ongoing project to understand molecular mechanisms initiated by Gs-coupled receptors (GsPCRs) that sensitize nociceptors, requiring large amounts of protein samples to characterize cAMP-mediated signaling pathways. To analyze receptor signaling in human cells, we are characterizing GsPCRs in the human male DRG neuron-derived cell line (HD10.6), which upon differentiation, displays a neuron-like fate with some characteristics of nociceptors, including expression of the ion channels TRPV1 and P2X3. We have systematically analyzed GPCR expression in published RNA-seq datasets from mouse and human DRG to identify GsPCRs highly expressed either in both species (potentially translationally relevant) or in human only (requiring analysis in human cells). We have observed through PCR validation an apparent underrepresentation of some GPCRs that may lead to the false exclusion of some receptors from further study, which others have also observed. Several receptors that are highly expressed in human DRG show low or no expression in mouse, including the only Gs-coupled purinergic receptor, P2YR11 (a gene absent in rodents), tachykinin receptor TACR2, lysophosphatidic acid receptor LPAR6, calcitonin gene-related peptide/adrenomedullin receptor CALCRL and beta-adrenergic receptor ADRB2. Other receptors are highly expressed in both species, including the prostaglandin receptors PTGIR, PTGDR, and PTGER2/4. All of these receptors are also expressed in HD10.6 cells. Next, we examined cAMP signaling pathways mediated by protein kinase A (PKA) or Rap guanine nucleotide exchange factors (RAPGEF/EPAC)) in mouse and human cells. We profiled total, PKA- or PKC-dependent phospho-protein by gel electrophoresis after stimulating cells with receptor agonists, including NF546 (P2YR11), neurokinin A (TACR2), isoproterenol (ADRB1/2), beraprost and PGE2 (prostaglandin receptors). Banding profiles differed across species and showed several bands unique to individual receptors. Thus, the results demonstrate the existence of some cAMP signaling components that differ between mouse DRG and HD10.6 cells. We propose that HD10.6 cells, in combination with human DRG RNA-seq data, provide a valuable resource to investigate intracellular signaling mechanisms involved in nociceptor sensitization in cells closely related to human nociceptors.

Disclosures: Z. Al-Abbasi: None. **R. Geguchadze:** None. **B.J. Harrison:** None. **D.C. Molliver:** None.

Poster

381. Human DRGs and iPSC Sensory Neurons

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 381.11

Topic: D.02. Somatosensation – Pain

Support: AHA Predoctoral Fellowship 828671

Title: Bradykinin receptor expression and functional modulation in human sensory neurons

Authors: *J. YI, Z. BERTELS, J. DEL ROSARIO, R. SLIVICKI, A. WIDMAN, M. PAYNE, H. SUSSER, R. W. GEREAU, IV;
Washington Univ. in St. Louis, Saint Louis, MO

Abstract: Bradykinin (BK), an inflammatory mediator, has been implicated in the pathogenesis of pain in both humans and rodents. At the cellular level, BK acts on G-protein coupled receptors (GPCR) to induce hyperexcitability in sensory neurons, which is thought to contribute to BK-induced allodynia and hyperalgesia in rodents. However, there is increasing evidence that suggests that human and rodent dorsal root ganglia (DRG) differ in expression and function of key GPCRs and ion channels, which may preclude the successful translation of preclinical analgesic targets to clinical therapy. Here, we used human DRG obtained from postmortem organ donors to characterize the distribution pattern of BK receptors in human sensory neurons. Our results indicate that BK receptors are expressed in both neuronal and glial subpopulations in the human DRG. Additionally, path clamp electrophysiology studies suggest that acute and prolonged application of BK modulates the excitability of various physiologically distinct subpopulations of human sensory neurons in vitro. The results of the study may help evaluate BK as a target for therapeutics development.

Disclosures: J. Yi: None. **Z. Bertels:** None. **J. del Rosario:** None. **R. Slivicki:** None. **A. Widman:** None. **M. Payne:** None. **H. Susser:** None. **R.W. Gereau:** None.

Poster

381. Human DRGs and iPSC Sensory Neurons

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 381.12

Topic: D.02. Somatosensation – Pain

Title: Human Induced Pluripotent Stem Cell-Derived Nociceptors on an Automated Patch Clamp System for High Throughput Pain Drug Discovery

Authors: T. STRASSMAIER[1], A. RANDOLPH[1], ***V. TRUONG**[2], R. HAEDO[1], P. WALSH[2];
[1]Nanion Technologies, Livingston, NJ; [2]Anatomic Inc., Minneapolis, MN

Abstract: There is still an unmet need for novel non-addictive pain analgesics as the opioid epidemic continues. The ability to functionally screen target compounds on human nociceptors in high throughput would increase both the efficiency and pace of preclinical pain drug discovery. We have previously demonstrated that human nociceptors can be generated in an accelerated, scalable method from human induced pluripotent stem cells (hiPSCs). These hiPSC-derived nociceptors (RealDRG™) share similarities to human dorsal root ganglia from a whole-transcriptome perspective and possess functional voltage and ligand-gated channels important for nociception via manual patch clamp. In this study, we have further developed on a novel dissociation method to electrophysiologically interrogate multiple ion channels in RealDRG™ cultures on the Nanion SyncroPatch 384 automated patch clamp system at 14, 21 and 28 days in

culture in high throughput 384 well format. The expression and properties of voltage-gated sodium (Nav) and potassium ion channels (Kv), transient receptor potential vanilloid 1 (TRPV1), GABA, and P2X ligand-gated ion channels, along with excitability properties in current clamp mode (resting membrane potential, spontaneous and evoked action potentials) were all explored. The percentage of cells with at least one evoked action potential increased from 42% to 77% over the course of maturation, though success rates decreased from 56 to 35% as the cells matured. There was also an increase in voltage-gated sodium (Nav) and potassium (Kv) currents as time progressed with success rates ranging from 94-98% and 93-100%, respectfully. Most neurons had tetrodotoxin-resistant (TTXr) sodium currents, with the trends of increasing number of cells with current and fraction of TTXr current per cell. A ligand puff protocol was developed to examine ligand-gated GABAR and P2X ion channels and responses to ATP and GABA currents were observed. Together, these findings demonstrate the ability to functionally screen multiple different targets in human nociceptors in a high throughput system.

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Poster

381. Human DRGs and iPSC Sensory Neurons

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 381.13

Topic: D.02. Somatosensation – Pain

Support: DOD (CDMRP) grant 13113162
Tonix Pharmaceuticals

Title: In Vitro Impact of Oxytocin on Human Sensory Neurons

Authors: *D. C. YEOMANS[1], V. BHARADWAJ[2];
[1]Stanford Univ., [2]Anesthesiol. and Pain Med., Stanford Univ., Stanford, CA

Abstract: *Background*: We and others have shown that oxytocin has potential as a non-opioid analgesic and, in fact clinical studies have confirmed this. Likely underlying this effect, we and others have shown that, when applied to rodent sensory neurons, oxytocin produces clear inhibition of excitability. In addition, we have shown that magnesium ions bind to a pocket in the oxytocin receptor which allows for greater potency of oxytocin as well as greater maximal inhibitory efficacy. However, all of this work has been performed either in transfected HEK cells or ex vivo in rodent dorsal root or trigeminal ganglia neurons. The purpose of this study is to determine whether similar phenomena are observable with human sensory neurons. The purpose of this study was to determine the impact of oxytocin plus or minus magnesium on human

derived dorsal root ganglia neurons. *Methods*: After enzymatic treatment and dissociation of neurons from human cadaveric DRGs, cells were mounted on coverslips and subjected to current clamp using standard methodology (10-15 cells per group). Prior to recording, cells were immersed overnight in a bath containing 500 nM IL-6 - which has been shown to increase expression of oxytocin receptors.!. Baseline rheobase and resting membrane potential (RMP) and pacing (response to repeated suprathreshold current injections) were assessed to determine excitability. 2. Bath fluid was exchanged for fluid containing 10 uM OT and these measures repeated after 5 min. 3. Bath fluid was exchanged for solution containing 10 uM OT in either minimal (0.5 mM) or high (1.75 mM) Mg^{2+} citrate buffer; after 5 min, excitability measures were reassessed. 4. In separate cells, the effect of 1.75 mM Mg^{2+} alone was assessed similarly. Coverslips were then immersed in 4% paraformaldehyde for fixation and processed for immunofluorescence to assesses neurons for immunoreactivity for oxytocin receptors. *Results and Significance:* While neither 10 uM oxytocin in a 0.5 mM Mg^{2+} buffer nor 1.75mM Mg^{2+} buffer alone had no significant effect on neuronal excitability, 10 uM OT, in the presence of a 1.75 mM Mg^{2+} buffer significantly reduced excitability as determined by increases in rheobase and RMP and by decreases in pacing. Normal physiologic range of blood Mg^{2+} is 0.75-0.95; thus, oxytocin, in a supraphysiologic concentration of Mg^{2+} produces robust inhibition of sensory neuron excitability. These results are consistent with our previous recordings in rat trigeminal ganglia wherein 1.75 mM Mg^{2+} proved to be maximal in terms of driving oxytocin's impact on sensory neurons. In addition, the presence of immunoreactive oxytocin receptors on DRG neurons was confirmed microscopically.

Disclosures: D.C. Yeomans: A. Employment/Salary (full or part-time):; Stanford University. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; ending Grant NS131176; DOD grant 13113162. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); A. D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); NA. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock Ownership: Tonix Pharmaceuticals; SiteOne; NewBio, Cyrtoinics: Royalties from USPatent 8,198,240. F. Consulting Fees (e.g., advisory boards); Tonix Pharmceuticals. Other; NA. **V. Bharadwaj:** None.

Poster

381. Human DRGs and iPSC Sensory Neurons

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 381.14

Title: WITHDRAWN

Poster

381. Human DRGs and iPSC Sensory Neurons

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 381.15

Topic: D.02. Somatosensation – Pain

Title: Pharmacology of TTX-resistant and TTX-sensitive sodium currents in non-human primate dorsal root ganglion sensory neurons

Authors: D. LIU, C. TIAN, A. HAZAN, M. SADEGHI, C. M. PETROSKI, ***R. E. PETROSKI**;
Neuroservice USA, Neuroservice USA, San Diego, CA

Abstract: Chronic pain is poorly managed by current therapies, and the current epidemic of opiate addiction has highlighted the need for alternative therapies for treating pain. Genetic and functional studies have established an important role of voltage-gated sodium channels in human pain disorders. Tetrodotoxin-sensitive (TTX-S) and tetrodotoxin-resistant (TTX-R) sodium channels expressed in dorsal root ganglion (DRG) sensory neurons are currently being pursued as promising therapeutic targets for treating human pain. Many drug discovery programs rely on pharmacology data from rodent neurons for generating the structure-activity relationships (SAR) during lead optimization. Frequently, the pharmacological activity of compounds on rodent targets differs from their human orthologs. This has been found to be true for voltage-gated sodium channels in rodent DRGs and poses a challenge for identifying the best pre-clinical candidates to advance to clinical trials. Here we describe the development of an assay of TTX-R and TTX-S sodium channels from non-human primate DRG sensory neurons. DRGs were harvested from adult cynomolgus monkeys. The animals were humanely euthanized in an AAALAC accredited facility using IACUC approved protocol to minimize pain and suffering. The tissue was dissociated using enzymatic and mechanical methods and individual sensory neurons were plated on poly-D-lysine and laminin coated glass coverslips in Neurobasal/B27 medium supplemented with NGF (25 ng/ml). Whole cell patch clamp recordings were conducted on sensory neurons at 1, 2, and 3 days in vitro. The composition of the external recording solution was: 110 mM choline-Cl, 30 mM NaCl, 2.5 mM KCl, 2 mM $CaCl_2$, 1.3 mM $MgCl_2$, 10 mM glucose,10 mM HEPES pH 7.3. The internal recording solution composition was: 70 mM CsCl, 70 mM CsF, 3 mM $MgCl_2$, 5 mM EGTA, 0.5 mM $CaCl_2$, 4 mM Na_2-ATP, 0.3 mM Li-GTP, 10 mM HEPES pH 7.3. Upon establishing the whole cell configuration, we measured the passive membrane properties for every cell (Cm, Rm, Ra). TTX-R sodium currents were measured after the TTX-sensitive sodium currents were blocked with 0.5 uM TTX. We show the concentration-response data from over fifteen compounds on TTX-R sodium currents from NHP DRG sensory neurons. In addition, we compare the activity of several of the compounds on TTX-R sodium currents from dog and rodent DRG sensory neurons. These results reveal the species differences in compound activity on native sodium channels.

Disclosures: **D. Liu:** A. Employment/Salary (full or part-time):; Author is an employee of Neuroservice USA, a contract research organization (CRO) providing functional data to support drug discovery programs. **C. Tian:** A. Employment/Salary (full or part-time):; Author is an employee of Neuroservice USA, a contract research organization (CRO) providing functional

data to support drug discovery programs. **A. Hazan:** A. Employment/Salary (full or part-time):; Author is an employee of Neuroservice USA, a contract research organization (CRO) providing functional data to support drug discovery programs. **M. Sadeghi:** A. Employment/Salary (full or part-time):; Author is an employee of Neuroservice USA, a contract research organization (CRO) providing functional data to support drug discovery programs. **C.M. Petroski:** A. Employment/Salary (full or part-time):; Author is an employee of Neuroservice USA, a contract research organization (CRO) providing functional data to support drug discovery programs. **R.E. Petroski:** A. Employment/Salary (full or part-time):; Author is an employee of Neuroservice USA, a contract research organization (CRO) providing functional data to support drug discovery programs..

Poster

381. Human DRGs and iPSC Sensory Neurons

Location: SDCC Halls B-H

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Program #/Poster #: 381.16

Topic: D.02. Somatosensation – Pain

Support: R01 CA231396
John J. Bonica trainee fellowship

Title: The role of the overexpressed protease, MMP1 in the activation of PAR1 and PAR2 receptors in oral cancer pain

Authors: *P. RAMIREZ GARCIA, Z. DUBEYKOVSKAYA, T. NGUYEN, B. L. SCHMIDT, D. ALBERTSON;
Bluestone Ctr. for Clin. Res., NYU Col. of Dent., New York, NY

Abstract: Oral cancer pain is the most prevalent and severe pain of all cancers [1,2]. Despite its severity, there is no effective and lasting treatment available to alleviate most forms of cancer pain. Moreover, patients with metastatic oral cancer (N+) experience greater pain than node-negative patients (N0) [3]. Recent work identified 40 genes overexpressed in N+ oral cancers from patients reporting high pain relative to N0 cancers and normal tissue [4], among them is Matrix metalloproteinase 1 (MMP1), an established (oral) cancer oncogene. Although the role of MMP1 in oral cancer pain is not established, a functional polymorphism in MMP1, associated with pain in non-cancer pain conditions [5], supports MMP1 as a pain mediator. Additionally, we observed that intraplantar administration of MMP1 in mice induces mechanical allodynia in a dose-dependent manner. MMP1 cleaves the N-terminus region of protease-activated receptor-1 and 2 (PAR_1 and PAR_2) [6-8] to expose a tethered ligand that can either activate or disarm the receptor by engaging with G-proteins. PAR_1 and PAR_2 are also found in nociceptive neurons and have a recognized role in pain [9,10]. Our hypothesis is that the overexpression of MMP1 in the cancer microenvironment may contribute to oral cancer-induced pain, by activation of PAR_1 and PAR_2. CRISPR PAR_1-KO and CRISPR PAR_2-KO HEK293 cell lines were used to study MMP1 (human recombinant) activation of PAR_1 and PAR_2 in isolated systems. Bioluminescence

resonance energy transfer (BRET) assays were used to examine PAR_1 and PAR_2 coupling to G-proteins ($G_{\alpha q}$, $G_{\alpha s}$, $G_{\alpha i}$ and $G_{\alpha 12/13}$). We found that MMP1 stimulation does not induce G-protein coupling to PAR_2. On the contrary, MMP1 triggers G-protein decoupling, suggesting a pre-assembly of PAR_2 with G-proteins, that is disengaged after MMP1 stimulation.The trafficking of PAR_2 from the plasma membrane (KRAS) to endosomes (Rab5) was examined by BRET assays. After MMP1 stimulation, PAR_2 distances from the plasma membrane, indicating internalization. However, PAR_2 does not engage with the endosomal Rab5 protein, present in early endosomes, suggesting that MMP1-activated PAR_2 could be internalized via a Rab5-independent mechanism. To examine the downstream signaling of PAR_2, we measured calcium influx using Fura-2/AM, and the CAMYEL BRET sensor to measure cAMP production. We observed an influx in Ca^{2+} triggered by MMP1, which was partially abolished when cells were preincubated with AZ3451 (PAR_2 antagonist). Interestingly, MMP1 triggered cAMP production, but this increase was not abolished by AZ3451. This data suggests that MMP1 may trigger the activation of multiple effectors and is not limited to PAR_2.

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Poster

381. Human DRGs and iPSC Sensory Neurons

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Program #/Poster #: 381.17

Topic: D.02. Somatosensation – Pain

Title: Proteostasis in Human Sensory Neurons: A Baseline for Modulation of Therapeutic Targets and Evaluation of Off-Targets

Authors: *P. WALSH[1], L. REICHART[2], V. TRUONG[1], A. KOPKE[2], H. HAHNE[2];
[1]Anatomic Inc., Minneapolis, MN; [2]OmicScouts GmbH, Freising, Germany

Abstract: Unlike the genome, the proteome is a highly dynamic system where multiple factors contribute to protein expression, but also protein stability including conformational states, protein-ligand interactions, and post-translational modifications. Recently, there have been an increasing number of novel therapeutics in the clinic that are developed to modulate target protein amounts. In the pain therapeutics field, many are developing similar strategies targeting sensory neurons. Sensory neurons convey various intrinsic and environmental cues related to actual or potential harm from the body to the central nervous system. Though the sensation of pain is helpful in survival[AK1] , chronic pain states are detrimental to quality of life. To better understand chronic pain and develop next-generation analgesics, a better understanding of proteomic changes in human sensory neurons is needed. In this investigation, we aimed to comprehensively define the baseline proteome of human induced pluripotent stem cell (hiPSC) derived sensory neurons. These sensory neurons are molecularly similar to primary human dorsal root ganglion and functionally respond to pain-specific compounds including capsaicin. Using

TurnoverScout™ proteome dynamics profiling, hiPSC-derived sensory neurons were cultured in Stable Isotope Labeled Amino acid Culture (SILAC) medium, allowing for the distinction between newly synthesized proteins from previously established proteins by mass spectrometry. The technology identified and quantified 7,792 proteins and their turnover rate in cultures of sensory neurons. The degradation and re-synthesis cycle of common pain targets, including the voltage gated sodium channels sub-types NaV1.7 and NaV1.8, ligand-gated purinergic receptors P2RX, mechanosensitive ion channel protein Piezo2, and the neuronal-type (N-type) voltage-gated calcium channel sub-type CaV2.2 were all identified. This study provides information on common pain target protein half-lives to support treatment strategy decisions including translation interference by siRNA or RNAi versus therapeutic target degradation using PROTACs or degraders. This ethical and human system can be used to investigate the effect of chronic pain stimuli, as well as therapeutic outcome of novel treatments on target abundance. It also allows for the testing of candidate drug selectivity and consequential regulatory effects that might reduce therapeutic efficacy. In summary, human sensory neuron proteome investigations offer insights into pain mechanisms and their possible reduction, all without the use of laboratory animals.

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Poster

381. Human DRGs and iPSC Sensory Neurons

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Program #/Poster #: 381.18

Topic: D.02. Somatosensation – Pain

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National Institute of Neurological Disorders and Stroke R01NS119476
National Institute of Drug Abuse DP1DA054343
Teva Pharmaceuticals
BWH Women's Brain Initiative

Title: Harmonized multi-species cell atlases of somatosensory ganglia

Authors: *S. BHUIYAN[1], M. XU[1], L. YANG[1], K. I. PANTALEO[2], W. RENTHAL[1]; [1]Brigham and Women's Hosp. & Harvard Med. Sch., Boston, MA; [2]Brigham and Women's Hosp. & Emmanuel Col., Boston, MA

Abstract: The trigeminal ganglion (TG) and dorsal root ganglion (DRG) allows mammals to sense the environment through touch, temperature, and pain, and these ganglia are comprised of a heterogeneous mixture of specialized neuronal and non-neuronal subtypes. The field has recently exploded with DRG and TG single-cell RNA-sequencing (scRNA-seq) studies to understand somatosensory cell development, regulation, and transcriptional response to injury. Currently, each study exists as separate scRNAseq datasets and each study has a different framework to annotate subtypes. Unifying these studies into one cell atlas would remove study-specific batch effects, validate cell identities across different studies, and characterize the rarer subtype-specific transcriptomes. To that end, we have integrated 14 DRG and 5 TG scRNA-seq mammalian studies using two independent computational approaches. We developed a hierarchical subtype annotation framework to manually annotate all clusters in each computational approach. We identified 17 neuronal and 14 non-neuronal subtypes across all studies, with ~85-93% agreement between cell type annotations using both tools. These harmonized cell atlases include 137,039 DRG cells and 153,152 TG cells. We then use these reference atlases to rapidly anchor snRNA-seq human DRG data and Drosopholid data to explore somatosensory cell type conservation between vertebrates and invertebrates. Finally, we provide our cell atlases as an Azumith web interface where future investigators can anchor their own data. Our work represents the first harmonized cell atlases for mammalian somatosensation, and our atlases will help the field dissect the genetic mechanisms responsible for maladaptive somatosensation such as chronic pain.

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Poster

381. Human DRGs and iPSC Sensory Neurons

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Topic: D.02. Somatosensation – Pain

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Title: Sustained depolarization induces homeostatic plasticity in mouse and human sensory neurons

Authors: *J. DEL ROSARIO[1], L. A. MCILVRIED[1], M. Y. PULLEN[1], A. WANGZHOU[2], T. D. SHEAHAN[1], R. A. SLIVICKI[1], T. J. PRICE[2], B. A. COPITS[1], Z. BERTELS[1], A. CHAMESSIAN[1], J.-N. LI[1], A. WIDMAN[1], J. YI[1], R. W. GEREAU[1];
[1]Washington Univ. in St. Louis, St. Louis, MO; [2]Univ. of Texas at Dallas, Dallas, TX

Abstract: Remodeling of the central nervous system (CNS) serves as a protective mechanism during abrupt disturbance of neuronal homeostasis. This includes both synaptic scaling and regulation of intrinsic neuronal excitability, an adaptive process known as homeostatic plasticity, to restore neuronal function and offer adequate maintenance of overall network activity. At the peripheral nervous system (PNS), alterations in neuronal excitability has been associated with the development of different forms of chronic pain. However, whether the involvement of homeostatic plasticity, in the context of prolonged depolarization of nociceptors or as a checkpoint to interfere in the pathophysiology of chronic pain, is poorly understood. Using a combination of pharmacological, and electrophysiological approaches, we show that sustained depolarization of cultured dorsal root ganglia (DRG) neurons, especially nociceptors, strongly reduces intrinsic excitability of mouse and human DRG neurons. These changes include decreases in input resistance, action potential (AP) fall time, AP half width, and an increase in rheobase associated with an overall decrease in AP firing, thus suggesting that mechanisms of homeostatic plasticity are engaged in mouse and human sensory neurons. Moreover, using voltage clamp experiments, we found that while voltage-gated potassium currents are not markedly affected after sustained depolarization, voltage-gated sodium currents are robustly inhibited contributing to the decrease in neuronal excitability and potentially serving as regulatory mechanism to drive homeostatic control of mouse sensory neurons. Our data reveal the presence of intrinsic homeostatic mechanisms that regulate neuronal excitability in response to sustained depolarization of mouse and human sensory neurons. Understanding the intrinsic molecular regulation of peripheral sensory neurons to noxious stimuli could help us dissect the mechanisms engaged during acute pain and the mechanisms lost or altered during the development of chronic pain.

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Poster

381. Human DRGs and iPSC Sensory Neurons

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Program #/Poster #: 381.20

Topic: D.02. Somatosensation – Pain

Title: A novel human dorsal root ganglion (hDRG) inflammatory pain model that detects neuronal spontaneous activity to facilitate drug development via a predictive machine learning algorithm

Authors: *A. GHETTI[1], Y. MIRON[1], J.-M. PACLEB[1], J. LIAW[1], F. CHEN[1], K. CARLIN[1], N. JUNG[2], H. ZHOU[3], J. SKOLNICK[3];
[1]AnaBios, San Diego, CA; [2]Karlsruhe Inst. of Technol., Karlsruhe, Germany; [3]Georgia Inst. of Technol., Atlanta, GA

Abstract: The effective treatment of pain remains a significant challenge affecting a large portion of the population. This is largely due the lack of safe and effective drugs to treat the various human pain conditions. Unfortunately, the effort to develop newer and more effective drugs is hampered by fundamental issues including the fact that there is an absence of preclinical models that accurately translate the requisite animal testing into actual human pain physiology. It is generally agreed that pain signaling is a complex process that often begins in the peripheral regions of an organism and follows a converging path of integration and modification until the signal is interpreted as pain in the brain. One of the initial sites of this signal convergence and processing is in the dorsal root ganglia (DRG), but the often-used rodent models have poor success for pain drug discovery, questioning its translatability to humans.As such, we propose a human ex vivo model of primary sensory neurons in culture isolated from organ donors. In this model, cultured hDRG are loaded with a calcium indicator and monitored by their fluorescence. To mimic an inflammatory state, cells are exposed to bradykinin and PGE2 altering the quiescent state of these neurons to a spontaneous firing of action potential detected by Fluo-8. The induced state is reproducible across donors and cultures. This induced inflammatory state is sensitive to common analgesics and anti-inflammatory drugs. Finally, there is flexibility for longer exposure to test compounds; as such, it can be a high throughput screening system.The utility of this novel model was demonstrated by using it in a small drug discovery screen. Using a novel machine learning algorithm, a small library of compounds was identified for high probability of non-addictive analgesic activity. This library was screened using our novel inflammatory model and resulted in the identification of 3 possible candidates with analgesic potency. Taken together, these results demonstrate that this hDRGs inflammatory model is a simple, robust, and flexible compound screening/profiling tool and can find a place in the pain drug discovery process given its obvious translational potential.

Disclosures: **A. Ghetti:** A. Employment/Salary (full or part-time):; AnaBios. **Y. Miron:** A. Employment/Salary (full or part-time):; AnaBios. **J. Pacleb:** A. Employment/Salary (full or part-time):; AnaBios. **J. Liaw:** A. Employment/Salary (full or part-time):; AnaBios. **F. Chen:** A. Employment/Salary (full or part-time):; AnaBios. **K. Carlin:** A. Employment/Salary (full or part-time):; AnaBios. **N. Jung:** None. **H. Zhou:** None. **J. Skolnick:** None.

Poster

381. Human DRGs and iPSC Sensory Neurons

Location: SDCC Halls B-H

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Program #/Poster #: 381.21

Topic: D.02. Somatosensation – Pain

Support: 212302/Z/18/Z

Title: Proteomic and functional analysis of exosomal communication in dorsal root ganglion

Authors: *V. PRATO[1], S. RASOOLI-NEJAD[2], S. MILNE[1], N. GAMPER[3];
[2]Fac. of Biol. sciences, [1]Univ. of Leeds, Leeds, United Kingdom; [3]Fac. of Biol. Sci., Univ. Leeds, Leeds, United Kingdom

Abstract: Chronic pain is a global clinical and societal burden affecting hundreds of millions of people worldwide. Current chronic pain treatments, such as opioids, tricyclic antidepressants and anticonvulsants are often inadequate due to either low efficacy or side-effects, including addiction. Most current analgesics act within the central nervous system, which is why psychotropic side-effects are a common issue. Therefore, it is of great importance to find new pro-analgesia targets outside the central nervous system. Dorsal Root Ganglia (DRG) is the site where the cell bodies of primary sensory neurons are located. DRGs are a part of the Peripheral Nervous System and are not protected by a blood-brain-barrier or blood-nerve-barrier making them an attractive target for pain relief. Satellite glial cells (SGCs) have an important role in the homeostasis of sensory neurons. One or more SGCs surround each DRG neuron forming units. Exosome communication have recently become of great interest in many biological settings, such as in cancer metastasis and in neurodegenerative diseases. The exosome cargo includes genomic material, proteins, microRNA and other mediators. Here we investigated the protein cargo of the exosomes released from the cultured DRG cells and also the potential mechanisms of exosome release. Separation of exosomes from cell culture media was achieved with an exosome isolation kit (Invitrogen) and confirmed by a presence of the exosome marker, CD63 via western blot. Exosome samples were lysed, alkylated and quantitative proteomic was obtained by liquid chromatography–mass spectrometry. Interestingly both neuronal and glial proteins were identified, suggesting that exosomes are released from both, neurons and SGC in the DRG. This may indicate a two-way communication process. Amongst the neuronal proteins identified were α2δ subunit of the voltage-gated Ca2+ channels, β-tubulin, Advillin, Synaptotagmin 2 (Syt2) and Neurofilament heavy polypeptide (NEFH). Amongst glial proteins Glia-derived nexin (GDN), S100 calcium-binding protein B (S100B), Fatty acid binding protein 7 (FABP7), Glial fibrillary acidic protein (GFAP), Glutamine synthetase (GS), and Apolipoprotein E were detected. We then used Western blotting, ELISA and Nanoparticle tracking analysis to show that exosomes are released from both cell types. Our ongoing experiments test involvement of Anoctamin6 (Ano6), a calcium-dependent lipid scramblase, in the exosome release from neurons and SGC. This research will help to uncover an underappreciated communication mechanism within the sensory ganglia.

Disclosures: V. Prato: None. **S. Rasooli-Nejad:** None. **S. Milne:** None. **N. Gamper:** None.

Poster

382. Muscle and Joint Pain

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 382.01

Topic: D.02. Somatosensation – Pain

Title: Dahl S rats: an innovative model of fibromyalgia

Authors: *J. ZICKELLA, L. F. FERRARI, N. E. TAYLOR;
Univ. of Utah, Salt Lake City, UT

Abstract: Fibromyalgia syndrome (FS) is characterized by widespread pain and is often associated with stress and anxiety. An obstacle in preclinical FS research is that current models do not reproduce key characteristics of the clinical disorder. All must be induced by repeated exposure to an environmental stressor and none consider genetic effects or comorbid symptoms. The Dahl S (SS) rat strain exhibits spontaneous hyperalgesia as a phenotype. We hypothesized this strain exhibits other features common to FS patients.
Hindpaw and gastrocnemius muscle nociceptive thresholds were evaluated in male and female SS, Sprague Dawley (SD) and Brown Norway (BN) rats, using the Randall-Sellito test. The strength of the diffuse noxious inhibitory controls (DNIC) in each strain was determined by evaluation of hindpaw withdrawal thresholds after subdermal capsaicin injection (125µg) into a single forepaw. To evaluate the levels of anxiety and depression in SS rats, separate groups underwent forced swim testing (FST) and elevated plus maze (EPM) behavioral studies. Plasma adrenocorticotrophic hormone (ACTH) and corticosterone levels were measured by radioimmunoassay. The effect of the pharmacological agents gabapentin (100mg/kg, i.p.), indomethacin (2mg/kg, s.c.) or dexamethasone (1mg/kg, s.c.) on hindpaw nociceptive thresholds of SS rats were evaluated by the Randall-Selitto method.
SS rats exhibited markedly low nociceptive thresholds in both skin (~82g) and muscle (~183g) compared to SD (~114g / ~357.5g) and BN (~135.4g / ~420.5g) rats. DNIC reflexes were absent in this strain ($p < 0.0001$ compared to SD and BN groups). SS rats demonstrated more depression and anxiety-like behaviors than SD and BN with decreased times frozen in the FST and open arm exploration in EPM. Lower diurnal variation in plasma ACTH (-16.9%) and corticosterone (47.1%) were found in the SS rats compared to BN rats (15% and 105.7%, respectively). With pharmacological treatments, gabapentin was the most effective in attenuating the SS hyperalgesia (40.5% increase in nociceptive threshold) with indomethacin (19.7%) and dexamethasone (10.2%) being mildly effective.
Our data show that SS rats exhibit many features found in FS patients, such as spontaneous/widespread pain, deficient endogenous pain control, anxiety, hypothalamic-pituitary-adrenal axis dysfunction, and systemic inflammation. As in FS patients, the analgesic effect of gabapentin was higher compared to indomethacin or dexamethasone, showing predicative validity and confirming this strain as an innovative preclinical model of FS.

Disclosures: **J. Zickella:** None. **L.F. Ferrari:** None. **N.E. Taylor:** None.

Poster

382. Muscle and Joint Pain

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Program #/Poster #: 382.02

Topic: D.02. Somatosensation – Pain

Support: NC12165994.0

Title: Opioid receptor antagonists induce pain episodes in a reserpine-induced fibromyalgia model in female rats.

Authors: C. JUAREZ[1], A. ALMANZA-GUTIERREZ[2], P. SEGURA-CHAMA[1], F. PELLICER[3], *F. MERCADO[1];
[1]Inst. Nacional de Psiquiatría Ramón de la Fuente Muñiz, Ciudad de Mexico, Mexico; [2]Inst. Nacional De Psiquiatria Ramon de la Fuente Muñiz, México DF, Mexico; [3]Inst. Nacional de Psiquatria, Huipulco, Mexico

Abstract: Fibromyalgia (FM) physiopathology is barely understood. Since the origin of the disease is unknown, a proper model for studying FM has been challenging. The reserpine-induced FM model resembles many of the main features of the disease, the most important is the induction of allodynia and hyperalgesia. An aspect absent in the pain models is the episodic nature of the disease, being relevant in the study of fibromyalgia because it is one of the main complaints of patients. In the reserpine-induced FM model the painful state is established immediately after reserpine administration, and allodynia and hyperalgesia remit after 21 days, all reports in the literature with this model have been carryout in this timeframe. In this work we show that, in the reserpine-induced FM model is possible to induce pain episodes based in the latent sensitization model.
Female rats were used, latencies to thermal (Hargreaves' apparatus) and mechanical (aesthesiometer) stimuli were taken before the induction of the FM model with reserpine (1 mg/kg, 3 consecutive days). Latencies were followed 2, 5, 7, 10, 15 and 21 days after reserpine. Then latent sensitization was tested with the intrathecal (i.t.) administration of Naltrexone (10 and 100 μg) and latencies were taken 15, 30, 60 and 120 minutes after i.t. administration. Additionally, specific μ and δ opioid receptor antagonists were tested by i.t. administration, and in some experiments subcutaneous (s.c.) Naloxone (1 mg/kg) was used. Reserpine by day 2 and 5 significantly reduced in half the latencies in thermonociceptive and mechanonociceptive tests compared with the latencies in rats injected with vehicle (VH). At day 21 the latencies in rats with reserpine back to basal values, and the i.t. administration of the opioid antagonist Naltrexone (at 10 and 100μg) in reserpine treated animals produced a significant shortening of the latencies (in thermo- and mechanonociceptive tests).To investigate if Naltrexone effect was due to the blockade of μ (MOR) or δ (DOR) opioid receptors, CTOP and Naltrindole were tested via i.t. Both antagonists produced a significant shortening of the latencies after their administration. Finally, to test how long latent sensitization last in this model, reserpine treated animals were injected with s.c. Naloxone the days 21, 24, 27, 30, 35 and 45 after reserpine administration; latencies were shortened significantly until day 30.
In conclusion, opioid antagonists produced painful episodes in the reserpine-induced FM model, the episodes were produced in the long term and could be useful to test pharmacological treatments for FM in preclinical stage.

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Poster

382. Muscle and Joint Pain

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Program #/Poster #: 382.03

Topic: D.01. Somatosensation

Support: NIH R01 NS097781
VA I01RX002316
Rebecca F. Hammond Endowment for Spinal Cord Injury Research

Title: Evidence that Group 2 Spindle Afferents Transmit Intramuscular Inhibition in Animals with Spinal Cord Injury

Authors: *A. DE BOEF[1], S. F. MCMURTRY[2], L. R. MONTGOMERY[3], R. L. VAN SANDT[6], A. TRELL[4], W. O'STEEN[4], D. R. HOWLAND[5], T. NICHOLS[1];
[2]Biol. Sci., [1]Georgia Inst. of Technol., Atlanta, GA; [3]Dept. of Physical Therapy and Rehabil. Sci., Univ. of Louisville, Philadelphia, PA; [5]Kentucky Spinal Cord Injury Res. Center, Depts of Neurolog. Surgery, Anat, [4]Univ. of Louisville, Louisville, KY; [6]Univ. of Wyoming, Laramie, WY

Abstract: Recent data (Niazi et al, 2020) obtained in control animals and those with spinal lateral hemisection (LHx) indicate inhibition between hindlimb extensor muscles is reorganized following injury, such that the inhibition onto ankle extensors is amplified and the inhibition from those muscles is reduced compared to the more balanced distribution observed in controls. This reorganization may contribute to the pronounced flexion exhibited by these animals during locomotion. The inhibition is force dependent, suggesting that it is mediated by pathways from Golgi tendon organs. However, the interneurons in the deep dorsal horn receive convergent input from tendon organs and secondary receptors of muscle spindles. In addition, group II afferents from secondary receptors, like group Ib afferents from tendon organs, project widely to muscles in the hindlimb, suggesting combined action from these two sensory sources. The purpose of this project was to test the hypothesis that pathways from group II afferents might contribute to the increased inhibition following LHx. Animals received a LHx or ventral quadrant lesion (VQx) and, following recovery periods of 7 or 12 weeks at the University of Louisville, underwent terminal experiments to map the distribution of intermuscular inhibition in the decerebrate state at Georgia Tech. Inhibition between limb extensors was measured by testing the effect of muscle stretch or intramuscular electrical stimulation of a donor muscle onto the stretch reflex of a recipient muscle. Muscle combinations were chosen to exclude muscles linked by feedback from group Ia afferents. The force in the donor muscle evoked by electrical stimulation was matched to the force evoked by stretch, keeping Ib input consistent between groups. Stretch was used to stimulate feedback from both spindle receptors and tendon organs, while intramuscular stimulation was expected to favor tendon organ pathways. In confirmation of previous studies (Lyle, 2019), both methods yielded similar inhibitory results in control animals, suggesting the inhibition was due mainly to pathways from tendon organs. Following LHx and VQx lesions,

however, the enhanced inhibition was less for intramuscular stimulation than for stretch, suggesting that feedback from group II afferents contributes to the amplified inhibition following spinal cord injury with ventral damage.
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Poster

382. Muscle and Joint Pain

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Program #/Poster #: 382.04

Topic: D.02. Somatosensation – Pain

Support: NIH Grant R01NS113965
NIH Grant R01NS105715

Title: Role of T-cell mobilizing chemokine, CCl27a, in muscle hypersensitivity after repetitive ischemia with reperfusion injury

Authors: *G. TRIPATHI[1], K. PROPSOM[1], K. KELLERMAN[1], M. JANKOWSKI[1,2,3];
[1]Anesthesia, Div. of Pain Mgmt., [2]Pediatric Pain Res. Ctr., Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH; [3]Dept. of Pediatrics, Univ. of Cincinnati, Cincinnati, OH

Abstract: Muscle pain is a significant clinical problem. Mechanisms of muscle pain development involve both peripheral and central nervous system changes in addition to immune modulation of cellular activity. Ischemic myalgia is a unique type of muscle pain due to transient reduction of the blood supply to a part of the body followed by reperfusion injury (I/R). Disorders characterized by I/R, such as sickle cell anemia, peripheral vascular disease, fibromyalgia or complex regional pain syndrome, are prevalent but currently have inadequate treatments for pain. Our model of prolonged ischemic myalgia which utilizes a repeated I/R injury to the forelimb has been shown to alter primary afferent function and immune signaling pathways. In addition, specific receptor mechanisms were thought to underlie observed peripheral hypersensitivity. Another factor that was found to be expressed in muscle afferents and could be contributing to these phenomena is expression of C-C motif chemokine ligand 27 (CCl27a) in the dorsal root ganglion (DRG). This chemokine acts upstream of or within positive regulation of T cell chemotaxis and positive regulation of actin cytoskeleton reorganization. To test whether CCl27a is involved in ischemia/reperfusion related hypersensitivity, we performed a brachial artery occlusion with reperfusion in mice, followed by a second I/R 7 days later to develop prolonged pain-like behaviors. We assessed CCl27a in the C8-DRG by RNAscope and also assessed CD3+ T cells in forepaw muscle through immunohistochemistry. We demonstrated

the presence of CCl27a in C8 DRG neurons. We also found a significant increased level of CD3 in forepaw tissue of mice with IR. Interestingly, 1d after the second I/R, both male and female mice display a significant upregulation of this chemokine in the DRGs. This data may support a role for nociceptor derived CCL27a in mobilizing T cells to the muscle to regulate I/R-related hypersensitivity. Results could provide evidence for development of novel treatment strategies for patients with IR-related pain.

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Poster

382. Muscle and Joint Pain

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Program #/Poster #: 382.05

Topic: D.02. Somatosensation – Pain

Support: NIH Grant R01NS105715

Title: Expression of STAT1 in the DRGs is Associated with Neonatal Nociceptive Behaviors after Inflammation

Authors: *A. O. FADAKA[1], A. DOURSON[1], M. JANKWOSKI[1,2,3];
[1]Dept. of Anesthesia, Div. of Pain Mgmt., Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH; [2]Pediatric Pain Res. Ctr., Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH; [3]Dept. of Pediatrics, Univ. of Cincinnati, Col. of Med., Cincinnati, OH

Abstract: Diverse noxious events that occur during neonatal development can significantly alter sensory, cognitive, and other health outcomes later in life. Neonatal pain remains a significant health concern and current pharmacological treatments are limited to agents that often produce more adverse effects than analgesic benefits in children. Data has suggested that the mechanisms by which neonates transduce noxious stimuli may be distinct from adults. Our recent work has linked the transcription factor serum response factor (SRF) downstream of local growth hormone (GH) signaling to incision-related hypersensitivity in neonates. However, it remains unclear if similar mechanisms contribute to inflammatory pain in neonates. Our preliminary data suggests that signal transducer and activator of transcription 1 (STAT1) was uniquely upregulated in the dorsal root ganglia (DRGs) of neonates after cutaneous and muscle inflammation. Therefore, we investigated if distinct transcription factors or GH may modulate nociceptive behaviors in neonatal pups following cutaneous or muscle inflammation. Inflammation was induced by 1 % carrageenan (in 0.9%NaCl) injection into either the right hairy hindpaw skin or hindpaw muscle at postnatal day 7 (P7) in Swiss Webster mice and compared to GH/ carrageenan treated and sham injected controls. Behavioral examination of spontaneous paw guarding, thermal hypersensitivity (cutaneous), and mechanical withdrawal thresholds using von Frey filaments (cutaneous), or muscle squeezing assays were then performed in our groups. Results were

correlated to gene expression in the L2/3 (skin) or L3/4/5 (muscle) DRGs at day one post injection using realtime PCR. Carrageenan injection significantly induced spontaneous paw guarding, and mechanical and/or thermal hypersensitivity after either skin or muscle inflammation. Results corresponded with significant edema in the hind paws of carrageenan injected groups compared to GH/ carrageenan treated and sham injected controls. Surprisingly, while there were no significant changes in a series of select transcription factors, STAT1 was differentially altered in the DRGs after both skin and muscle inflammation. In addition, co-injection of GH/ carrageenan suppressed STAT1 expression. Data suggest that STAT1 upregulation may be downstream of GH signaling and contribute to neonatal nociception specifically during skin and muscle inflammation. Results could uncover new ways to treat inflammatory pain in neonates.

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Poster

382. Muscle and Joint Pain

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Topic: D.02. Somatosensation – Pain

Support: NIH Grant R01NS113965

Title: Sex specific role of RNA-binding protein, AUF1, on somatosensory responses from muscle after repetitive ischemia with reperfusion injury

Authors: *M. QUIJAS[1], D. P. JOSHI[2], A. A. WEYLER[2], L. F. QUEME COBAR[3], M. P. JANKOWSKI[4];
[1]Univ. of Cincinnati Neurosci. Grad. Program, [2]Univ. of Cincinnati, Cincinnati, OH; [3]Cincinnati Children's Hosp. Med. Ctr., Cincinnati Children's Hospit, Cincinnati, OH; [4]Cincinnati Children's Hosp. Med. Ctr., Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH

Abstract: Myalgia is experienced worldwide more than any other type of pain. One cause of myalgia is ischemia with reperfusion injury (I/R). Chronic ischemic myalgia develops in diseases such as sickle cell anemia or peripheral vascular disease. There are currently limited treatment strategies for pain in these conditions. Further, females with ischemic myalgia have lower pain tolerance and heightened sensitivity compared to males. We have recently developed a model of prolonged ischemic myalgia in mice whereby animals experience repeated I/R injury leading to behavioral and afferent hypersensitivity. We have identified sensitization after repeated I/R to be associated with an increase in glial cell line-derived (GDNF) family receptor α1 (GFRα1) and acid sensing ion channel 3 (ASIC3) expression in male dorsal root ganglia (DRG) and interleukin 1 receptor type 1 (IL1r1) and transient receptor potential cation channel (TRPV1) in female DRGs. In addition, affected muscle from females after I/R exhibit higher amounts of interleukin 1 beta (IL1β) whereas males only display increased GDNF. To determine possible

mechanisms by which distinct sex specific gene expression patterns modulated IR-related hypersensitivity in mice, we performed unbiased and targeted screening strategies in male and female DRGs with or without I/R. Several distinct proteins were found to be differentially expressed including AU-rich element RNA binding protein (AUF1). AUF1 is specifically upregulated in female DRGs when compared to males and has the ability to stabilize RNAs. Since AUF1 is known to have several binding sites on the IL1r1 mRNA, we hypothesized that AUF1 can sustain the expression of IL1r1 leading to prolonged hypersensitivity specifically in females with repeated I/R. To test this, we performed nerve targeted AUF1 knockdown in males and females with repeated I/R injury and assessed behavioral responses and gene expression. No effect of AUF1 knockdown was observed on male DRG gene expression or behavior, however, in females the knockdown reduced hypersensitivity and prevented the upregulation of IL1r1 and TRPV1 in the DRGs. Using an adeno-associated virus to overexpress AUF1 in males, we then observed an increase in hypersensitivity similar to females after I/R injury. Data suggests that RNA binding proteins like AUF1 may underlie the sex specific effects on DRG gene expression that modulate behavioral hypersensitivity after repeated I/R injury. This study may aid in finding distinct receptor differences related to the evolution of acute to chronic muscle pain development between sexes.

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Poster

382. Muscle and Joint Pain

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Topic: D.02. Somatosensation – Pain

Support: NIH (R01NS113965)

Title: Role of TACAN ion channel in mediating muscle afferent sensitization

Authors: *A. ADLAKHA[1], L. F. QUEME[1,2,3], R. SHARIF-NAEINI[5], M. P. JANKOWSKI[1,2,3,4];
[1]Dept. of Anesthesia, [2]Pediatric Pain Res. Ctr., Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH; [3]Dept. of Anesthesiol., [4]Dept. of Pediatrics, Univ. of Cincinnati, Col. of Med., Cincinnati, OH; [5]Dept. of Physiol. and Cell Information Systems group, McGill Univ., Montreal, QC, Canada

Abstract: The process of converting mechanical forces into electrical stimuli, which is termed mechanotransduction, underlies a diverse variety of vital physiological functions such as pain, touch and proprioception. Interestingly, the sensitization of nociceptive neurons to mechanical stimuli is associated with the majority of pain disorders. Nevertheless, the exact mechanisms underlying mechanotransduction of noxious stimuli from the muscle is not well understood.

TACAN (Tmem120A), a putative mechanotransducing ion channel which is highly expressed in a subset of nociceptive neurons, was recently reported to contribute to detection of noxious mechanical stimuli. Therefore, in the present study, we evaluated the role of TACAN ion channel in muscle mechanosensory function. To test this, *in vivo* knockout of TACAN was carried out by unilaterally injecting an adeno-associated virus, serotype 9 (AAV9) expressing Cre recombinase or a scrambled, non-targeting control sequence into the right tibial nerve at post-natal day 14 in TACAN $^{fl/fl}$ mice. Withdrawal thresholds and nocifensive behaviors were then recorded. Results from behavioral tests were then compared to single unit electrophysiological recording of muscle afferents using our *ex vivo* hindpaw muscle/tibial nerve/dorsal root ganglion/spinal cord preparation. The control and experimental mice showed no differences in their withdrawal thresholds to subthreshold paw squeezing or the von Frey test. However, control mice showed a significantly greater display of nocifensive behaviors during suprathreshold paw squeezing as compared to the TACAN knockout mice. The decrease in nocifensive behaviors in TACAN knockout mice correlated with a decrease in number of mechanically sensitive cells that responded to a force threshold less than two grams. This behavioral difference also correlated with a decrease in the mean firing rate response to more noxious mechanical forces. This finding led to the conclusion that knocking out the TACAN ion channel leads to muscle hyposensitivity to noxious stimuli in mice. Altogether, this data strengthens the evidence for a role of the TACAN ion channel in mediating muscle mechanosensory function. These results may have implications for future development of pain therapeutics for muscle pain.

Disclosures: A. Adlakha: None. **L.F. Queme:** None. **R. Sharif-Naeini:** None. **M.P. Jankowski:** None.

Poster

382. Muscle and Joint Pain

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Topic: D.02. Somatosensation – Pain

Support: NIH Grant NS108087
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Title: The Role of the Nociceptor Scaffold Protein Magi-1 in Monoiodoacetate-induced Osteoarthritis Pain

Authors: *R. RODRIGUEZ[1], A. BHATTACHARJEE[2];
[1]State Univ. of New York, Buffalo, Buffalo, NY; [2]Pharmacol. and Toxicology, SUNY-Buffalo, Buffalo, NY

Abstract: Osteoarthritis (OA) chronic pain is one of the most common causes of disability in the elderly population. Current approaches to treat osteoarthritis (OA)-related pain have relied on the use of systemic anti-inflammatory drugs to reduce pain sensation, however, these analgesics have been associated with multiple adverse effects, as well as lack of efficacy after long-term use. Alternatively, local approaches, such as intraarticular injection of glucocorticoids are often used, however a randomized clinical trial showed that local glucocorticoid injection resulted in significantly greater cartilage volume loss and no significant difference in joint pain (PMID: 28510679). Due to the heterogeneity of OA pain mechanisms, developing novel efficient local pain medications requires the identification of specific targets at joint nociceptors. Our previous studies identified the PDZ and WW domain-containing protein Magi-1 as a key scaffold for voltage-gated ion channels in dorsal root ganglion neurons. Our data showed that Magi-1 regulated Nav1.8 channel trafficking and localization at the plasma membrane, which altered sensory neuronal excitability. Here we aimed to characterize Magi-1 expression in joint afferent neurons and determine its effect on OA-related pain hypersensitivity. Immunostaining analysis of subchondral bone showed that Magi-1 and Nav1.8 channel are both expressed in joint nociceptors. These results led us to explore the effects of Magi-1 deficiency on pain behavior in the monoiodacetate (MIA) model of OA . To achieve this, Magi-1 shRNA-based in vivo sciatic nerve transfection was performed to downregulate Magi-1 expression in mouse joint nociceptors prior to MIA intraarticular injection. Dynamic weight bearing and the von Frey assay were used to asses pain behavior on OA pain development in scrambled-treated and Magi-1 shRNA treated mice. Our results showed that Magi-1-deficient mice experienced a reduction in pain sensitivity compared to scrambled shRNA treated mice post MIA injection. Altogether, this study seeks to add to the current knowledge on the diversity of OA pain mechanisms, and to identify novel local analgesic targets to treat joint chronic pain.

Disclosures: R. Rodriguez: None. **A. Bhattacharjee:** None.

Poster

382. Muscle and Joint Pain

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Program #/Poster #: 382.09

Topic: D.02. Somatosensation – Pain

Support: NIH Grant NS108087
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NIH Grant NS128543

Title: Targeting the nociceptor endocytosis AP2 complex affects MIA-Induced Osteoarthritis pain behavior

Authors: *A. COOPER[1], R. RODRIGUEZ[2], R. G. POWELL[3], A. BHATTACHARJEE[4]; [1]State Univ. of New York, Univ. at Buffalo, Buffalo, NY; [2]Pharmacol. and Toxicology, State

Univ. of New York, Buffalo, Buffalo, NY; [3]Boston Children's Hosp., Boston, MA; [4]Pharmacol. and Toxicology, SUNY-Buffalo, Buffalo, NY

Abstract: Targeting the nociceptor endocytosis AP2 complex affects MIA-Induced Osteoarthritis pain behavior Authors: A Cooper, Jessica Tabman, R Rodriquez, R Powell, V Young, A Bhattacharjee; Neuroscience University at Buffalo, Buffalo, NY Pharmacology and Toxicology, University at Buffalo, Buffalo, NYDisclosures: A Cooper NONE J Tabman NONE R Rodriquez NONE R Powell NONE V Young NONE A Bhattacharjee NONE Osteoarthritis (OA), is a degenerative condition that leads to chronic pain and a need for pain relief. Pain management for OA pain is fraught with efficacy issues and adverse effects. For example, although injection of corticosteroids into arthritic joints is a mainstay therapeutic approach to treat arthritic pain, clinical studies have raised questions on the efficacy of steroid therapy and have also shown treatment caused significantly greater cartilage volume loss (PMID: 28510679). Our previous studies have indicated that inhibition of the nociceptor endocytotic adaptor protein complex 2 (AP2) inhibits neuronal hyperexcitability and inflammatory pain behavior (PMID: 34608164). Here we show the AP2A2 subunit localized to CGRP-containing large dense core vesicles (LDCVs) in human and mouse DRG neurons. We further demonstrate that pain behavior in arthritis is reduced by local pharmacological inhibition of the nociceptor AP2 complex using a lipidated AP2 peptide inhibitor. Monoiodoacetate (MIA) was used to induce knee joint OA in Sprague-Dawley rats. Four days later, upon verification of pain, a one-time intra-articular injection to the arthritic knee of either the AP2 inhibitor peptide or a scrambled peptide control was administered. Joint pain was assessed via a dynamic weight-bearing (DWB) assay. Pain behavior was monitored over the course of 28 days post MIA-induced OA. DWB analyses showed the typical decrease in weight bearing in the group whose arthritic knees were injected with scrambled peptide; however, an increase in weight bearing, persisting up to 24 days, was observed in the AP2 peptide injected group. Furthermore, micro-computed tomography and histological analyses were performed on MIA injected knee joints as well as the contralateral healthy knee joints at end of 28 days to evaluate the effects on disease progression. Pathological analyses indicated the typical reduction of subchondral bone content and cartilage formation in scrambled peptide-injected MIA-treated joints, however intra-articular AP2 inhibitor peptide injections surprisingly resulted in a significant retention of bone content and cartilage. This data suggests that inhibition joint nociceptor endocytosis can decrease OA pain and pathology.

Disclosures: A. Cooper: None. **R. Rodriguez:** None. **R.G. Powell:** None. **A. Bhattacharjee:** None.

Poster

382. Muscle and Joint Pain

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 382.10

Topic: D.02. Somatosensation – Pain

Support: P20-GM-103643
P30-AR-079206

Title: Analysis of mid-stage and advanced OA pain states in chemical and surgical murine osteoarthritis models

Authors: *V. EATON[1], C. QUATTROCHIO[2], N. ADAMCZYK[4], R. MILLER[4], T. KING[3,1]; [1]Ctr. of Excellence in the Neurosciences, [2]Col. of Arts and Sci., [3]Col. of Osteo. Med., Univ. of New England, Biddeford, ME; [4]Dept. of Intrnl. Medicine, Div. of Rheumatology, Rush Univ. Med. Ctr., Chicago, IL

Abstract: Osteoarthritis (OA) is one of the most prevalent causes of chronic pain in US adults suffering with OA. OA pain can be characterized into three stages: early stage, mid-stage, and advanced- stage with advanced-stage OA often accompanied by constant dull, aching pain as well as intermittent bouts of intense pain. Although NSAIDs are commonly prescribed for OA pain and help mitigate the predictable episodes of pain associated with early and mid-stage OA, evidence has shown that NSAIDs are not sufficient in treating chronic advanced OA pain. It is imperative to develop new and improved treatments for advanced OA pain. To achieve this, a better understanding of how the mechanisms driving advanced OA pain differ from mid-stage OA pain is required. Here we compared behavioral readouts of mid-stage and advanced OA pain between the monosodium iodoacetate -induced (MIA) murine OA model and a surgical partial meniscal excision (PMX) murine OA model. The MIA model allows for phenotypic and mechanistic study of different stages of OA 14 days post-induction, a major benefit of the model, while the PMX surgical models trauma-induced OA, with behaviors indicating mid-stage OA joint pain developing over a 12-week time course. However, whether advanced OA develops in the PMX model of OA pain is unknown. Animals underwent behavioral testing to assess mid-stage or advanced OA pain phenotypes between the models, including measurements of weight asymmetry, conditioned place preference (CPP), and locomotor assays (LMA). Both models induce weight-asymmetry with MIA-induced weight asymmetry observed in a dose-dependent manner and PMX-induced weight asymmetry observed in a time-dependent manner. PMX induced weight asymmetry by week 12 post-surgery was similar to weight asymmetry values seen in the advanced stage MIA model. In the MIA model, OA-induced changes in movement, distance moved and rearing, were not observed at concentrations that produce advanced OA pain as indicated by weight asymmetry and ongoing pain. PMX animals demonstrated diminishment of these same measures at 8 weeks post-surgery, a time-point associated with weight asymmetry. These results indicate that both models induce joint pain. Moreover, different pain phenotypes can be observed in these models. Mid-stage and advanced OA pain are concentration dependent in the MIA model and time dependent in the PMX model. This work has been supported by the NIH through a National Institute of General Medical Sciences COBRE grant P20-GM-103643 at UNE and a National Institute for Arthritis and Musculoskeletal and Skin Disease grant, P30-AR-079206).

Disclosures: V. Eaton: None. **C. Quattrochio:** None. **N. Adamczyk:** None. **R. Miller:** None. **T. King:** None.

Poster

382. Muscle and Joint Pain

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Topic: D.02. Somatosensation – Pain

Support: NIH Grant P20GM103643

Title: Sex differences in knee joint pain in relation to joint pathology

Authors: *T. VESEY, N. SIAN, M. MUETH, V. EATON, P. CARADONNA, T. KING; Univ. of New England, Biddeford, ME

Abstract: Osteoarthritis (OA) is characterized by progressive articular cartilage loss, new bone formation, and synovial proliferation resulting in pain, knee joint damage, and a lower quality of life. Patients with osteoarthritis can develop different pain phenotypes. Many patients report pain during joint use that abates during rest and that can be managed with non-steroidal anti-inflammatory agents (NSAIDs), described as mid-stage osteoarthritis. Some patients report development of persistent ongoing pain that is resistant to NSAIDs, termed advanced OA. We examined potential changes in the OA joint that may contribute to differences in these pain states in a mouse model of osteoarthritis. We tested the hypotheses that mid-stage and advanced OA pain have different levels of joint pathology and pathological sprouting of sensory fibers. Notably, females develop advanced OA pain at lower MIA concentrations compared to males. We examined the hypothesis that the sex differences in pain phenotype is independent of joint pathology suggesting central mechanisms mediating these sex differences. Nav1.8-tdTomato male mice were injected (10 µl) with monosodium iodoacetate (MIA) to produce pain phenotypes corresponding to mid-stage (16 mg/ml) or advanced OA (80 mg/ml) pain to examine innervation within the knee joint and surrounding tissue. MIA induced increased tdTomato in the knee joint in an intensity dependent manner. Follow up experiments were conducted with male and female C57bl/6 injected with MIA to induce mid-stage or advanced OA pain phenotypes. Females developed advanced OA pain phenotype at a 5-fold lower dose compared to males. H&E and toluidine blue staining demonstrate that males and females both demonstrate concentration dependent signs of knee joint OA including cartilage loss, synovitis, and bone remodeling. Immunofluorescent staining using the pan neuronal marker beta tubulin 3 (BT3), CGRP and Tyrosine Hydroxylase demonstrate concentration dependent changes in joint innervation corresponding to changes in pain phenotype in males and females. These data suggest that females have a greater susceptibility to develop ongoing knee joint pain with less joint pathology compared to males. Supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences (NIGMS) of the National Institutes of Health (NIH). Behavioral analysis performed in the COBRE Behavior Core, Histology and Imaging done in the COBRE Histology and Imaging Core supported by P20GM103643. TV supported by Kahn Family Foundation Research Fellowship.

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Poster

382. Muscle and Joint Pain

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 382.12

Topic: D.02. Somatosensation – Pain

Support: LSA contract number PD41886

Title: Protoxin II, a Nav 1.7 channel antagonist, promotes analgesia in the early and later phase allodynia in the K/BxN arthritis model

Authors: *K. FRANCO MALANGE[1], K. DORE[2], G. GONCALVES DOS SANTOS[1], M. YUN[1], J. BORGES PAES LEMES[1], M. CORR[3], H. ZHAO[4], P. ALVAREZ[4], T. YAKSH[1]; [1]Dept. of Anesthesiol., [2]Dept. of Neurosciences, [3]Div. of Rheumatology, Allergy & Immunol., UCSD, San Diego, CA; [4]IONIS Pharmaceuticals, Carlsbad, CA

Abstract: In rodents and humans, the Nav 1.7 isoform of the voltage-gated sodium channels (VGSCs) has been shown to be expressed in nociceptive primary afferent neurons. The relevance of these channels to the pain phenotype in inflammatory and nerve injury has been supported by the respective effects of gain and loss of function mutations. Our work with the K/BxN serum transfer murine model has shown that it mimics the characteristics of rheumatoid arthritis (RA), resulting in a long-lasting (15-20 days), but reversible articular inflammation, joint degeneration, dorsal horn glial activation, and a robust post-inflammatory pain phenotype that mimics a polyneuropathy. In this study, we aimed to investigate the spinal effects of the Nav 1.7 antagonist Protoxin II (PTxII) in the tactile allodynia observed in the inflammatory and post-inflammatory phase of the K/BxN model. Adult, male, K/BxN mice displayed a prominent paw tactile allodynia on days 6-7 (0.16 ± 0.14 vs 0.90 ± 0.27 gm; $P<0.001$; K/BxN vs naïve mouse; two-way ANOVA, Tukey test) and day 25-26 (0.35 ± 0.17 vs 0.91 ± 0.16 gm; $P<0.001$; K/BxN vs naïve mouse; two-way ANOVA, Tukey test). The intrathecal delivery in mice of Nav 1.7 antagonist Ptx II (10µM; 5µL) in a limited but significant way increases the mechanical threshold of K/BxN mice in the early (0.16 ± 0.15gm vs 0.86 ± 0.53 and 0.91 ± 0.60 gm; $P<0.05$; baseline vs 1h and 2h after PTx II; two-way ANOVA, Tukey test) and late phase (0.27 ± 0.14gm vs 0.62 ± 0.19 gm and 0.59 ± 0.14 gm; $P<0.05$; baseline vs 1/2h and 4h after PTx II; two-way ANOVA, Tukey test). To confirm the effects of Protoxin II on primary afferent neuron excitability, we performed voltage-clamp recordings. Sodium currents induced by -80mV to -20mV voltage steps were significantly reduced by 10nM Protoxin II. We also used 10ms puffs (delivered with a picospritzer) of 1µM OD1, a Nav 1.7 agonist, to elicit sodium currents in these neurons. Fast and slow currents were observed and 75nM Protoxin II could block these two types of sodium currents. These results are consistent with the contribution of Nav 1.7 to the facilitated pain state observed in the early and late phase K/BxN mouse. This work was supported by IONIS pharmaceuticals (LSA contract number PD41886).

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Poster

382. Muscle and Joint Pain

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 382.13

Topic: D.02. Somatosensation – Pain

Support: NIH/NINDS 1 R01 NS099338 (TY)
CONACYT-PDCPN-2015-01-191 (JMJA)

Title: Role of Toll-like receptor 4 in joint remodeling, sprouting, and allodynia in the K/BxN serum transfer model of arthritis

Authors: *G. GONCALVES DOS SANTOS[1], J. M. JIMÉNEZ-ANDRADE[3], E. MUNOZ-ISLAS[4], M. RAMIREZ-ROSAS[4], M. E. CANDANEDO-QUIROZ[4], B. DRUMMOND[2], T. L. YAKSH[2], M. CORR[2];
[1]Univ. of California San Diego, Univ. of California San Diego, La Jolla, CA; [2]Univ. of California San Diego, San Diego, CA; [3]Unidad Académica Multidisciplinaria Reynosa-Aztlán, UAT, Reynosa Tamaulipas, México., Reynosa, Mexico; [4]Unidad Académica Multidisciplinaria Reynosa-Aztlán, Reynosa, Mexico

Abstract: Rheumatoid arthritis (RA) is a chronic autoimmune disorder characterized by joint inflammation and chronic pain that negatively affects quality of life, restricts movement, and contributes to depression. The murine K/BxN serum transfer model capitulates many of the features of RA but the inflammation is self-remitting. Using this model we previously described that WT males show a persistent allodynia while WT females partially resolve their allodynia during the post-inflammatory phase. Despite comparable inflammatory profiles in Toll-like receptor 4 (TLR) deficient mice, males show a significant reversal of allodynia while females an earlier and more robust reversal of allodynia. We sought to characterize the involvement of TLR4 in the K/BxN model with a focus on the peripheral phenotype. Groups of recipient mice (WT and *Tlr4*-/-) received intraperitoneal (IP) injection (100 μL on days 0 and 2) of sera from adult K/BxN transgenic mice. Mechanical allodynia and joint inflammation (scoring of clinical signs) were assessed on days 0-6, 9, 12, 15, 18, 21, 24, and 28. At day 28, spinal cord and legs were harvested to characterize peripheral innervation and bony pathology. *i) Joint Inflammation:* Both male and female WT and $Tlr4^{-/-}$ mice showed comparable RA inflammatory profiles. *ii) Allodynia:* Confirmed the rapid resolution of allodynia in $Tlr4^{-/-}$ males and females, and the persistence of allodynia in WT males and only partial resolution in WT females. *iii) Joint innervation:* Both, male and female WT K/BxN mice displayed increased $CGRP^{+}$, TH^{+}, and $GAP\text{-}43^{+}$ fiber synovial innervation. Male $Tlr4^{-/-}$ mice showed decreased TH and GAP43 staining, while females revealed decreases in TH and CGRP. *iv) Bone density:* Lower bone density was observed only in female WT mice and the bone phenotype is rescued in $Tlr4^{-/-}$. v) *Osteoclast numbers*: WT K/BxN males and females had increased numbers of osteoclasts which were normalized in $Tlr4^{-/-}$ mice. These results show a key role of TLR4 function associated with

the evolution of a chronic pain phenotype and the constellation of changes (peripheral sprouting, osteoclast activity and bone density) observed in the K/BxN mouse.

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Poster

382. Muscle and Joint Pain

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 382.14

Topic: D.02. Somatosensation – Pain

Support: NIH 75N95019D00026

Title: Evaluation of behavioral pain phenotype in the rat monoiodoacetate and medial meniscal tear models of osteoarthritis pain

Authors: *M. URBAN[1], E. DUGAN[1], K. BUBAN[1], J. HAGEDORN[1], S. A. WOLLER[2], S. IYENGAR[2], T. HANANIA[1];
[1]PsychoGenics, Inc., Paramus, NJ; [2]NIH/NINDS, Rockville, MD

Abstract: In collaboration with the NIH HEAL Initiative Preclinical Screening Platform for Pain (PSPP), we examined a variety of pain behaviors in the rat monoiodoacetate (MIA) and medial meniscal tear (MMT) models of osteoarthritis to validate these models for the evaluation of novel assets. Adult male and female Sprague Dawley rats (n=10, each sex) were used in these studies and behavioral pain responses were evaluated for a period of 4 weeks. For the MIA model, intraarticular injection of MIA (1, 3 mg) into the hindlimb knee joint produced unilateral hind paw tactile hypersensitivity in male and female rats which was maximal at Week 2. Unilateral knee joint hypersensitivity to pressure and pinch stimuli was observed in female, but not male, rats at Week 2. Weight bearing deficits associated with the affected hind limb were modest when measuring static weight bearing, and were more pronounced when measuring dynamic weight bearing in male and female rats, with maximal effects observed at Week 1. Changes in gait were also observed in male and female rats following MIA injection, with significant differences observed in hind paw speed, rhythmicity, and paw position at Weeks 1 and 2. The optimal dose of MIA to examine pharmacological effects in this model was determined to be 1 mg, based on lower variability in weight bearing responses. Single administration of morphine (3 mg/kg s.c.) reduced hind paw tactile hypersensitivity and weight bearing deficits in male and female rats in the MIA model, whereas single administration of ketoprofen (6 mg/kg s.c.) or duloxetine (60 mg/kg p.o.) was partially effective or ineffective. In contrast, repeated administration of ketoprofen or duloxetine (4 days, b.i.d.) significantly reduced tactile hypersensitivity and weight bearing deficits. For the MMT model, male rats that had received MMT surgery displayed unilateral hind paw tactile hypersensitivity that was maximal at

Week 3, while no changes in hind paw tactile sensitivity were observed in female rats. Knee joint sensitivity to a pressure stimulus was unaffected in male and female MMT rats, and dynamic weight bearing was also unaffected in male and female rats that had received MMT surgery. The results from this study demonstrate that a variety of pain behaviors associated with knee joint osteoarthritis can be measured using the rat MIA model, while pain behaviors in the MMT model were less robust or not observable. Evaluation of novel assets following single and repeated administration in the MIA model using multiple pain endpoints may be a viable strategy to accelerate the development of non-opioid, non-addictive therapeutics for the treatment of osteoarthritis pain.

Disclosures: **M. Urban:** A. Employment/Salary (full or part-time):; PsychoGenics, Inc.. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; PsychoGenics, Inc. **E. Dugan:** A. Employment/Salary (full or part-time):; PsychoGenics, Inc.. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; PsychoGenics, Inc. **K. Buban:** A. Employment/Salary (full or part-time):; PsychoGenics, Inc.. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; PsychoGenics, Inc. **J. Hagedorn:** A. Employment/Salary (full or part-time):; PsychoGenics, Inc.. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; PsychoGenics, Inc.. **S.A. Woller:** None. **S. Iyengar:** None. **T. Hanania:** A. Employment/Salary (full or part-time):; PsychoGenics, Inc.. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; PsychoGenics, Inc..

Poster

382. Muscle and Joint Pain

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 382.15

Topic: D.02. Somatosensation – Pain

Title: NIP-322, a novel sepiapterin reductase specific inhibitor without tolerance and safety concerns for the treatment of osteoarthritis pain

Authors: *K. KAGAYA[1], Y. ITO[1], A. DATE[1], K. GORYO[1], D. TAKAHASHI[1], S. HAYASHI[2], Y. HOSOZAWA[3], M. HAYASHI[3], Y. INABA[3], Y. SHINTANI[3], M. KAMAURA[3], J. KAMON[1];
[1]Medicinal Res. Department, Biol. Res. Labs., [2]Toxicology & Envrn. Sci. Department, Biol. Res.

Labs., Nissan Chem. Corp., Shiraoka-shi, Saitama, Japan; [3]Pharmaceut. Res. Department, Chem. Res. Labs., Nissan Chem. Corp., Funabashi-shi, Chiba, Japan

Abstract: ***Background.*** Tetrahydrobiopterin (BH_4) levels are critical for processes related to pain sensitivity in human and rodent models. Sepiapterin reductase (SPR) is reported to be a promising drug target to regulate BH_4 production (Neuron.86:1393-1406, 2015). NIP-322 has a potent analgesic effects after a single dosing, and appropriately alters two kinds of biomarkers based on SPR inhibition in rat osteoarthritis (OA) model in our previous report (WO2021210586, IASP 2022 world congress on pain/in preparation) In this study, we examined the enzyme selectivity of NIP-322 and prior SPR inhibitors (Q-1169, Q-1195, and Q-1245; Q-compounds). In addition, we investigated the possible development of tolerance to repeated administration of NIP-322 and its effect on motor coordination at high doses for the potential as a treatment for OA pain. ***Methods.*** Enzyme assays were conducted using spectrophotometric systems. The putative binding mode of NIP-322 and Q-compounds to BH_4 related enzymes were predicted by *in-silico* molecular docking analysis using previously reported co-crystal structure (PDB code; SPR/4J7U, CBR/3BHJ). SD rats were injected with monoiodoacetate (MIA, 2 mg/50μL) into the infrapatellar ligament at day 0. After repeated administration of NIP-322 (1.5 mg/kg, p.o., q.d.) from day10 to 15, the analgesic effect was evaluated by grip strength test in the blind manner. Histological examination was performed on the joint tissues for disease-modifying effects. Motor coordination were assessed using rota-rod after single dosing of NIP-322 (5-50 mg/kg, p.o.) in rat. ***Results.*** The IC_{50} of NIP-322, Q-1169, Q-1195 and Q-1245 for human SPR enzyme were 0.73, 1.0, 0.90 and 0.82 nM, respectively. NIP-322 had no inhibitory effect against CBR enzyme, which related to the salvage pathway of BH4 production, even at 10 μM. Q-1169, Q-1195 and Q-1245 had inhibitory effect for human CBR enzyme and the IC_{50} were 1.1, 0.72 and 0.41 μM, respectively. These enzyme selectivity data were supported by molecular docking studies. The plasma exposure of Q-compounds to rats had the potential to CBR inhibition. Repeated administration of NIP-322 demonstrated a clear analgesic effect without tolerance, but not had disease-modifying effects in histology. NIP-322 had no significant effect on the rota-rod performance at 5-50 mg/kg. ***Conclusions.*** These studies demonstrate that NIP-322 has the difference from prior Q-compounds in chemical and biological property, suggesting a rationale for no toxicological findings in 4-week dog toxicity study. We concluded that NIP-322 will be a promising candidate option for OA patients with a favorable profile that avoids the tolerance and dyscoordination.

Disclosures: **K. Kagaya:** A. Employment/Salary (full or part-time):; Nissan Chemical Corporation. **Y. Ito:** A. Employment/Salary (full or part-time):; Nissan Chemical Corporation. **A. Date:** A. Employment/Salary (full or part-time):; Nissan Chemical Corporation. **K. Goryo:** A. Employment/Salary (full or part-time):; Nissan Chemical Corporation. **D. Takahashi:** A. Employment/Salary (full or part-time):; Nissan Chemical Corporation. **S. Hayashi:** A. Employment/Salary (full or part-time):; Nissan Chemical Corporation. **Y. Hosozawa:** A. Employment/Salary (full or part-time):; Nissan Chemical Corporation. **M. Hayashi:** A. Employment/Salary (full or part-time):; Nissan Chemical Corporation. **Y. Inaba:** A. Employment/Salary (full or part-time):; Nissan Chemical Corporation. **Y. Shintani:** A. Employment/Salary (full or part-time):; Nissan Chemical Corporation. **M. Kamaura:** A. Employment/Salary (full or part-time):; Nissan Chemical Corporation. **J. Kamon:** A. Employment/Salary (full or part-time):; Nissan Chemical Corporation.

Poster

382. Muscle and Joint Pain

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Program #/Poster #: 382.16

Topic: D.02. Somatosensation – Pain

Support: FAPESP grant 2019/26414-2
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NIH grant R01AR077019-01A1

Title: The sodium channel tamoxifen receptor as a novel target for pain control

Authors: M. MCCOLLUM[1], M. LARMORE[1], S. ISHIHARA[2], L. NG[1], ***L. KIMURA**[3], E. GUARDARRAMA[1], C. TA[1], T. VIEN[1], G. FROST[1], K. SCHEIDT[1], R. MILLER[2], P. DECAEN[1];
[1]Northwestern Univ., Chicago, IL; [2]Rush Univ., Chicago, IL; [3]Butantan Inst., São Paulo, Brazil

Abstract: Voltage-gated sodium channels (Navs) initiate action potentials required for the transmission of painful stimuli by nociceptors. Targeting Navs with drugs to produce analgesic effects for pain management is an active area of research. Recent advances in structural biology tools have enabled the resolution of several subtypes of Navs, providing a blueprint for drug design. Previously, we determined a novel receptor site for several tamoxifen analogs, including N-desmethyl tamoxifen (ND-Tam), within a prokaryotic Nav channel. Here we characterized the pharmacology of ND-Tam against eukaryotic Navs natively expressed in sensory neurons. We tested the potency of ND-Tam against endogenously expressed Navs in cultured DRG sensory neurons isolated from Nav1.8-tdTomato mice by conducting whole cell voltage clamp recordings. Sodium currents were activated by a 0.2 Hz train of -10 mV depolarizations from a holding potential of -100 mV. To determine if there was any specificity in the potency of ND-Tam against Nav subtypes, we heterologously expressed human orthologues in HEK cells and conducted voltage-clamp experiments. We used an osteoarthritis mouse model where destabilization of the medial meniscus (DMM) induces osteoarthritis and associated pain to investigate whether ND-Tam had local analgesic effects in vivo. The half maximal potency of INa inhibition (IC50) for ND-Tam was 1.7 μM ± 0.2. Interestingly, the majority (86 ± 5 %) of INa did not recover after removing ND-Tam from the bath and waiting for 5 minutes which suggests that the drug-receptor interaction has a slow dissociation constant. Electrophysiology of heterologously transfected Navs subtypes into HEK cells showed no selectivity amongst Nav1.1, Nav1.6, Nav1.7 and Nav1.8. Interestingly, when locally injected (knee joint) in mice subjected to an osteoarthritis model of pain, ND-Tam exhibited analgesic effects comparable to lidocaine, even though ND-Tam binds to a different site within the channel. Taken together, this work supports the potential of the newly characterized ND-Tam binding site within the mamalian Navs for pain control.

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Poster

382. Muscle and Joint Pain

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Program #/Poster #: 382.17

Topic: D.02. Somatosensation – Pain

Support: R01NS105715
R01NS113965
CTF-2022-01-007

Title: Schwann cell specific deletion of Nf1 leads to pain-related hypersensitivity in mice.

Authors: *N. G. R. RAUT[1], A. ADLAKHA[3], L. MAILE[5], I. MITXELENA[1], L. F. QUEME[1], L. M. OSWALT[1], L. BOKROS[1], T. A. RIZVI[4], N. RATNER[2], M. P. JANKOWSKI[4];
[2]Cincinnati Children's Hosp, [1]Cincinnati Children's hospital medical center, Cincinnati, OH; [3]Cincinnati Children's Hosp. Med. Ctr., CINCINNATI, OH; [4]Cincinnati Children's Hosp. Med. Ctr., Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH; [5]Univ. of Cincinnati Neurosci. Grad. Program, Univ. of Cincinnati, Cincinnati, OH

Abstract: Neurofibromatosis type 1 (NF1) is a genetic disorder that predisposes individuals to benign tumors in the peripheral nervous system (PNS). NF1 patients display multiple clinical manifestations including cutaneous and plexiform neurofibromas and café au lait spots; however, pain is a major contributor to their decreased quality of life. The *Nf1* gene encodes neurofibromin, a negative regulator of Ras-GTP signaling. Tumor development is thought to be due to Schwann cell (SC) specific loss of *Nf1*, but the role of SC *Nf1* in nociception and pain development is not known. Here, we wanted to determine whether SCs, sensory neurons, or the interactions between these cells, contributed to neuropathic pain-like behaviors in an animal model of NF1. We performed behavioral assessments of mechanical sensitivity, and novel single unit electrophysiological recordings of sensory neurons using our *ex vivo* hairy skin/saphenous nerve/DRG/spinal cord preparation from mice with SC specific (DhhCre;Nf1f/f) or sensory neuron specific (PirtCre;Nf1+/f) deletion of *Nf1* and compared results to Nf1+/- mice and littermate controls. We then performed similar assessments in animals' wildtype for *Nf1* with chemogenetic manipulation of SC calcium activity. Data was correlated with DRG gene expression using realtime PCR and nerve structure using electron microscopy. *Nf1* deletion in SCs produced mechanical hypersensitivity, while *Nf1* deletion in sensory neurons did not. Behavioral hypersensitivity in the SC specific *Nf1* mutants, correlated with sensitization of myelinated A-fiber nociceptors and unmyelinated polymodal C-fibers (CPM) to mechanical stimuli. This occurred with Remak bundle disruption but not tumor formation. Chemo-genetic manipulation of SC calcium activity in mice wildtype for *Nf1*, induced mechanical

hypersensitivity at the behavioral level and produced sensitization of CPM neurons to mechanical stimuli compared to controls. The observed peripheral sensitization correlated with elevation of various cytokines and growth factors in the L2/L3 DRGs that have been linked to both tumor development and pain. This suggests that alterations in SCs are at least partially responsible for changes in specific sensory neuron subtypes that possibly modulate neuropathic pain development in NF1. This study may help to identify novel cell-specific treatment strategies to ameliorate pain in NF1 patients. **Funding:** This work was supported by grants from the NIH (R01NS105715 and R01NS113965) to MPJ and the Children's Tumor Foundation (CTF-2022-01-007) to NGRR. **Keywords:** neurofibromatosis 1, behavior, electrophysiology, gene expression, electron microscopy, chemogenetics

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Poster

383. Thalamic and Cortical Processing in the Somatosensory System

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 383.01

Topic: D.03. Somatosensation – Touch

Support: Brain Korea 21 PLUS and This work was supported by the National Research Foundation of Korea(NRF) grant funded by the Korea government(MSIT)(2021R1A2C3007164)

Title: Measuring tactile discrimination performance using closed loop whisker stimulation system

Authors: *H. JEONG[1], H. KWAK[1], C. PARK[1], E. CHEONG[1], E. LEE[2];
[1]biotechnology, yonsei university, Seoul, Korea, Republic of; [2]Anat., Col. Of Medicine, Yonsei Univ., Seoul, Korea, Republic of

Abstract: Mice use active whisking combined with free movement in order to discriminate objects. In sensory discrimination, the level of motivation strongly influences sensorimotor strategy and sensory acuity. The classical head fixed, go/no-go task enables the evaluation of sensory discrimination performances of mice. However, the task does not consider the trial-by-trial variations in motivation levels. To evaluate discriminative abilities while regarding both motivation and locomotion, we developed a closed loop tactile discrimination task for head fixed mice to analyze discrimination performances and neuronal encodings only in motivated trials—meaning the subjects are involved in discrimination trials only when they intently move forward (i.e. motivated to move). Using textures with various roughness levels, we found that the closed loop task showed discriminatory performance levels comparable to those of passive stimulation discrimination tasks. These results indicate that our discrimination task system is a more

comprehensive method for measuring the level of discrimination that reflects motivation and locomotion.

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Poster

383. Thalamic and Cortical Processing in the Somatosensory System

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Title: Coordination of head, eye, and vibrissae motor actions in a goal-directed orientation behavior used by rat.

Authors: *A. FASSIHI[1], J. DUCKWORTH[3], D. KLEINFELD[2];
[1]UCSD Dept. of Neurosciences, San Diego, CA; [2]UCSD Dept. of Neurosciences, La Jolla, CA; [3]UCSD, San Diego, CA

Abstract: Animals, including humans, actively move their sensory organs to allow them to explore their environment. In particular, rats tactually perceive their local environment by actively moving their vibrissae back and forth in combination with head motion. Concurrent visual perception employs eye movements combined with head motion. While vibrissa, head, and eye can move independently, these motions coordinate during goal-directed orientation behavior. Here, stable perception of the sensory input is accomplished by concurrent adjusting of the head, eye, and vibrissae.To study how the brain executes and coordinates these orofacial movements, we developed a behavioral paradigm in which the rat uses its head and vibrissa movement to find the location of the object that is the reward spout and receives a water reward. The animal can perform excellently after two to five training sessions.To explore how the rat detects the location of a reward spout and uses the fourth sense, licking, to obtain the reward, we randomly varied the spout location every 10-15 trials. The animal produces a well-controlled head, eye, and vibrissa movement that is highly reproducible within and between animals. The motion trajectory and temporal structure of orofacial movements anticipate the endpoint trajectory of the snout, that is, the reward spout location.The temporal and spatial structure of the vibrissae motion is precisely controlled to achieve high acuity goal direction movement and is aligned to the head and eye movement onset. Vibrissae and head movement are arranged to maximize the detection probability while minimizing the movement. This implies a high-level control of vibrissa and head movement, similar to head and eye movement observed in primates. We are currently recording and manipulating, both optogenetically and pharmacologically, the activity of the superior colliculus, which is a hub for high-level control, in behaving animals.

These recordings should lead to a better understanding of how high-level control of orofacial behavior is implemented at the high-level neuronal circuit level.

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Poster

383. Thalamic and Cortical Processing in the Somatosensory System

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Topic: D.03. Somatosensation – Touch

Support: F32MH122995
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Title: Geometric Deep Learning Reveals Posture Representations in Somatosensory Cortex

Authors: *K. SEVERSON[1], J. LU[1], W. XIAO[2], H. JIANG[1], S. CHOI[1], T. DUNN[3], F. WANG[1]; [1]Brain and Cognitive Sci., MIT, Cambridge, MA; [2]Neurobio., [3]Biomed. Engin., Duke Univ., Durham, NC

Abstract: The brain contains an internal model of the spatial configuration of the body, called the "body schema". Generation of this internal model likely results from a combination of central (internal processes) and peripheral (sensory) components. Body schema representations have been observed in higher order cortical areas such as posterior parietal and premotor cortices. Proprioceptive information represented in primary somatosensory cortex (S1) is a likely "sensory origin" involved in constructing the body schema. Here, we investigated the sensory origins of body schema in mice using simultaneous electrophysiological and synchronized, 3D-calibrated multi-camera recordings. We tracked full body posture from the multi-camera video using a geometric deep learning approach called "DANNCE". DANNCE accurately tracks dozens of 3D keypoint positions, including all major joints in the limbs, in freely moving mice. Furthermore, we utilized anatomical skeletal models to extract all relevant joint angles from predicted keypoint positions. We investigated coding properties of large S1 single unit activity to combinations of joint angle and kinematic variables. S1 units showed strong correlations with posture and kinematics. Advances in pose tracking tools reveal how body posture and movement are represented in S1 during free moving behavior, which may be critical for understanding neural mechanisms underlying postural control and body schema.

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Poster

383. Thalamic and Cortical Processing in the Somatosensory System

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Title: Brain networks associated with selective activation of sensory afferents investigated by spinal cord optogenetic-fMRI

Authors: *S. KAUL[1], S. RANA[2], A. D. MICKLE[3], D. D. FULLER[2], S. VAHDAT[4];
[1]Univ. of Florida, [2]Physical Therapy, [4]Dept. of Applied Physiol. & Kinesiology, [3]Univ. of Florida, Gainesville, FL

Abstract: Background: The muscle spindles (MS) and Golgi tendon organs (GTO) give rise to group Ia and Ib afferent fibers, respectively transmitting muscle length/velocity and contractile force information to the central nervous system. Although the spinal cord circuits innervated by group Ia and Ib fibers are extensively studied, little is known about the brain circuits associated with selective activation of each of these group of fibers. Here, we aim to identify brain areas activated by selective excitation of Ia and Ib fibers from upper limb muscles in mice. We developed a novel multimodal technique that incorporates virus vector transduction via peripheral injections in the associated muscle or tendon, optogenetic stimulation of neuronal populations in the spinal cord, and functional magnetic resonance imaging (fMRI) of the whole brain.**Method:** We injected viral vector AAV9-FLEX-rev-Chr2(H134R)-mCherry in the right biceps belly (MS group) or tendon (GTO group) of PV-Cre transgenic mice that express Cre recombinase in parvalbumin-expressing neurons. PV is specifically expressed in both group Ia and Ib sensory fibers, and injections in muscle belly or tendon can selectively target these fibers. Next, an optic fiber inside a cannula was implanted at the cervical C6 level on the right dorsal horn. Brain activation using optogenetic-fMRI method (Vahdat et al., 2021) was performed (10 Hz stimulation, 6 blocks of 15 s stimulation) on a 11T Bruker scanner after 4-5 weeks of virus injection. The tissues from the dorsal root ganglion and spinal cord were extracted and stained with NeuN antibody and Anti-mCherry to confirm ChR2 expression. **Results:** Optogenetic stimulation of afferent fibers in the dorsal horn resulted in significant ($p < 0.05$, corrected) activation clusters in: 1) thalamus, SI and SII, and cerebellum and ipsilateral brainstem in the MS group, and 2) bilateral thalamus, S1, SII, M1, retrosplenial cortex, and cerebellum in the GTO group. Our results show colocalization of ChR2-mCherry expressing cells with NeuroTrace, confirming the expression of ChR2 in the cell bodies of the C6 dorsal root ganglions. **Conclusion:** Our study shows the feasibility of a novel imaging technique for mapping brain activations in response to selective stimulation of genetically defined sensory fibers using spinal cord optogenetic-fMRI. Our results provide evidence for overlapping but distinct brain networks

in response to activation of group Ia and Ib afferent fibers. Future work can use this method to examine the brain networks activated by specific groups of afferent fibers and their functional role during sensorimotor behavior.

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Poster

383. Thalamic and Cortical Processing in the Somatosensory System

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Title: Cell-type specific wiring between Ventral Posterior Thalamic nucleus neurons and Somatosensory cortices

Authors: *M. RUBIO-TEVES[1], P. MARTIN-CORREA[1], D. CASAS-TORREMOCHA[2], N. TIMONIDIS[3], R. BAKKER[3], M. GARCIA-AMADO[1], C. ALONSO-MARTINEZ[1], P. TIESINGA[3], C. PORRERO[1], F. CLASCA[1];
[1]Autónoma de Madrid Univ., Madrid, Spain; [2]Inst. d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain; [3]Radboud Univ., Nijmegen, Netherlands

Abstract: The ventral posterior complex of the thalamus (VP) relays several parallel lines of somatosensory information to the cerebral cortex. A medial nucleus (VPM) relays trigeminal information, whereas a lateral nucleus (VPL) relays spinal signals. There is limited evidence for morphological and functional heterogeneity among VP cells in separate regions of the nucleus known to relay different somatosensory submodalities. However, these differences have not been systematically explored beyond the barreloid-to-barrel pathway that conveys mechanoreceptive information about the mystacial vibrissae. Here, we examined in adult mice the fine morphology of thalamocortical (TC) VP axons innervating different regions of the somatosensory cortex that represent the mouth, tongue, lips, face, vibrissae, limbs and trunk. To this end, we made iontophoretic microinjections of biotynilated dextranamine (BDA) in multiple selected spots of VP and mapped their tangential and laminar distribution in the cortex. In addition, we analyzed isolated VP neurons we transfected with Sindbis pal-eGFP vectors as well as similar morphologies available from open source databases (MouseLight, Braintell). VP axons varicosity sizes were measured and compared between different areas and laminae. As there is some evidence for histological inhomogeneities within VP, we applied a series of immunolabeling methods to investigate the existence of marker-specific subregions that could be

correlated to the patterns of TC innervation.
Our results show that projection neurons throughout VP predominantly target a single point domain within the primary somatosensory cortex layer 4 (L4). These axons arborize densely in layer 4 with additional branches in L6, and strictly avoid L1. Some VP axons arborize in L4 of the adjacent secondary somatosensory cortex. In addition, neurons situated along the border between the VPM and VPL (that were previously identified as an input specific subdomain, VPMvl) innervate the primary somatosensory cortex (S1) layers 2,3 and 5 in loose fashion, while densely targeting the secondary somatosensory cortex (S2) L4. We observed consistent differences in the size (measured as maximum projection area) of VP axon varicosities (putative synaptic boutons) between the different subareas and layers of S1 and S2.
Our findings reveal a diverse array of thalamic output pathways directed from VP to cortex. Specific axon wiring motifs may reflect the specialized processing of information along different sensory relay routes.

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Poster

383. Thalamic and Cortical Processing in the Somatosensory System

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Topic: D.03. Somatosensation – Touch

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Neurodegenerative Consortium

Title: Heterogenous neuronal firing properties in the adult thalamic reticular nucleus

Authors: *N. RIVERA-RAMIREZ[1], R. JAGIRDAR[2], J. R. CAMPBELL[3], M. SILVA-PÉREZ[2], J. CHIN[4], M. BEIERLEIN[1];
[1]McGovern Med. Sch. at UTHealth, HOUSTON, TX; [2]Neurosci., Baylor Col. of Med., Houston, TX; [3]Neurosci., Baylor Col. of Med., HOUSTON, TX; [4]Dept. of Neurosci., Baylor Col. of Med., Houston, TX

Abstract: GABAergic neurons in the thalamic reticular nucleus (TRN) provide the main source of inhibition to thalamic nuclei, thereby controlling sensory processing, attention, and sleep-related rhythmic activity. TRN dysfunction and the resulting disruption of thalamocortical circuit activity has been implicated in neurodevelopmental disorders including schizophrenia and autism. Additionally, we previously found reduced TRN activity and sleep deficits in amyloid precursor protein transgenic mice (APP mice), a model commonly used to study Alzheimer's disease (AD), suggesting a key role of TRN dysfunction in AD progression. To probe the underlying mechanisms, we investigated the intrinsic firing properties of TRN neurons in mature

(3-4 months) APP mice, which have deficits in sleep and memory but no plaque deposition yet, and their wildtype littermates. Studies in younger wildtype adult mice (<1 month) have shown that TRN neurons in a given functional modality are heterogenous in their molecular and electrophysiological properties, and form discrete functional sub-circuits based on their location within distinct TRN laminae: i) TRN neurons in the central core express calbindin, display rebound bursting, and reciprocally connect with first-order thalamic nuclei, while ii) TRN neurons in the shell express somatostatin, lack robust bursting and interconnect with higher order thalamic nuclei. In agreement with these findings, we found that core and shell neurons show dramatically different intrinsic properties, with core neurons exhibiting a lower rheobase, action potentials with faster kinetics, and more rebound spiking activity evoked by hyperpolarizing current steps. However, in contrast to previous studies in younger animals, core neurons displayed a higher diversity of firing patterns evoked by hyperpolarizing current steps, including rhythmic bursting, persistent firing (PF), and sparse firing. As core neurons are directly involved in the generation of sleep-related rhythmic activity, we determined their properties in APP mice. We found that TRN heterogeneity remains high in APP mice, with fewer neurons generating PF. Furthermore, core neurons generated lower peak firing rates at threshold and lower maximum firing rates compared to wildtype littermates. Together, our studies highlight extensive TRN neuronal diversity in fully mature animals, which might enable the TRN to carry out a variety of computational tasks during distinct behavioral states. Furthermore, our observation of reduced excitability of TRN core neurons in APP mice indicates that specific TRN sub-networks might be particularly affected during pre-clinical stages of AD.

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Poster

383. Thalamic and Cortical Processing in the Somatosensory System

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Topic: D.03. Somatosensation – Touch

Support: NS103993
PR201842

Title: Cortical and thalamic input to pairs of parvalbumin positive interneurons and pyramidal excitatory neurons is correlated

Authors: *R. GOZ, N. SCHNEIDER, M. ARNOLD, R. WILLIAMSON, B. HOOKS;
Univ. of Pittsburgh, The Univ. of Pittsburgh, Pittsburgh, PA

Abstract: In mammalian cortex, feedforward excitatory connections invariably recruit feedforward inhibition. This is often carried by fast-spiking (parvalbumin, PV+) interneurons, which potentially connect densely to local pyramidal (Pyr) neurons. Whether this inhibition

generically inhibits all local excitatory cells or is targeted to specific subnetworks is unknown. Here, we test how feedforward inhibition is recruited by cortical and thalamic afferents by using 2-channel circuit mapping to excite (S1 and PO) inputs to PV+ interneurons and pyramidal neurons of mouse motor cortex. We find that connected pairs of PV+ interneurons and excitatory pyramidal neurons receive correlated cortical and thalamic inputs. This suggest that excitatory inputs to M1 target inhibitory networks in a specific pattern which permits recruitment of feedforward inhibition to specific subnetworks within the cortical column. We then develop methods for in vivo circuit mapping to study changes in the connection strength of cortical and thalamic inputs to PV+ and Pyr neurons. This will enable a temporal understanding of how synaptic connectivity changes in cortical circuits during learning and disease.

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Poster

383. Thalamic and Cortical Processing in the Somatosensory System

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Topic: D.03. Somatosensation – Touch

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Title: Burst firing, cortical connectivity, and oscillatory behavior of thalamocortical neurons driven by the thalamus-specific transcription factor, Shox2

Authors: *I. G. FEBBO[1], L. A. MARTINEZ[3,4], V. WARKINS[1], N. VARGAS[1], J. LIANG[1], A. E. ANDERSON[3,4], L. A. SCHRADER[2,1];
[1]Neurosci. Brain Inst., [2]Cell and Mol. Biol., Tulane Univ., New Orleans, LA; [3]Pediatrics-Neurology, Baylor Col. of Med., Houston, TX; [4]The Cain Fndn. Labs. and the Jan and Dan Duncan Neurolog. Res. Inst., Texas Children's Hosp., Houston, TX

Abstract: Recent studies have revealed that a biomarker for both autism and schizophrenia is a reduction in sleep spindle oscillation density. Sleep spindles are thought to originate in the thalamus and have been shown to be important for memory consolidation during sleep. Here, we study whether the thalamus-specific transcription factor, *Shox2*, plays a role in the development and maintenance of thalamocortical neuron (TCN) characteristics that are critical to these sleep spindle oscillations, which would suggest a link between *Shox2* and sensory perception disorders. We surgically injected a Cre virus into the ventrobasal (VB) nucleus of the thalamus of *Shox2 fl/fl* mice at P6 and P21 and analyzed effects from cellular to behavioral levels. Specifically, we investigated: TCN ion currents and burst firing using patch clamp electrophysiology, TCN connectivity to the cortex and VB TCN cortical target organization using immunochemistry, thalamocortical network spindles using in vivo electrophysiology, sensory perception, and sleep spindle related behavior. Consistent with our previous findings, we

discovered that *Shox2* regulates key ion channels, the T-type Ca^{2+} and hyperpolarization-activated, cyclic-nucleotide gated (HCN) channels that underlie TCN burst firing. In addition, we found that in P21 KO vs. CTL TCNs, area under the post-anodal burst curve and number of action potentials per burst are significantly reduced. Cytochrome oxidase and VGlut2 staining of VB cortical targets (cortical barrels) revealed that *Shox2* KO at P6, but not P21, disrupted barrel organization. Further, P21 KO significantly reduced sleep spindle oscillation density in adult mice. These cellular and network effects culminated into significantly impaired whisker somatosensation and significantly impaired memory consolidation in these mice. These data strongly indicate a critical role for *Shox2* in development and maintenance of proper thalamic activity, specifically, sensory perception and memory consolidation.

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Poster

383. Thalamic and Cortical Processing in the Somatosensory System

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Topic: D.03. Somatosensation – Touch

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Title: Cortical control of thalamocortical cell burst/tonic firing mode in open- and closed-loop circuit organizations

Authors: *J. MOREIRA[1], B. KOCAOGLU[2], S. AHMAD[3], C. VARELA[2], S. DURA-BERNAL[1,4];
[1]Physiol. and Pharmacol., SUNY Downstate Hlth. Sci. Univ., BROOKLYN, NY; [2]Psychology, Florida Atlantic Univ., Boca Raton, FL; [3]Numenta, Redwood City, CA; [4]Ctr. for Biomed. Imaging and Neuromodulation, Nathan Kline Inst. for Psychiatric Res., Orangeburg, NY

Abstract: The thalamus is a key router of information in and out of the cerebral cortex. It is mostly composed of excitatory thalamocortical (TC) cells, segregated in different functionally organized nuclei, and projects to the different cortical regions. TC cells fire in tonic- (Na+-mediated) and burst-mode (Ca2+-mediated, T-channels). Burst firing is ubiquitous during sleep, and tonic firing dominates during awake/attentive behavior. However, burst firing can still occur in awake/attentive conditions, and it is thought to act as a wake-up call to cortex, redirecting the focus of the network to an incoming stream of information. Despite the intricate patterns of information processing in the cortex, the structure of the corticothalamic (CT) and TC projections follow a consistent organization. CT cells from cortical layer 6 send excitatory projections that branch out to the thalamic reticular nucleus (TRN), which is composed exclusively of inhibitory neurons and provide a source of disynaptic inhibition to the thalamus. Although the precise organization of these CT and TRN projections is still an open question,

recent studies show that they innervate nearby areas and provide further insight into the wiring of this circuitry. This leads to new research questions regarding 1) whether CT/TRN projections follow an open- or closed-loop organization and 2) how the mechanisms of cortical modulation might regulate the "gain" of driver inputs into the TC cells, making the cell more susceptible to fire in burst- or tonic-mode. The size of the TC cell ensemble recruited in the process of forwarding a message to the cortex is also undetermined. An understanding of the processing at the single-cell level could help determine the role of ensembles, and whether the dendritic computations within a single TC cell can by itself carry out this function. In this study we evaluated the ability of modulatory inputs from CT and TRN targeting the branches of TC cells to regulate the switch between burst and tonic firing in response to driver inputs. Using a three-compartment model of a TC cell, we demonstrate that differential depolarization/hyperpolarization of the dendritic branches can prime different segments of the neuron to a tonic-ready/burst-ready mode with localized differences in dendritic resting membrane potential and local deinactivation of T-channels. This "gain" mechanism can also be implemented using different connectivity schemes, previously proposed in the literature. The existence of such a mechanism expands the computational power currently attributed to TC cells, providing an ability to process information at the single-cell level.

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Poster

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Title: Dynamic corticothalamic gain modulation of the somatosensory thalamocortical circuit during wakefulness

Authors: *E. DIMWAMWA[1], A. PALA[2], V. CHUNDRU[3], N. C. WRIGHT[2], G. B. STANLEY[1];
[1]Georgia Inst. of Technol., [3]Biomed. Engin., [2]Georgia Inst. of Technol., Atlanta, GA

Abstract: Amid the traditionally studied feedforward neuronal pathways that enable perception through our senses are numerous feedback processes. Corticothalamic feedback from layer 6 of cortex (L6CT) is one such process that provides extensive input to the thalamus, in addition to direct intracortical inputs. L6CT neurons are well-positioned to play an important role in thalamocortical sensory signaling. Yet, their function remains elusive. Using the vibrissa system

of awake, NTSR1-cre mice selectively expressing channelrhodopsin-2 in L6CT neurons, we conducted extracellular recordings of populations of individual neuron spiking in the primary somatosensory cortex (S1) as well as in the ventral posteriomedial (VPm) and reticular (TRN) nuclei of the thalamus. We investigated how optical activation of L6CT neuron spiking modulated thalamic and cortical activity.
We found that with increasing optogenetic drive of L6CT neurons, VPm ongoing activity switched from net suppression to net enhancement of spiking. This result could not be explained by the effect of L6CT activation onto TRN because we did not find a decrease in TRN drive under conditions where VPm was enhanced versus suppressed.
A possible explanation for the bidirectional effect of L6CT activation onto VPm could be something akin to the window of opportunity (WOO) observed in cortical neurons receiving thalamic input. The WOO is the period in which monosynaptic excitation summates before being dwarfed by strong, disynaptic inhibition. Extensive work has shown that such integration is highly sensitive to the synchrony of the inputs. Therefore, in order to increase the synchrony of L6CT neurons while keeping their firing rates constant, we delivered noisy optical stimuli of constant mean and increasing variance. We found that such optogenetic stimuli were able to elicit bidirectional changes in VPm activity, and thus concluded that L6CT synchrony is likely a primary driving factor in regulating VPm activity.
We then explored the effect of L6CT activation on sensory responses evoked by single whisker stimulation in VPm and S1. In both areas, we found heterogeneous effects of L6CT drive on stimulus-evoked responses, which we are further investigating in ongoing work. Altogether our work suggests a rate and synchrony-dependent role of L6CT neurons in shaping ongoing and stimulus-evoked activity in the thalamocortical circuit.

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Poster

383. Thalamic and Cortical Processing in the Somatosensory System

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Transition Grant Support; Office of Research and Graduate Education, WVU Health Sciences Center

Title: Fast (400 Hz) network oscillations evoked by optogenetic thalamocortical stimulation

Authors: H. HU, R. HOSTETLER, ***A. AGMON**;
Neurosci., West Virginia Univ., Morgantown, WV

Abstract: Network oscillations, the extracellular manifestation of synchronous, rhythmic activity in large but localized neuronal assemblies, are a ubiquitous phenomenon in multiple

mammalian species. Frequencies of brain oscillations span 3 or more orders of magnitude, and different frequency bands are often hallmarks of specific brain or behavioral states. At the higher end of the scale, brief bouts of 150-200 Hz “ripples” in the hippocampus occur during quiet immobility and slow-wave sleep, and are thought to be crucial for memory consolidation, while “fast ripples”, at 250-500 Hz, are considered biomarkers of epileptogenic tissue. We describe here “ripplets”, a novel, transient (<25 ms), high-frequency (>400 Hz) network oscillation in thalamorecipient cortical layers 4 and 5B. Ripplets were elicited by brief (as short as 1 ms) optogenetic stimulation of thalamocortical axons and terminals, in brain slices from mice genetically expressing ChR2 in the ventrobasal thalamus. Although in the “fast ripple” frequency band, ripplets were clearly a non-paroxysmal oscillation; epileptiform activity was observed only when fast inhibition was blocked pharmacologically. A ripplet consisted of 2-5 negative waves in the local field potential (LFP). Simultaneously recorded fast-spiking (FS) inhibitory interneurons fired a highly reproducible burst of 3-6 spikes, almost exactly out of phase with the LFP. Pairs of FS cells fired in exquisitely precise, sub-millisecond synchrony, regardless of electrical or chemical coupling between them. Excitatory, regular-spiking cells rarely fired more than 1-2 spikes per ripplet; however an alternating sequence of subthreshold EPSCs and IPSCs, entrained to the ripplet oscillation, was evident under voltage-clamp at a membrane potential of -50 mV. Bursts in presynaptic thalamocortical axons (when evident) were slower than the ripplet frequency, suggesting that ripplets were generated de-novo within the local cortical network, through a reciprocating synaptic exchange between excitatory and inhibitory neurons. The necessity of recurrent excitatory connections was demonstrated by the abolishment of FS bursts in layer 5B when layer 5 was disconnected by a cut from its strong source of excitatory input in L4. Our experiments were conducted in brain slices; however similar oscillations were sporadically reported previously in vivo, suggesting that ripplets are a physiological response of sensory cortices to a strong, transient sensory input. The functional significance of ripplets, and whether they contain (like hippocampal ripples) precise spike sequences which encode sensory experience, remains to be determined.

Disclosures: **H. Hu:** None. **R. Hostetler:** None. **A. Agmon:** None.

Poster

383. Thalamic and Cortical Processing in the Somatosensory System

Location: SDCC Halls B-H

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Program #/Poster #: 383.12

Topic: D.03. Somatosensation – Touch

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Title: Stimulated emission depletion (STED) super-resolution imaging of thalamocortical synapses on layer 4-targeting somatostatin interneurons

Authors: *R. HOSTETLER, M. HRUSKA, A. AGMON;
Neurosci., West Virginia Univ., Morgantown, WV

Abstract: Somatostatin-positive (SOM) interneurons are the second largest group of inhibitory interneurons in the neocortex. They are involved in complex cognitive tasks such as sensory processing, learning, and memory, and their dysfunction is implicated in disorders with sensory and cognitive deficits such as schizophrenia and Alzheimer's. Sensory information enters the neocortex in layers 4 and 5 through projections from the sensory thalamus. In the rodent somatosensory "barrel" cortex, these projections come from the ventral posteromedial nucleus (VPM). Previous ultrastructural and electrophysiological work by multiple labs has confirmed that VPM projections make monosynaptic connections on excitatory "regular spiking" cells and parvalbumin-containing (PV) "fast spiking" inhibitory interneurons. However, there are conflicting reports regarding thalamocortical synapses on SOM cells. These differing results could reflect the often overlooked diversity within the SOM cell population. Previous electrophysiological work from our lab has shown monosynaptic thalamocortical inputs on a subset of layer 4-targeting SOM cells which express GFP in the X94 transgenic mouse line. Our aim in the current project was to confirm the presence of thalamocortical synapses on X94 SOM cells anatomically, using STimulated Emission Depletion (STED) Microscopy. STED microscopy has an X-Y resolution of 50 nm, 5x higher than the limit of conventional light microscopy. Male and female mice were used, age 6+ weeks. Using immunostaining for vesicular glutamate transporter 2 (VGluT2), a marker of thalamocortical terminals, bassoon, a marker of presynaptic active zones, and PSD95, a major component of the excitatory postsynaptic density, we identified close appositions of all three markers on the cell bodies and dendrites of X94 SOM cells in layers 4 and 5. Ongoing experiments are examining the spatial distribution of these synapses and comparing them to thalamocortical synapses on PV interneurons. Through these experiments, we have confirmed a previously disputed monosynaptic thalamocortical input onto SOM interneurons. Elucidating thalamocortical circuitry is critical for gaining a better understanding of sensory processing and neuropsychiatric disorders with sensory processing deficits.

Disclosures: R. Hostetler: None. **M. Hruska:** None. **A. Agmon:** None.

Poster

383. Thalamic and Cortical Processing in the Somatosensory System

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Program #/Poster #: 383.13

Topic: D.03. Somatosensation – Touch

Support: Department of Pharmacology and Nutritional Sciences Chair's Pilot Research Award

Title: Neuronal calcium network alterations in layers 2/3 of somatosensory cortex mediate age-dependent impairments in tactile encoding and reflect on gait dysregulation

Authors: *S. L. CASE, R.-L. LIN, H. N. FRAZIER, O. THIBAULT;
Dept. of Pharmacol. & Nutritional Sci., Univ. of Kentucky Col. of Med., Lexington, KY

Abstract: Over the past 30 years, the calcium (Ca^{2+}) hypothesis of brain aging has suggested that neuronal Ca^{2+} dysregulation is a key biomarker of aging. Indeed, age-dependent Ca^{2+}-mediated changes in intrinsic excitability, synaptic plasticity, and environmental mapping activity (i.e., using electrophysiological or imaging techniques) have helped identify some of the mechanisms engaged in memory and cognitive decline. Here, we used two-photon (2P) imaging techniques *in vivo*, paired with a genetically encoded, fluorescent Ca^{2+} nanosensor (GCaMP8f) to characterize neuronal networks in the primary somatosensory cortex (S1), an area involved in proprioception and tactile discrimination. A Morse continuous wavelet transform (CWT) analysis was developed (MATLAB) to extract network communication variables (overall activity, connectivity, synchronicity & connection length) and graph correlogram data, in order to detect both aging- and surface-related changes in the network performance. Extraction of GCaMP8f data in S1 cortex of C56BL/6J mice during ambulation across surfaces of varying textures revealed alterations with aging. In young and aged mice, and compared to smooth surfaces, a surface that provided more points of contact on the paw pad yielded greater network activity. Additionally, measures of gait (deviation from center, variability, paw precision) were obtained while mice ambulated down a 3-plane visualization task as well as measures of forelimb and hindlimb grip strength. The work highlights central differences in neuronal Ca^{2+} network encoding with age in S1 and may reflect on the reduced locomotor stability seen in aging.

Disclosures: **S.L. Case:** None. **R. Lin:** None. **H.N. Frazier:** None. **O. Thibault:** None.

Poster

383. Thalamic and Cortical Processing in the Somatosensory System

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Title: Effects on neocortical inhibitory circuits underlying sensory representation in adults after developmental excitation of neocortical pyramidal neurons

Authors: *E. L. CRESPO[1], G. FOLKERT[2], J. W. MURPHY[4], C. I. MOORE[4], U. HOCHGESCHWENDER[3];

[1]Biochemistry, Cell. and Mol. Biol. Program - Col. of Med., [2]Col. of Med., [3]Neurosci., Central Michigan Univ., Mount Pleasant, MI; [4]Neurosci., Brown Univ., Providence, RI

Abstract: Disturbing spontaneous neural activity during developmental processes through genetic or environmental causes is linked to altered adult behavior in neurodevelopmental psychiatric disorders. However, it is difficult to identify a link to specific cell types due to the complexity of underlying etiological susceptibilities. To experimentally address the question of whether early life alterations in neural activity can induce permanent behavioral phenotypes and related circuit changes, we systematically and selectively enhanced pan-neocortical Emx1+ pyramidal activity levels during early postnatal development. We have previously found that early hyperexcitation of pyramidal neurons alone, and for a limited time window, is sufficient to create adult neurophysiological and behavioral signatures of autism spectrum disorder (ASD) phenotypes in the absence of other insults (e.g. genetic or environmental). The observed changes replicated those seen in other models of ASD and in ASD patients: social deficits, stereotypic movements, hyperexcitable pyramidal neurons, and deficits in inhibitory neuron function.Our prior in vivo electrophysiology studies showed changes in the prefrontal cortex, a target motivated by our observed behavioral deficits in social interaction. However, several studies suggest that primary sensory neocortex may also be transformed by this manipulation. We are systematically testing the parallel hypothesis that sensory hypersensitivity and related electrophysiological changes occur in our established experimental model. To this end, we are testing the key prediction that increasing activity of pyramidal neurons during early postnatal development leads to persistent alterations in firing properties of inhibitory fast spiking interneurons (FS), and deficits in sensory processing in adults. We are conducting simultaneous in vivo electrophysiology recordings in mouse vibrissa somatosensory cortex (vSI) while presenting fixed-frequency vibrissa stimuli at varying amplitudes as a probe to relate neocortical dynamics with sensory processing. Our results further dissect basic mechanisms of neocortical sensory processing and conceptually broaden discussions of ASD pathophysiology by considering developmental neural hyperexcitation as a convergent driver across etiologies of ASD and other neurodevelopmental disorders.

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Poster

383. Thalamic and Cortical Processing in the Somatosensory System

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Program #/Poster #: 383.15

Topic: D.03. Somatosensation – Touch

Support: Deutsche Forschungsgemeinschaft (DFG) GR3757-4/1

Title: Neuronal circuits for salience encoding and cognitive flexibility in freely moving mice

Authors: *F. HEIMBURG, J. TIMM, N. SALUTI, L. EMBRAY, M. KLUMPP, A. GROH; Univ. Heidelberg, Univ. Heidelberg, Heidelberg, Germany

Abstract: The original conception, according to which peripheral impressions are conducted almost unchanged to the cortex, has been increasingly refuted in recent years. Instead, it has been recognized that complex thalamocortical interactions exert a significant influence on our perception and actions. We address this framework in a mouse model (C57BL/6, n=6), by simultaneously recording location-recovered extracellular signals from the whisker-related higher-order posterior nucleus of the thalamus (POm), its first-order counterpart, the ventral posteromedial nucleus (VPM), the strong inhibitory zona incerta (ZI), and the primary somatosensory barrel cortex (BC). In a newly developed tactile behavioral paradigm, mice learn to discriminate between two different apertures, linked to either a reward or a punishment. Mice learned the first rule set faster than the rule change (mean 388 trials SD 53 trials vs. 1077 ± 330, respectively). Inhibiting the tactile inputs, by applying lidocaine onto the whisker pads, deteriorated the performance (median d-prime 3.01 vs. d-prime 0.67, respectively, Paired t-Test p-value ≤ 0.001). A “neutral” aperture, without outcome, elicited an intermediate lick response, in between lick rates for rewarded and punished apertures, proving that the mice were able to discriminate multiple aperture states. The amount of POm units (19.15% in the first two vs. 50% in the last two learning sessions, Paired t-Test p-value ≤ 0.05), but not of VPM units (20,42% in the first two vs. 34,38% in the last two learning sessions, Paired t-Test n.s.), which respond to the touch onset with the apertures, significantly increases with the learning progression of the tactile task. Moreover, the data suggests a biphasic activation of POm during learning, with two peaks in the response upon whisker contact with the apertures. The response of POm units upon whisker contact is pronounced at the beginning of learning, but less so during consolidation of the newly learned rule. This pattern might reflect encoding of novelty and contextual relevance emerging with task-expertness. The pronounced recruitment of POm units during learning is paralleled by a marked disengagement of the zona incerta, rendering incerto-thalamic interactions a possible checkpoint controlling thalamocortical remodeling during learning. The bursting ratio of POm units was higher compared to VPM units, making bursting behavior a valuable candidate for transmitting task-relevant contextual information. Taken together, POm dynamics strongly reflect learning dynamics, suggesting an implication of higher-order thalamic pathways in the context of valence-driven learning.

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Poster

383. Thalamic and Cortical Processing in the Somatosensory System

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Topic: D.03. Somatosensation – Touch

Support: NINDS Grant R01NS101325
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Title: Texture coding in higher order somatosensory cortices

Authors: *K. H. LONG[1], C. M. GREENSPON[4], A. VAN DRIESCHE[2], S. J. BENSMAIA[3]; [1]Med. Scientist Training Program, [2]Dept. of Organismal Biol. & Anat., [3]Dept. of Organismal Biol. and Anat., Univ. of Chicago, Chicago, IL; [4]Dept. of Organismal Biol. & Anat., Chicago Univ., Chicago, IL

Abstract: Our sense of touch confers to us a sensitivity to textures that spans six orders of magnitude in spatial scale from tens of nanometers to tens of millimeters. A texture sensation can be described using a broad range of qualitative descriptors, implying a complex sensory space. We have previously characterized the coding of natural textures in the somatosensory nerves, and in the cutaneous modules of somatosensory cortex (SC), including Brodmann's area 3b, 1, and 2. In the present study, we wished to extent this analysis to higher somatosensory cortex, including the secondary somatosensory cortex (S2) and the parietal ventral area (PV). To this end, we recorded single-unit activity in S2/PV while animals performed a judged whether two textures sequentially scanned across the skin were the same or different. We then characterized these neural responses and compared them to their counterparts in SC. First, we found that neurons in S2/PV tend to respond less strongly and reliably to textures than do neurons in SC. Secondly, we found that the dimensionality of S2/PV responses was substantially higher than that of SC. That is, S2/PV neurons exhibited much more heterogeneous responses than did their SC counterpart. Moreover, the dominant axis of variance of the population response in S2/PV was not strongly related to roughness as it is in SC. Finally, we found that S2/PV neurons carry non-texture related signals that relate to decision making or task variables, unlike SC neurons. The present study constitutes the first attempt to characterize the sensory representations in S2/PV using complex, naturalistic stimuli.

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Poster

383. Thalamic and Cortical Processing in the Somatosensory System

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Title: Tactile stimuli-delivering systems allowing active touch for fMRI study with humans and electrophysiology with non-human primates

Authors: *J.-W. LEE[1], D. PARK[2], S. HWANG[1], S.-H. LEE[3], H. F. KIM[1];
[1]Seoul Natl. Univ., Seoul, Korea, Republic of; [2]Bio and Brain Engin., [3]Dept. of Bio and Brain Engin., Korea Advanced Inst. of Sci. and Technol., Daejeon, Korea, Republic of

Abstract: Primates have well-developed hands and fingers and actively move their fingers to recognize an object, comprehend its current condition, and make an appropriate decision. It is therefore necessary to investigate the brain processes of tactile perception through active touch for understanding our perception and behavior. To examine the process of tactile perception, we developed two systems for delivering tactile stimuli, which are compatible to functional magnetic resonance imaging (fMRI) with human subjects and electrophysiology with non-human primates (NHP). In the MR-compatible system for the delivery of tactile stimuli, a pneumatic stepper actuator moves tactile stimuli blocks on a rail and locates a certain stimulus among them at a presentation hole. Participants actively touched a tactile stimulus through the hole, which was detected by a photosensor. To test the system, we measured BOLD responses while the participants were instructed to move their fingers in and out in response to the color changes of a fixation cross on the screen, and they had to determine whether the braille pattern in the current trials matched that presented in the previous trial. To identify the brain regions that selectively process the tactile perception, brain responses to the braille stimuli were compared to the responses to the visual fractal objects. In our new tactile system, stronger BOLD responses were found during active tactile perception compared to visual perception in the middle part of the contralateral primary somatosensory cortex. In the electrophysiology-compatible system for NHP, two braille units are located in the holes, and photosensors are placed at the entrance of holes to detect monkeys' active finger movements. To test whether a monkey can discriminate different braille patterns and remember values of stimuli, we used Tactile Value Discrimination Task (TVDT). In this TVDT, the monkey actively touched and discriminated two different braille patterns presented in the two holes. Each braille pattern was associated with a high or low reward value (e.g. pattern1-water reward and pattern2-no reward). Through the active tactile perception, the monkey not only discriminated braille patterns but also learned which is the high-valued braille and chose it with a successful 79.286 ± 0.088% correct rate. Our tactile stimuli-delivery systems allow humans and NHPs to actively touch and recognize tactile stimuli and their values. Using these systems, we will investigate the neural mechanisms of tactile perception, tactile valuation, tactile memory, and multisensory integration with humans and non-human primates.

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Poster

383. Thalamic and Cortical Processing in the Somatosensory System

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Topic: D.03. Somatosensation – Touch

Support: DoD SCIRP SC180308
VAMR 5I01RX002654

Title: Cortical representation of single-location tactile indentation of the human fingertip

Authors: *B. C. HUTCHISON[1], P. BHAT[1], J. T. KRALL[1], A. KETTING-OLIVIER[1], W. D. MEMBERG[1,3], R. F. KIRSCH[1,3,2], J. P. MILLER[4,3,2], E. L. GRACZYK[1,3,2], B. AJIBOYE[1,3,2]; [1]Biomed. Engin., [2]Sch. of Med., Case Western Reserve Univ., Cleveland, OH; [3]FES Ctr. of Excellence, Rehab. R&D Service, Louis Stokes Cleveland Dept. of Veterans Affairs Med. Ctr., Cleveland, OH; [4]Univ. Hosp., Cleveland, OH

Abstract: Loss of somatosensation and movement following high cervical spinal cord injury (SCI) compromises the ability of individuals to dexterously interact with objects. Our long-term goal is to develop a bi-directional brain-machine interface (BMI) controlled neuroprosthesis for motor and sensory restoration of hand function. Towards that end, the present study investigates the cortical representation of single location touch in the human sensorimotor cortex, including the primary somatosensory cortex (S1), primary motor cortex (M1), inferior frontal gyrus (IFG), and anterior intraparietal cortex (AIP). A human participant with sensory incomplete C4 AIS B SCI enrolled in the Reconnecting the Hand and Arm to the Brain (ReHAB) Clinical Trial and received penetrating microelectrode arrays in S1, M1, IFG, and AIP. A linear actuator was used to indent the ring fingertip to various depths and at various rates; each condition was repeated 10 times. For each electrode with adequate signal-to-noise ratio, the mean multi-unit firing rate (FR) was calculated during a 100 ms window after indentation onset and normalized to the baseline quiescent period of two seconds before indentation onset. The mean normalized FR were compared across conditions on an electrode-by-electrode basis. The mean normalized FR was significantly different for 19/46 (41%) electrodes in S1 (17 excitatory, 2 inhibitory), 4/9 (44%) of electrodes in M1 (1 excitatory, 3 inhibitory), 19/33 (58%) electrodes in AIP (7 excitatory, 12 inhibitory), and 25/34 (74%) electrodes in IFG (16 excitatory, 9 inhibitory) (t-test, $p < 0.05$). Further analysis was only conducted on S1 and IFG electrodes. S1 FRs were modulated by rate for 10 electrodes and/or depth for 3 electrodes. IFG FRs were modulated by rate for 3 electrodes and/or depth for 3 electrodes (2-way ANOVA, $p < 0.05$). For electrodes that modulated to rate and/or depth, linear regression models correlated the FR on the electrode to the indentation rate and depth. In S1, the mean R^2 of the models was 0.18 +/- 0.11. In IFG, the mean R^2 of the models was 0.1 +/- 0.04. Preliminary results demonstrate that information about tactile stimuli in the periphery can be detected in the cortex in humans with sensory incomplete SCI. The low R^2 values suggest that additional terms need to be added to the linear model to explain the variance in the data. Future studies will examine cortical responses to tactile stimuli applied to other fingers and to combinations of fingers simultaneously. This information will be important for the development of an intracortical sensory neuroprosthesis to provide multi-finger touch feedback during use of brain computer interface (BCI) systems.

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Poster

383. Thalamic and Cortical Processing in the Somatosensory System

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Topic: D.03. Somatosensation – Touch

Support: DoD SCIRP SC180308
DARPA INI HR00111990044

Title: Representation of peripheral nerve stimulation-evoked neural activity in the somatosensory cortex

Authors: *P. R. BHAT[1,3], B. C. HUTCHISON[1,3], W. D. MEMBERG[1,3], R. F. KIRSCH[1,3,2], J. P. MILLER[3,4,2], B. AJIBOYE[1,3,2], E. L. GRACZYK[1,3,2];
[1]Biomed. Engin., [2]Sch. of Med., Case Western Reserve Univ., Cleveland, OH; [3]FES Ctr. of Excellence, Rehab. R&D Service, Louis Stokes Cleveland Dept. of Veterans Affairs Med. Ctr., Cleveland, OH; [4]Neurol., Univ. Hosp. Cleveland Med. Ctr., Cleveland, OH

Abstract: Tactile sensation is important for hand function because it helps with object manipulation and interpersonal connection. One promising avenue for supplying sensation to individuals with somatosensory deficits is peripheral nerve stimulation (PNS). We do not currently understand how PNS is processed in the brain, yet this critical information could help researchers design optimal PNS paradigms. The purpose of the study was to examine the psychophysical and cortical response to PNS in a human subject with sensory-incomplete tetraplegia.
PNS was delivered through electrode contacts of a Composite Flat-Interface Nerve Electrode (C-FINE) implanted around the median nerve in the upper arm. The cortical response was measured via two 64-channel intracortical microelectrode arrays placed in the primary somatosensory cortex (S1) of the contralateral hemisphere. The pulse width (PW) of the PNS was varied over a large dynamic range including 3 subthreshold and 6 suprathreshold values. To establish the PW conditions, we used a staircase approach to find the sensory detection threshold and maximum comfortable limit for target contacts. We presented the PW conditions and a control condition without stimulation to the subject for 2 seconds and 15 times each in a random order. We calculated firing rate (FR) from multi-unit activity and normalized it to baseline. We also collected the perceived location and intensity of all sensory percepts evoked by PNS.
The participant reported sensation on the index finger from PNS on 6 out of 15 C-FINE contacts. The normalized perceived intensity increased linearly as the suprathreshold PW increased (R^2=.64). The mean intensity ratings were significantly different among all the suprathreshold PW conditions (ANOVA, $p<0.001$) but not between any of the subthreshold conditions. Individual S1 array channels demonstrated an increase in FR during the onset of stimuli (one sample t-test, $p<0.001$). The peak normalized firing rate during the trial increased as the PW increased (ANOVA, $p<0.001$). Channels were labelled as “responsive” if the peak normalized firing rate exceeded 2 SD above baseline. There were more responsive channels present as PW increased (ANOVA, $p<0.01$). The number of responsive channels and the normalized perceived intensity had a weak but significant (F-test, $p<0.001$) positive correlation (R^2=.12).

This study demonstrates that PNS produces detectable cortical activity that is scalable to PW modulation. These findings can inform the design of PNS paradigms that will be more functional for sensory applications, such as touch restoration for individuals with spinal cord injury, limb loss, or stroke.

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Poster

383. Thalamic and Cortical Processing in the Somatosensory System

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Topic: D.03. Somatosensation – Touch

Support: NIH Grant DC014519

Title: Voice-related cortical activity is associated with the onset of glottal adduction and responds to rapid laryngeal somatosensory perturbation

Authors: H. SAKE, T. FRODEL, A. KESTOL, M. SMARZINSKI, A. GERNENZ, E. HUFF, O. KORZYUKOV, ***M. HAMMER**;
Wisconsin Airway Sensory Physiol. Lab., Whitewater, WI

Abstract: Vocalization is a complex sensorimotor task involving precisely coordinated movements of respiratory, laryngeal, and supralaryngeal structures. It would be an advantage to provide the nervous system with the wealth of available movement detail related to vocalization including movement and position of the vocal folds and other laryngeal structures, movement of air flow through the larynx, and any unexpected somatosensory perturbations that may occur during vocalization. Although previous studies suggest that laryngeal afferent mechanisms are important for voice production and voice disorders, no previous studies known to these authors have directly examined cortical responses to phonatory onset or to a rapid somatosensory perturbation during vocalization. Therefore, our two-fold goal was (a) to determine the cortical activity associated with the onset of phonation, timed precisely to the onset of vocal fold adduction and (b) to provide a somatosensory perturbation during ongoing vocalization that would yield a reliable robust cortical response to determine the cortical processing of laryngeal sensory input. We tested a group of 12 healthy young right-handed adults using whole-head electroencephalography (EEG). EEG data were aligned to the onset of vocal fold adduction to examine the association of cortical activity with the motor action of the larynx for phonatory onset. EEG data were aligned to the onset of somatosensory perturbation to examine the association of cortical activity with somatosensory input. Consistent with our hypotheses, we found an orderly pattern of activity in the sensorimotor cortices, consistent with cortical control of vocalization and processing of voice-related somatosensory input. For the onset of vocal fold adduction, we found a significant increase in the amplitude of the ~10Hz spectral band of

cortical activity in electrodes specifically overlying sensorimotor regions, with significantly greater changes in the left vs. right hemisphere. For responses to the somatosensory perturbation, we observed a significant initial event-related response with a peak latency of approximately 35-40ms, with significant increases in the amplitude of the ~10Hz spectral band of sensorimotor cortical activity.These findings suggests (a) an initial decoupling of voice-related sensorimotor regions prior to the onset of phonation, perhaps enabling adjustments based on thalamocortical inputs and (b) the rapid transmission of unexpected sensory input to sensorimotor cortical regions, perhaps enabling laryngeal cortical regions to monitor and - if necessary - to adjust to unexpected perturbations.

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Poster

383. Thalamic and Cortical Processing in the Somatosensory System

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Topic: D.03. Somatosensation – Touch

Support: UH Health Research Institute

Title: Hand sensory function in heart failure

Authors: *H. HIBINO, S. L. GORNIAK;
Hlth. and Human Performance, Univ. of Houston, Houston, TX

Abstract: Heart failure (HF) is a complex syndrome characterized by structural or functional defects of ventricular filling or ejection fractions of the heart that do not meet metabolic requirements. While dyspnea, fatigue, and fluid retention are symptoms commonly reported by persons with HF (PwHF), reports demonstrating declined independence in activities of daily living (ADLs) are increasing. ADLs are associated with fine motor skills, which, in part, depend on somatosensory inputs: tactile and proprioceptive inputs. Thus, HF may negatively affect the sensory inputs of the hand. To test this hypothesis, we compared hand sensory function between PwHF and age- and sex-matched control subjects. In our pilot study, we have tested eight PwHF and four age- and sex-matched control subjects. The threshold of tactile registration was tested via the Semmes-Weinstein monofilament test. The threshold of tactile registration was evaluated on digits 1, 2, 5, hypothenar eminence, and 1st dorsal interosseus of both hands. The smallest pressure the subjects recognized three consecutive times was recorded as the threshold of tactile registration. The sense of object recognition arising from tactile and proprioceptive inputs was tested via stereognosis, in which the subjects had to answer the combination of two numbers comprised of raised dots on dominos. Both hands were tested; a total of five dominos were delivered to each hand. Response accuracy and response time were recorded. The mean of the response accuracy and response time were quantified as the score of stereognosis. In addition to

the sensory assessments, we evaluated general cognitive function and age-adjusted processing speed via Montreal Cognitive Assessment (MOCA) and NIH Toolbox Pattern Comparison Processing Speed Test, respectively. Mann-Whitney U test suggested that the executive component of MOCA and the processing speed in PwHF are lower and slower compared to control subjects, respectively (MOCA Executive: $U = 4.0$, $z = -2.23$, $p < 0.05$, Sensory Processing Speed: $U = 3.5$, $z = -2.14$, $p < 0.05$). Across both hands, comparable thresholds of tactile registration were found between PwHF and control subjects. The response accuracy of stereognosis did not differ between the two groups across hands. PwHF had slower response time compared to control subjects in the left hand, but not in the right hand (Right Hand: $U = 7.0$, $z = -1.53$, $p = 0.13$, Left Hand: $U = 1.0$, $z = -2.55$, $p < 0.05$). The results of this study suggest that HF does not affect the threshold of registering subtle touch, but negatively affects the speed to integrate sensory inputs of the hands to sense objects.

Disclosures: H. Hibino: None. **S.L. Gorniak:** None.

Poster

384. Olfaction: Behavior and Perception I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 384.01

Topic: D.04. The Chemical Senses

Support: University of Chicago Social Sciences Division and Institute for Mind and Biology Seed Grant

Title: The role of gamma oscillations in contextual and cognitive load influences on odor discrimination in rats

Authors: *H. LI[1,2], A. STUART[2], J. ZENG[3], L. M. KAY[1,3];
[1]Dept. of Psychology, [2]Inst. for Mind and Biol., [3]The Col., The Univ. of Chicago, Chicago, IL

Abstract: Neural oscillations play an important role in both sensory and cognitive processes. Understanding the role neural oscillations play in odor discrimination helps us further the inquiries of both chemosensation and learning. In the rat olfactory bulb (OB), gamma oscillations (65-100 Hz) in Local Field Potential (LFP) are elevated during fine odor discrimination. These oscillations are necessary and sufficient for fine odor discrimination in rats, mice and honeybees. However, we do not know what aspects of the odor discrimination task drive increased gamma. Studies suggest that cognitive load may be a factor, with easier loads making discrimination of similar odorants simpler than in high load conditions. We address interactions between cognitive load and odorant similarity using a paradigm in which context cues make an odor discrimination task easier or harder; furthermore, we investigate the timepoints and timescale at which gamma oscillations increase during the behavioral tasks by presenting the tasks in interleaved and block designs. We use a 2-alternative choice task, during which rats discriminate a pair of very different odorants (coarse) and a pair of relatively similar

odorants (fine) in block and interleaved fashion for sucrose reward. We record LFPs from the OB, piriform cortex and hippocampus, and respiratory activity during active tasks. We test the hypotheses that a) gamma is elevated in anticipation of a difficult discrimination, with difficulty levels varying in odor similarity and other cognitive load requirements, and b) informative vs. uninformative context cues decrease or increase the difficulty of the fine discrimination requiring modulation of gamma power to do well in the task.

Disclosures: H. Li: None. **A. Stuart:** None. **J. Zeng:** None. **L.M. Kay:** None.

Poster

384. Olfaction: Behavior and Perception I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 384.02

Topic: D.04. The Chemical Senses

Support: Division of the Social Sciences and Institute for Mind and Biology, The University of Chicago.

Title: Sex differences in rat olfaction : An oscillatory approach

Authors: *K. VM[1,2,3], A. STUART[1], L. M. KAY[1,2];
[1]Inst. for Mind and Biol., [2]Dept. of Psychology, [3]Master of Arts Program in the Social Sci., The Univ. of Chicago, Chicago, IL

Abstract: Females across several species have olfactory abilities superior to males. Studies in humans show that women have lower olfactory thresholds, and better odor discrimination and identification abilities than men. In mice, odorants elicit faster responses from a greater number of olfactory sensory neurons in females relative to males. Circulating sex hormones might govern these differences. Gonadectomized female mice have glomerular odor activation patterns comparable to control males, while gonadectomized males have activation maps comparable to control females. We explore sex differences in olfaction from an oscillatory perspective. Local Field Potentials (LFP) in the olfactory bulb (OB) represent the coordinated activity of bulbar neurons. Both gamma (40-110 Hz) and beta (15-30 Hz) oscillations have been linked to odor discrimination ability. Spontaneous and odor-evoked OB LFPs are recorded from awake rats at the same time for 12 days. Odors used include urine of both sexes and monomolecular odorants characterized previously for correlation of volatility with behavior and OB oscillations. Sampling duration, spectral composition, and beta and gamma rhythm power are analyzed; also accompanied by estrus staging through visual inspection in females. Preliminary data show no significant differences in oscillatory power in the beta or gamma band. However, sniff duration in females is shorter relative to males, suggesting differences in odor sampling behavior.

Disclosures: K. Vm: None. **A. Stuart:** None. **L.M. Kay:** None.

Poster

384. Olfaction: Behavior and Perception I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 384.03

Topic: D.04. The Chemical Senses

Support: NIDCD R01-DC014367

Title: The effects of retronasal discrimination learning on olfactory gamma and beta oscillation patterns

Authors: *R. HE[1], A. STUART[2], J. ZENG[1], L. M. KAY[3];
[1]The Univ. of Chicago, Chicago, IL; [2]St. John's Col., Annapolis, MD; [3]Dept. of Psychology, Univ. of Chicago, Chicago, IL

Abstract: Olfactory stimuli reach the olfactory epithelium in the nose via both orthonasal (nasal breathing) and retronasal (oral eating and drinking) routes. Our study aimed to understand the neural dynamics underlying retronasal olfactory learning as it involves reward, gustatory, and learning circuits. Local field potentials were recorded from the ipsilateral olfactory bulb, olfactory tubercle, anterior piriform cortex, gustatory cortex and hippocampus in eight rats (4M/4F). The results showed a significant decrease in gamma band (40-110Hz) power during the first part of retronasal odor sampling in multiple brain regions. Beta band (15-30Hz) power and coherence significantly increased later in odor sampling, in a pattern similar to orthonasal olfaction. Sensorimotor inputs driven by nasal respiration and licking also contributed to LFP signals in the olfactory system as indicated by high coherence between the bulb and both licking and breathing signals. In addition, a hidden-Markov model was used to identify different stages of sleep using the multisite LFP recordings. This allowed us to examine the same circuits pre- and post-training to track memory consolidation at the system level.

Disclosures: R. He: None. **A. Stuart:** None. **J. Zeng:** None. **L.M. Kay:** None.

Poster

384. Olfaction: Behavior and Perception I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 384.04

Topic: D.04. The Chemical Senses

Support: OIST Corporation

Title: Holding information over time: Investigation of brain areas involved in olfactory delayed-actions and working memory

Authors: *J. K. REINERT, J. REUSCHENBACH, I. FUKUNAGA;
Okinawa Inst. of Sci. and Technol., Okinawa Inst. of Sci. and Technol., Onna, Japan

Abstract: Delaying actions and retaining sensory information over time is integral to the behavioral repertoire of most species and signatures of such functions, including persistent activity, have been observed in the prefrontal cortex. Recent studies indicate the involvement of more peripheral regions, for example, the piriform cortex in odor-based working memory tasks, though optogenetic silencing of this region only results in a partial decrease in behavioral performance. We thus aimed to resolve the circuitry involved in retaining olfactory information. We first investigated delayed actions, by modifying a Go/No-Go olfactory discrimination task by delivering a water reward with a prolonged delay to train mice to delay the onset of anticipatory licks. Shortening the delay to reward delivery, from 4.2 s to 2.3 s or 0.8 s after odor onset, revealed mice responding with a progressively faster onset of anticipatory licks (mean onsets: 1578 ms ± 149; 999 ms ± 71; 657 ms ± 44; n = 33, 18 and 12 mice, respectively; $F(2, 60) = 10.59$, $p = 0.001$), indicating that mice can hold task related information and delay actions well beyond the time needed to make decisions.

To identify brain regions involved in short-time retention of task information, we used mice expressing the light-activated ion channel channelrhodopsin-2 in GABAergic neurons to perturb key brain regions during different timepoints of the delay phase. Perturbing the piriform cortex or the dorsal striatum greatly reduced the lick response of the mice in both light intensity and time point dependent manner (maximal lick change: piriform cortex: -66 % ± 7, n = 8 mice; striatum: -50 % ± 6, n = 7 mice). Conversely, this was not observed when silencing the olfactory tubercle (maximal lick change: -20 % ± 5, n = 4 mice).

To further distinguish whether the effect in the dorsal striatum reflects retention of task information during delayed actions, rather than motor planning activity, we combined an olfactory delayed non-match to sample (DNMS) task with optogenetic perturbation. Perturbing the dorsal striatum during the delay period had a devastating impact on the lick response of the mice (mean licks: -88 % ± 7, n = 7 mice). Further, perturbations during the late delay phase had a greater impact compared to the early delay period (mean licks: early delay: -50 % ± 14; late delay: -83 % ± 9, n = 7 mice) or perturbation during either the sample or test odor presentation (mean licks: sample -9 % ± 10; test: -55 % ± 10, n = 7 mice), indicating striatal involvement may be critical, especially during the late delay period.

Together our results suggest that the basal ganglia may be an integral part of the circuitry holding olfactory-task-related information over time.

Disclosures: **J.K. Reinert:** None. **J. Reuschenbach:** None. **I. Fukunaga:** None.

Poster

384. Olfaction: Behavior and Perception I

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Program #/Poster #: 384.05

Topic: D.04. The Chemical Senses

Support: NIH Grant R01-DC-016364
NIH Grant R01-DC-018539
NIH Grant T32 NS047987

Title: Fast sniffing as a potential mechanism for stimulation of human hippocampal oscillations

Authors: *A. SHERIFF[1], G. ZHOU[1], J. JAMKA[1], J. ROSENOW[2], S. U. SCHUELE[1], G. LANE[1], C. ZELANO[1];
[1]Neurol., [2]Neurosurg., Northwestern Univ., Chicago, IL

Abstract: Human nasal breathing stimulates neural oscillations across a wide range of brain areas, thus modulations of respiratory behavior could modulate neural oscillations. The impact of respiratory modulations on brain oscillations is relatively unexplored; in particular, the impact of fast sniffing behaviors on neural oscillations in humans is unknown. Rodents sniff fast (6-9 Hz) during olfactory discrimination, with performance directly related to the degree of interaction between the olfactory bulb, which tracks respiratory frequency, and ongoing hippocampal theta oscillations (~8 Hz). Although humans breathe an order of magnitude slower than rodents at rest, human inspiration is accompanied by increased oscillatory power across a broad range of frequency bands and limbic areas that dissipates during exhalation. We hypothesized that fast sniffing in humans would 1. impact the frequency of respiratory-aligned oscillations in the human brain, 2. stimulate oscillations that persist through exhalation, and 3. induce oscillations across a wider range of brain areas compared to natural breathing. To test these hypotheses, we collected intracranial electrophysiological data from surgical patients with medically intractable epilepsy. Participants were presented with plain or odorized air and instructed to sniff fast or slow. Hippocampal theta and gamma oscillations were increased following the initiation of fast compared to slow sniffing. Further, hippocampal theta exhibited increased phase reset following initiation of fast sniffing. These data help consolidate findings between rodent and human studies of respiratory entrained neural oscillations and suggest a potential translational mechanism of fast sniffing to engage limbic and cortical circuits. Future work will use these methods to compare differences in olfactory processing during fast versus slow sniffing, and whether fast sniffing modulates subsequent hippocampal-dependent behaviors including virtual navigation.

Disclosures: A. Sheriff: None. **G. Zhou:** None. **J. Jamka:** None. **J. Rosenow:** None. **S.U. Schuele:** None. **G. Lane:** None. **C. Zelano:** None.

Poster

384. Olfaction: Behavior and Perception I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 384.06

Topic: D.04. The Chemical Senses

Support: BRAIN Initiative R01NS123903

Title: The impact of hallucinogens on active sampling and the olfactory bulb

Authors: *A. C. WELCH[1], T. M. FINDLEY[2], R. MARSDEN[2], K. R. JONES[2], T. A. TARVIN[2], M. A. BROWN[2], M. C. SMEAR[1];
[1]Psychology & Inst. of Neurosci., [2]Inst. of Neurosci., Univ. of Oregon, Eugene, OR

Abstract: Olfactory hallucinations occur in many disorders, including Parkinson's disease, epilepsy, schizophrenia, and migraines, but the neural mechanisms underlying these hallucinations are not completely understood. Mechanistic studies of hallucination in animal models are fundamentally limited, since animals do not verbalize what they perceive. However, in lieu of a verbal report, internal states can be inferred from an animal's externally observable behavior. Using computational tools, our lab has shown that a mouse's perceptual states can be inferred from close analysis of strategic sniffing behavior. Further, we have found that behaviorally-inferred states can predict the population structure of spontaneous activity in the olfactory bulb. We now leverage these advances to infer hallucinatory states from mouse behavior. Importantly, many hallucinogens act on serotonin pathways, which feed heavily into the mouse olfactory system. Further, we have found that injection of the hallucinogen DOI alters the rhythmic structure of sniffing behavior. In ongoing work, we are investigating how DOI impacts population dynamics in the olfactory bulb. This work will provide fresh insights into the link between active sampling, olfaction, and hallucinations.

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Poster

384. Olfaction: Behavior and Perception I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 384.07

Topic: D.04. The Chemical Senses

Support: DBT/Wellcome Trust India Alliance intermediate grant (IA/I/14/1/501306)
DST-Cognitive Science Research Initiative (DST/ CSRI/2017/271)

Title: Mouse olfactory system acts as anemo-detector and discriminator

Authors: S. MAHAJAN, S. TAMBOLI, A. BHATTACHARJEE, M. PARDASANI, P. SRIKANTH, ***N. ABRAHAM**;
Lab. of Neural Circuits and Behaviour (LNCB), Dept. of Biol., Indian Inst. of Sci. Educ. and Res. (IISER), Pune, Pune, India

Abstract: The sense organs are dedicated to collect information from the external environment and convert the incoming physical/chemical energy into neural representations. While most of the sensory systems encode various features of a single sensory stimulus through multiplexing, the rodent olfactory sensory neurons (OSNs) can process completely distinct stimuli - the mechanical stimulation[1, 2] associated with the airflow that carries odor molecules and the chemical sensation of odors themselves[3]. Although the neural mechanisms of the latter have been

investigated in detail, pathways processing the mechanical information remain largely elusive. Therefore, probing the multimodal aspects of olfactory information processing is fundamentally important. Here, we investigated the role of rodent olfactory system in detecting and discriminating airflow rates in presence and absence of odorant molecules. Our results showed that mice can learn to discriminate a range of airflow rates even in the absence of whiskers, which are known to be specialized sense organs that processes airflow-related information. Further, the discrimination abilities of the animals were lost when OSNs were ablated or after the olfactory bulbectomy. As GABAergic interneurons of the olfactory bulb were activated during airflow discriminations, we modified the function of this inhibitory circuit in a bidirectional way using optogenetics. While the enhancement of inhibition resulted in a poor discrimination of airflow rates, decrease of inhibition exhibited a contrasting behavioral phenotype. However, the same optogenetic modulations during odor discriminations ensued opposite behavioral phenotypes compared to airflow discriminations, proving that optimal synaptic inhibition needed for refining olfactory and mechanosensory information varies. By altering airflow and odorant components, the opposing effect of optogenetic modifications was nullified, thus confirming the association between mechanical and odorant information in olfactory perception.

1. Connelly T, *et al.* G protein-coupled odorant receptors underlie mechanosensitivity in mammalian olfactory sensory neurons. *P Natl Acad Sci USA* **112**, 590-595 (2015).
2. Grosmaitre X, *et al.* Dual functions of mammalian olfactory sensory neurons as odor detectors and mechanical sensors. *Nat Neurosci* **10**, 348-354 (2007).
3. Buck L, Axel R. A Novel Multigene Family May Encode Odorant Receptors - a Molecular-Basis for Odor Recognition. *Cell* **65**, 175-187 (1991).

Disclosures: S. Mahajan: None. **S. Tamboli:** None. **A. Bhattacharjee:** None. **M. Pardasani:** None. **P. Srikanth:** None. **N. Abraham:** 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.; DBT/Wellcome Trust India Alliance.

Poster

384. Olfaction: Behavior and Perception I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 384.08

Topic: D.04. The Chemical Senses

Support: European Research Council StG SociOlfa
Doctoral fellowship from the French Ministry of Research and Education
Bordeaux University Initiative of Excellence Junior Chair Program
ATIP-Avenir grant
Neurocampus chair supported by the Region Nouvelle Aquitaine

Title: High-precision monitoring of nasal pressure in freely moving mice provides state-specific respiratory features and vigilance state prediction

Authors: *C. MIERMON, G. CASALI, N. CHENOUARD, G. TERRAL, T. DOLIQUE, E. LESBURGUERES, F. GAMBINO, L. ROUX;
IINS, Bordeaux, France

Abstract: In olfaction, odor sampling is controlled by respiratory/sniffing behaviors, which determine the dynamics of odor stimulation. The temporal pattern of airflow in the nasal cavity is therefore a fundamental parameter to consider when studying brain processing of odorant stimuli. Increasing evidence indicates that the olfactory system uses a precise temporal code which requires the detection of sniff onsets with millisecond precision. Yet, it remains unclear whether other characteristics of the respiratory cycle waveforms (such as airflow direction and respiratory pauses) can impact olfactory coding. These considerations prompted us to develop a new method that allow recording nasal airflow in freely moving mice with an enhanced level of precision as compared to methods used previously such as thermocouples. We have adapted an approach which allows measuring variations in the air pressure within the nasal cavity of freely moving mice. It relies on light (<1g) pressure sensors that can be carried by small rodents and combined with in vivo chronic neuronal recordings. Our data provides significant improvement in precision (e.g., better access to amplitude variations, cycle onset and inhalation/exhalation timing) compared to classical thermocouple recordings**.** We combined nasal pressure monitoring with hippocampal recordings in both male and female mice from different strains and developed an analysis pipeline partially based on the Breathmetrics toolbox created by the Zelano Lab (Northwestern University). Based on these data, we provide an in-depth characterization of the respiratory signal across brain states (Wake, REM and Non-REM Sleep). The respiratory signal in each brain state is characterized by a specific proportion of each component (inhalation, exhalation, pauses) and specific cycle features (amplitude, durations⋯). Thanks to these specific features, we have built a classifier to predict brain states based on respiratory signal features. Overall, this new method of respiration monitoring could be useful for neuroscientists interested in olfactory coding in naturalistic (freely moving) conditions as well as those studying the impact of respiratory rhythms on cognition.

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Poster

384. Olfaction: Behavior and Perception I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 384.09

Topic: D.04. The Chemical Senses

Support: ARMENISE EPFD0111

Title: A sampling-invariant code in the olfactory cortex

Authors: F. MICHELON[1,2], A. ABOLGHASEMI[1,2], E. PIASINI[3], *G. IURILLI[1];
[1]Inst. Italiano di Tecnologia, Rovereto, Italy; [2]Cimec, Univ. degli Studi di Trento, Trento, Italy; [3]Intl. Sch. for Advanced Studies (SISSA), Trieste, Italy

Abstract: Active sampling behaviors introduce stimulus variability that can influence cortical representations. In olfaction, sniffing increases the airflow velocity inside the nostrils and the number of odor molecules that contact the olfactory epithelium during each inhalation. Nevertheless, animals can discriminate odor concentrations regardless of the velocity of their inhalation. We show that sniffing and regular breathing differently influence the activity of the olfactory cortex during a single inhalation. However, such influence is quasi-orthogonal to the encoding of odor concentration. Therefore, the same population code can be used to decode odor concentration regardless of whether an animal is sniffing or not. This simple, sampling-invariant population code is guaranteed by a linear and interaction-free encoding of airflow velocity and odor concentration by individual cortical neurons. In summary, the activity of the olfactory cortex differs between regular breathing and sniffing, and provides a sampling-invariant representation of odor concentration.

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Poster

384. Olfaction: Behavior and Perception I

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Program #/Poster #: 384.10

Topic: D.04. The Chemical Senses

Support: NARSAD Young Investigator Grant 30288

Title: Nose and mouth breathing differentially entrain ripples and neuronal spiking in humans

Authors: *A. R. CARDENAS[1,2], J. F. RAMIREZ-VILLEGAS[3], F. HEIDARI[1,2], K. D. MENGUC[4], J. L. CSICSVARI[3], T. A. VALIANTE[5,1,2];
[1]Krembil Brain Inst., Toronto, ON, Canada; [2]CRANIA, Toronto, ON, Canada; [3]Inst. of Sci. and Technol. (IST) Austria, Klosterneuburg, Austria; [4]Univ. of Toronto, Toronto, ON, Canada; [5]Toronto Western Hosp., Toronto, ON, Canada

Abstract: An emergent hypothesis is that respiration entrains and coordinates the network dynamics across different subsystems in the brain. Animal studies have shown that respiratory modulation of brain activity may rely on the olfactory bulb stimulation by nasal airflow. The extent and reach of this dependence are still controversial. We recorded LFP and spiking activity in surgical epilepsy patients during awake resting state while heart and respiratory activity were monitored. We compared neural activity in mesio-temporal and frontal areas during nose and mouth breathing (nose clipped). We show that hippocampal ripples and high frequency oscillations (HFOs) in the amygdala are entrained by respiration. Moreover, breathing modulates single neuron activity in the human hippocampus, amygdala, insula and prefrontal cortex.

Finally, the respiratory entrainment of ripples and spiking activity decreases but does not disappear during mouth breathing. These findings offer potential insights on how respiration modulates emotion and memory in humans.

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Poster

384. Olfaction: Behavior and Perception I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 384.11

Topic: D.04. The Chemical Senses

Support: NIH (NIDCD) R01-DC-016364
NIH (NIDCD) R01-DC-018539

Title: Humans can sniff at speeds overlapping with neural oscillatory frequency band theta (4-8Hz)

Authors: *J. JAMKA, A. SHERIFF, G. ZHOU, T. NOTO, G. LANE, C. ZELANO; Northwestern Univ., Chicago, IL

Abstract: Sniffing is an active odor sampling behavior that is exhibited by all terrestrial vertebrates. Sniffing speeds vary across species (Spencer et al., 2021) and impact neural oscillations in olfactory cortex, hippocampus, and neocortical areas (Martin et al., 2007; Wróbel et al., 2020). Rodents sniff at frequencies ranging from 6 to 9Hz during odor sampling (Kepecs et al., 2007; Macrides et al., 1982; Rajan et al., 2006; Uchida and Mainen, 2003; Youngentob et al., 1987), which overlaps with the classic neural oscillatory EEG frequency band theta (4 to 8Hz). These fast-sniffing bouts expedite odor delivery (Wesson et al., 2009) and impact the magnitude of coherence between the olfactory bulb and hippocampus (Kay, 2005). Human sniffing behavior and how it impacts neural oscillations in the brain is less well understood. In humans, a single sniff is considered sufficient for optimal odor perception (Laing, 1986), though the impact of sniffing speed on odor perception and neural activity/coherence has not been fully characterized in humans. In particular, we do not know how fast humans are capable of sniffing, and whether they can achieve speeds that are comparable to neural oscillatory frequency bands, as in rodents. In this study, we aim to determine how fast humans are capable of sniffing as a first step towards characterizing effects of sniff rate on olfactory perception and related behaviors. 26 healthy human participants performed an incentivized fast-sniffing task in which they were instructed to perform sniffing bouts at the fastest speed they could achieve while nasal airflow was recorded with a pneumotachometer. Participants also completed a standard olfaction function test (UPSIT) and anxiety survey (GAD-7). Preliminary results suggest that humans can sniff as fast as 6.3Hz, which is within theta range. These results show that humans are capable of sniffing at speeds comparable to those observed in macrosmatic animals. Though fast sniffing

may not be a common behavior in humans, these results open up the possibility that fast-sniffing bouts in humans could impact olfactory and limbic activity in a manner similar to that observed in rodents.

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Poster

384. Olfaction: Behavior and Perception I

Location: SDCC Halls B-H

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Program #/Poster #: 384.12

Topic: D.04. The Chemical Senses

Support: NIH Grant 1R01DC019405-01A1

Title: Human olfactory navigation recruits grid-like representations in entorhinal, prefrontal and piriform cortices

Authors: *C. U. RAITHEL[1,2], A. J. MILLER[2], R. A. EPSTEIN[1], T. KAHNT[3,4], J. A. GOTTFRIED[1,2,4];
[1]Dept. of Psychology, [2]Dept. of Neurol., Univ. of Pennsylvania, Philadelphia, PA; [3]Intramural Res. Program, Natl. Inst. on Drug Abuse, Baltimore, MD; [4]Dept. of Neurol., Northwestern Univ., Chicago, IL

Abstract: Olfactory navigation is a behavioral phenomenon observed extensively across the animal kingdom. One species that is barely considered in this context is the human species. In our experiment, we used a combination of air-dilution olfactometry techniques, Virtual Reality (VR) software applications and neuroimaging methods to investigate whether humans can navigate an olfactory landscape by learning about the spatial relationships among discrete odor cues and integrating this knowledge into a spatial map. Our data show that, across the time course of the experiment, participants improved their performance on the odor navigation task, i.e., took more direct paths toward the target, and completed more trials within a given time period. These behavioral results suggest that participants can successfully navigate a complex odorous environment, reinforcing the notion of olfactory navigation in humans. Functional Magnetic Resonance Imaging (fMRI) data collected during olfactory navigation revealed the presence of grid-like representations in entorhinal, prefrontal and piriform cortices. The engagement of a primary olfactory region within the grid network suggests that the sensory modality relevant to a navigational task determines the neural correlates of the cognitive map, in line with reports from the rodent literature. In next steps, we plan to examine the interactions between olfactory and/or other sensory (e.g., visual) inputs to gain a better and more ecologically valid understanding of spatial navigation at both the neural and behavioral levels.

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Poster

384. Olfaction: Behavior and Perception I

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Program #/Poster #: 384.13

Topic: D.04. The Chemical Senses

Title: Combining physiological measurements and virtual reality to decipher emotions evoked by scents

Authors: X. MELEQI[1], A. PEGARD[2], U. MEIERHENRICH[1], *J. TOPIN[1];
[1]Inst. de Chimie de Nice, Univ. Côte d'Azur, Nice, France; [2]R&D, Robertet Groupe, Grasse, France

Abstract: Introduction: The analysis of human physiological responses to stimuli is a topic of interest to both academic and industrial researchers. Emotion identification remains a challenge due to: i) the difficulty of labeling an odor-induced emotion, ii) the lack of a reference database that can be used to associate physiological data with the emotional label. We developed a new methodology based on the combination of a self-report survey and physiological analysis. The identification of an emotion is determined by a comparison of olfactory and visual responses, the latter being previously associated with an emotion. Our protocol paves the way for accurate labeling of odor-induced emotions.
Method: 30 participants without smell disorder (mean age 37.6 +/- 10.4, 26 females) were recruited. They were stimulated by 22 selected raw materials, 31 fragrances and 20 virtual reality movies. Physiological data such as heart rate, skin conductance, and respiratory volume were collected from the participants. Participants were also asked to rate the valence, arousal, intensity, and naturalness of each stimulation. A principal component analysis (PCA) was performed with the olfactory and visual stimuli. Finally, the stimuli were clustered using k-means analysis and spatially represented using multidimensional scaling analysis.
Results: Using the k-means clustering methodology, 13 relevant groups of responses were obtained. The intra-class variation is about 1.90 and the inter-class variation is about 6.43. The principal component analysis of the fragrance and virtual reality stimuli reached a score of 68.55% on the 2 main axes. The same analysis on the ingredient and virtual reality stimuli reaches a score of 67.18% on both principal axes. Videos with the same emotional label were associated in the PCA as well as in the k-means analysis. Each cluster is defined by at least one emotional term.
Discussion: The results show that the response patterns following an olfactory stimulation are comparable to those obtained during virtual reality stimulation. We were also able to highlight the importance of the subjective perception of the stimulation on the responses and finally on the triggered emotion.
Conclusion: This methodology involving virtual reality allows to easily create databases of emotional references. Finally, our protocol opens the way to a relatively fast evaluation of odor-induced emotions, based on a combination of subjective and objective criteria.

Disclosures: **X. Meleqi:** A. Employment/Salary (full or part-time):; Robertet Groupe. **A. Pegard:** None. **U. Meierhenrich:** None. **J. Topin:** None.

Poster

384. Olfaction: Behavior and Perception I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 384.14

Topic: D.04. The Chemical Senses

Support: DST Cognitive Science Research Initiative (DST/CSRI/2017/271)
Council of Scientific & Industrial Research, Govt. of INDIA.

Title: Recovered COVID-19 subjects show altered breathing patterns during olfactory matching test.

Authors: *S. MAHAJAN, G. NAIR, M. PARDASANI, N. M. ABRAHAM;
Indian Inst. of Sci. Educ. and Res., Pune, India

Abstract: Loss of olfaction has been reported as one of the early and prevalent symptoms for COVID-19 infection[1]. Owing to varying experimental evidences on the neurotropic nature of the virus[2, 3], it is critical to quantify the extent of neurocognitive deficits even after the recovery. Recently, we have custom-built an instrument with built-in safety precautions to prevent cross-contamination to quantify olfactory fitness in COVID-19 patients and assessed the extent of olfactory deficits. While only 15% of tested individuals reported unfavorable changes in their smelling abilities upon subjective evaluations, more than 80% of asymptomatic carriers were indeed found to be suffering from olfactory deficits using our method[4, 5]. To quantify neurocognitive impairments of recovered COVID-19 subjects, we used our custom-built olfactory-action meter (OAM) to collect behavioral readouts while subjects were tested with olfactory matching. We used varying number of stimuli, depending on the detectabilities shown by the subjects, to quantify their olfactory matching skills. During the test, we also quantified their breathing patterns using a flow sensor with a resolution of 30-milliseconds. Our quantification of the sniff dynamics during olfactory matching for control subjects proved the following. 1. In presence of odour stimuli, longer inhalations and shorter exhalations resulted in a concomitant increase in breathing frequency. 2. The amplitudes of inhalation and exhalation were higher during the olfactory matching. The same analysis on the recovered COVID-19 subjects reveled alterations in the breathing patterns. This paradigm enables us to probe neurocgnitive deficits along with sampling strategies while subjects are challened with complex decision-making based on olfactory matching. Further, the same experimental strategy may allow to diagnose obstructive pulmonary disorders as well as cognitive dysfunctions.
References:1. Menni C, *et al.* Real-time tracking of self-reported symptoms to predict potential COVID-19. *Nat Med* **26**, 1037 - (2020). **2.**Khan M, *et al.* Article Visualizing in deceased COVID-19 patients how SARS-CoV-2 attacks the respiratory and olfactory mucosae but spares the olfactory bulb. *Cell* **184**, 5932 - (2021). **3.**Douaud, *et al.* SARS-CoV-2 is associated with

changes in brain structure in UK Biobank. *Nature* https://doi.org/10.1038/s41586-022-04569-5, (2022). **4.**Bhattacharjee, *et al.* Quantitative assessment of olfactory dysfunction accurately detects asymptomatic COVID-19 carriers. *Eclinicalmedicine* **28**, (2020). **5.**Li JW, *et al.* Affected olfaction in COVID-19: Re-defining "asymptomatic" Comment. *Eclinicalmedicine* **29-30**, (2020).

Disclosures: S. Mahajan: None. **G. Nair:** None. **M. Pardasani:** None. **N.M. Abraham:** None.

Poster

384. Olfaction: Behavior and Perception I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 384.15

Topic: D.04. The Chemical Senses

Title: Intergenerational inheritance of conditioned taste aversion

Authors: *W. FOSTER[1], C. LIFF[1], E. R. SHERMAN[2], E. JAEGER[3], J. J. SUN[4], B. J. MARLIN[1];
[1]Columbia Univ. Zuckerman Inst., New York, NY; [2]Cambridge Univ., Cambridge, United Kingdom; [3]Columbia Univ., New York, NY; [4]Caltech, Pasadena, CA

Abstract: A parent's experience can have lasting effects on their naïve offspring. In humans, following the Dutch Hunger Winter famine of 1944-1945, the children and grandchildren of survivors — who had not experienced this stress directly — exhibited elevated rates of both metabolic and psychological illness. The phenomenon of physical or emotional trauma being transmitted to offspring via epigenetic modifications, called transgenerational epigenetic inheritance (TEI), is a process by which an *acquired* trait in a parent can become *innate* in their offspring. Our work and others (Dias and Ressler, 2014) has demonstrated that pairing an odor with foot shock leads to an increase in the number of olfactory sensory neurons sensitive to the paired odor in both the paired mice *and* their untrained offspring. However, little is known about TEI in other sensory systems. Here, we explore the heritability of a conditioned taste aversion (CTA). To explore the intergenerational effects of CTA, we induce CTA in male C57BL/6J mice (F0) by pairing consumption of 5.5mM saccharin with injection of either lithium chloride (LiCl), which induces nausea, or PBS. Males are mated with female C57BL/6J mice and removed from the cage before the litter is born to prevent father-offspring interaction. F0 saccharin preferences are tested in a 2-choice test. The offspring (F1) are exposed to 5.5mM saccharin for 10-minutes to eliminate neophobia effects, and later given a 2-choice test. Our results demonstrate a highly significant difference ($p<0.0001$) between the taste preferences of F0 PBS-injected and LiCl-injected male mice, with PBS mice exhibiting a preference for saccharin and LiCl mice showing a strong aversion (F0 PBS n = 12, F0 LiCl n = 11). We also found a very significant difference ($p=0.0002$) between the saccharin preferences of PBS-F1s and LiCl-F1s, with the F1 of LiCl males demonstrating an innate aversion to what is appetitive in PBS controls (F1 PBS n = 12, F1 LiCl n = 23). Our findings show that a *learned* taste aversion becomes *innate* in the next

generation. Future studies will examine the high speed orofacial responses to conditioned tastants in F0 and naïve F1. Insight into the inheritance of an aversive experience will provide insight into adaptive epigenetics.

Disclosures: W. Foster: None. **C. Liff:** None. **E.R. Sherman:** None. **E. Jaeger:** None. **J.J. Sun:** None. **B.J. Marlin:** None.

Poster

384. Olfaction: Behavior and Perception I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 384.16

Topic: D.04. The Chemical Senses

Title: Changes in Olfactory Sensory System during Motherhood

Authors: *V. ANDREU[1], B. J. MARLIN[2];
[1]Columbia Univ., New York, NY; [2]Columbia Univ. Zuckerman Inst., New York, NY

Abstract: Motherhood is characterized by some of the most dramatic physical, emotional and behavioral changes that an organism will undergo. Pregnancy is paired with drastic hormonal shifts that affect the brain, including sensory inputs that trigger maternal behavior. There is evidence that olfactory projections to the brain are necessary for maternal behaviors, although the exact effects of pregnancy on the olfactory system are still unknown. We developed a behavioral paradigm based on pup odor cues and showed that female mice pup odor preference shifts with pregnancy. We focus on the neuromodulator oxytocin which is essential for pregnancy and parturition. We have labeled oxytocinergic neurons using Oxt-Ires-Cre-Ai9, a transgenic mouse line that labels oxytocinergic neurons, as well as Oxtr-T2A-Ai9, another transgenic mouse line labeling oxytocin receptors. We have identified a subset of oxytocinergic neurons in the main olfactory epithelium (MOE). Using phosphorylated P6 ribosomal protein (phospho-P6), a marker of neuronal activity, we identified a group of oxytocinergic neurons in the MOE active during pup odor exposure. Understanding the role of oxytocin in olfactory driven parental behavior will help us better understand how motherhood may modulate the olfactory processing of pup chemosensory cues at the level of primary sensory neurons in the main olfactory epithelium and adapt to the internal and external changes associated with motherhood.

Disclosures: V. Andreu: None. **B.J. Marlin:** None.

Poster

384. Olfaction: Behavior and Perception I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 384.17

Topic: D.04. The Chemical Senses

Support: NSF GRFP

Title: Determining the molecular mechanisms of heritable stress-induced bias in olfactory neuron differentiation

Authors: *R. STECKY, S. LOMVARDAS, B. J. MARLIN;
Columbia Univ., New York, NY

Abstract: From the Dutch Hunger Winter to the Holocaust, human history gives us myriad examples of intergenerational inheritance of traumatic experiences (Heijmans et al., 2008; Yehuda et al., 2016). More recently, these population-wide examples have been supported with experimental data that suggest stress-induced somatic changes can be inherited across generations (Dias and Ressler, 2014; Shea et al., 2015). Using the mouse olfactory system as our model, the Marlin lab has found that pairing odor with a mild foot shock induces an upregulation in the birth of olfactory sensory neurons that respond to the paired odor. Moreover, this phenotype has been demonstrated not only in the conditioned mice, but also in their unconditioned progeny. How does odor-shock pairing bias the differentiation of newly born neurons? Here I aim to identify molecular mechanisms of our observed neuronal upregulation using bulk RNA-sequencing. After olfactory fear conditioning with the P2 ligand, I sort odor-sensing mature neurons (P2+) and progenitor neurons from the main olfactory epithelium and perform transcriptional profiling on these neuronal populations. Due to the regenerative nature of the main olfactory epithelium, I reiterate this experimental paradigm over a period of 14 days after conditioning to survey transcriptomic changes in the main olfactory epithelium as it undergoes one full round of cell turnover - the result of which produces a new layer of neurons enriched for the paired odor's receptor. Comparing the odor-shock paired group and the unpaired control group (n = 2 per group per time point), differential gene expression analysis shows opposite-valence gene regulation between mature and progenitor neurons over this time course. One day after conditioning, we observe striking upregulation of extracellular matrix and exosomal genes *Col6a3* and *Thsd4* (log2foldchange > 4; p-value < 0.05) in the mature neuronal population. These genes and others in the same gene families become broadly downregulated in the progenitor neuronal population later in the time course. These preliminary data suggest a potential exosomal mechanism of intercellular communication between mature neurons and the undifferentiated stem cell population - the exact nature of which requires further dissection. Our findings have profound implications in helping us understand how the olfactory system integrates salient environmental information and how traumatic experiences can regulate gene expression in a heritable manner.

Disclosures: R. Stecky: None. **S. Lomvardas:** None. **B.J. Marlin:** None.

Poster

384. Olfaction: Behavior and Perception I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 384.18

Topic: D.04. The Chemical Senses

Title: Disease diagnostics in the mouse olfactory bulb

Authors: *J. S. HARVEY[1], B. A. KIMBALL[2], D. RINBERG[3,1];
[1]NYU Langone Hlth., New York, NY; [2]Monell Chem. Senses Ctr., Philadelphia, PA; [3]Ctr. for Neural Sci., NYU, New York, NY

Abstract: Animals exhibit the remarkable ability to detect disease states in other animals via olfactory cues. This is evidenced by innate avoidance behaviors, as well as an extensive literature on detection training. Despite their ubiquity and clear medical relevance, most diagnostic odor features—or 'odorprints'—remain unknown, as do the neural mechanisms by which olfactory systems recognize them. We conducted an exploratory study into the representation of a range of disease states in the mouse olfactory bulb (OB), using wide-field calcium imaging to record from populations of olfactory sensory neurons and mitral cells.
Two studies were carried out to image responses to human blood plasma, obtained from patients diagnosed with either ovarian cancer or lung cancer, as well as healthy controls. All samples evoked responses in the dorsal OB. Low-rank representations of glomerular responses rendered cancer and non-cancer samples separable, despite large within-condition variance.
We also imaged the glomerular responses to urine samples collected from mice injected with lipopolysaccharide (LPS), a bacterial membrane component that induces inflammation and sickness. Those responses were compared to those for control samples: urine collected from mice injected with phosphate buffer solution (PBS). Again, all samples evoked responses, despite previous imaging studies reporting no glomerular responses to mouse urine in the dorsal OB. Glomerular responses to LPS- and PBS-injected mouse urine were separable after nonlinear dimensional reduction.
Initial results indicate that across different diseases in both human and mouse, glomerular responses in the dorsal OB may provide a readout of diagnostic features for detecting disease states.

Disclosures: J.S. Harvey: None. **B.A. Kimball:** None. **D. Rinberg:** Other; Founder and a chief scientific adviser of the company Canaery, Inc..

Poster

384. Olfaction: Behavior and Perception I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 384.19

Topic: D.04. The Chemical Senses

Support: NIH BRAIN Initiative Grant U19NS112953

Title: Measuring odorant concentrations that evoke intensity-matched olfactory percepts in mice

Authors: *B. BARRA[1], D. DEMETERFI[1], N. CHAUHAN[1], J. WITZTUM[1], D. RINBERG[1,2]; [1]Neurosci. Inst., New York Univ. Langone Hlth., New York, NY; [2]Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Perception of intensity is crucial across all sensory modalities. Without intensity information, most sensations would be uninterpretable and performing most daily tasks would be difficult. However, how the brain encodes intensity is currently unknown. The mammalian olfactory system presents an optimal framework to study the neural encoding of intensity, due to its anatomical organization which is simple and largely known. Interestingly, in the olfactory system, perceived intensity does not merely reflect odor concentration: at similar concentrations, some odors evoke very strong sensations, while others are merely perceivable. The investigation of how different concentrations of a wide variety of odorants generate percepts of different intensity can offer insights into the neural mechanisms that encode intensity information. Moreover, availability of modern neuroscience tools, such as fluorescence imaging and optogenetics stimulation, constitutes an optimal opportunity to study neural signatures of perception in mammals. However, the study of perception in animal models presents inherent challenges, as it is difficult to obtain perceptual reports, such as measures of intensity. Here, we approached this problem by adopting a behavioral paradigm previously developed in a rat animal model (1). This allowed us to measure differences in perceptual intensity, and find which odorant concentrations produced indistinguishable intensity percepts in mice. The paradigm allows to build concentration-dependent psychometric curves for each odor. Interestingly, whenever two odors are presented to the animal in the same session and one of them is perceived as more or less intense, a shift in the psychometric curves is observed (ΔI). This shift reflects misclassifications due to a difference of perceived intensity between the two odors. Conversely, overlapping psychometric curves would indicate that the odorants at those concentrations were perceived as equally intense. We used this paradigm to determine odor concentrations that produced intensity-matched percepts for more than 6 odor pairs. Concentrations ranged between 10^{-5} and 10^{-9} M. ΔI was linearly dependent on the difference in concentration on a logarithmic scale, across several orders of magnitude. We plan to leverage these results to record glomerular activations with wide field calcium imaging at intensity-matched concentrations as well as at intensity mismatched concentrations, to gain insights into the neural correlates of intensity in the mouse olfactory system.
(1) Wojcik, P. T. & Sirotin, Y. B. Single Scale for Odor Intensity in Rat Olfaction. Curr. Biol. 24, 568-573 (2014).

Disclosures: **B. Barra:** None. **D. Demeterfi:** None. **N. Chauhan:** None. **J. Witztum:** None. **D. Rinberg:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-founder of the company Canaery, Inc..

Poster

384. Olfaction: Behavior and Perception I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 384.20

Topic: D.04. The Chemical Senses

Title: Emergence of value encoding through a striatopallidal circuit

Authors: *D. LEE, C. ROOT;
UCSD, San Diego, CA

Abstract: The process through which the vertebrate nervous system learns the association between a conditional stimulus (CS) and reward has been the subject of extensive research in the last century. Though much progress has been made in understanding the behavioral learning algorithm at play, the mechanism through which different brain nuclei work together to implement said algorithm is poorly understood. We used 2-photon Ca2+ imaging to record activity from neurons of the olfactory tubercle (OT) and its downstream target, the ventral pallidum (VP) during 7 days of a 6-odor classical conditioning task. In the VP, but not in the OT, the magnitudes of ΔF/F were significantly larger for rewarded odors than unrewarded odors. Through auROC analysis, we observe that activities of single VP neurons contain information about the reward contingency of the odors; activities of single OT neurons do not. At the population level, however, both VP and OT activities were able to distinguish rewarded odors from unrewarded odors through support vector machine (SVM) classifiers. VP activities induced by two different reward-predicting odors could not be easily distinguished through a SVM whereas the OT activities could (VP accuracy: 60% with 50 neurons. OT accuracy: 90% with 50 neurons). Lastly, chemical silencing of OT neurons through viral expression of tetanus toxins delays the expression of anticipatory licking to rewarded odors (n=4). Taken together, these data demonstrate that while the feature of reward-contingency is derived in the VP, downstream of the OT, the OT is still required for learning the association between olfactory CS and reward.

Disclosures: D. Lee: None. **C. Root:** None.

Poster

385. Auditory: Plasticity, Multimodality, Modulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 385.01

Topic: D.05. Auditory & Vestibular Systems

Support: NIH/NIDCD R01 DC-018561

Title: Listening for learned sound-reward cues in a background of noise impacts the enhancing effects of epigenetic mechanisms on the formation of highly precise auditory memory and cortical plasticity

Authors: *N. ATESYAKAR[1], A. SHANG[1], K. M. BIESZCZAD[1,2,3];
[1]Behavioral and Systems Neuroscience, Dept. of Psychology, [2]Rutgers Ctr. for Cognitive Sci. (RuCCS), Rutgers Univ., Piscataway, NJ; [3]Dept. of Otolaryngology-Head and Neck Surgery, Rutgers Robert Wood Johnson Med. Sch., Rutgers Univ., New Brunswick, NJ

Abstract: Experience-dependent representational plasticity in the adult auditory system is integral to long-term memory formation for sounds. Representational plasticity, here, refers to any form of experience-dependent neurophysiological plasticity that leads to prolonged changes in neurophysiological activity (e.g. receptive field shifts and tonotopic map expansion) that represents a dimension of a sound. Epigenetic mechanisms, which regulate gene expression essential for long-term memory, have been identified as robust modulators of experience-dependent neurophysiological plasticity in the auditory cortex that underlie the formation of memory for signal sounds (Shang & Bieszczad, 2022). For example, inhibition of the epigenetic enzyme, histone deacetylase 3 (i-HDAC3) promotes signal-specific tuning bandwidth reduction and tonotopic map expansion for a signal acoustic frequency that predicts reward, while also increasing behavioral signal-specific responding to the trained frequency (relative to non-signal frequency cues) (Bieszczad et al., 2015; Shang et al., 2019; Rotondo & Bieszczad, 2020; Rotondo & Bieszczad, 2021). However, these results were observed under optimal (silent background) listening conditions that are often irrelevant to real-world experiences. Indeed, the extent to which the effects of i-HDAC3 to increase signal-specific neurophysiological and behavioral responses persist in novel backgrounds of noise is currently unknown. Thus, findings are presented that replicate a rodent (rat) model of sound-reward learning for a 5.0 kHz (60 dB) pure tone frequency cue with *in vivo* auditory cortical (A1) multiunit electrophysiological recordings (as in Rotondo & Bieszczad, 2020) to examine the impact of background noise on the frequency-specificity of behavioral and cortical responses mediated by training with or without i-HDAC3. A1 frequency-tuning is known to depend on signal-to-noise ratios in naïve rats (Teschner et al., 2016). To determine the effect of sound-reward learning and i-HDAC3 on this relationship, we assessed behavioral and A1 responses to signal and non-signal frequency cues presented under different signal-to-noise ratios (+0, +20 and +40dB SNR). The results reveal how noisy backgrounds can ultimately impact the behavioral expression of highly frequency-specific memory. Our attempt to build a comprehensive model of the epigenetic regulation of neuroplasticity in A1 and long-term memory for sounds will further improve the ability to achieve successful hearing-related therapeutics in real world environments where sounds are seldom encountered in isolation.

Disclosures: **N. Atesyakar:** None. **A. Shang:** None. **K.M. Bieszczad:** None.

Poster

385. Auditory: Plasticity, Multimodality, Modulation

Location: SDCC Halls B-H

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Program #/Poster #: 385.02

Topic: D.05. Auditory & Vestibular Systems

Support: R01: NIH/NIDCD R01 DC-018561

Title: Experience-dependent massive transcriptional changes within the auditory system that contribute to temporal information encoding for auditory memory

Authors: *A. GUNGOR AYDIN[1], M. S. CHIMENTI[3], K. L. KNUDTSON[3], K. M. BIESZCZAD[1,2];
[1]Psychology- Behavioral and Systems Neurosci., Rutgers Univ., Piscataway, NJ; [2]Dept. of Otolaryngology-Head and Neck Surgery, Rutgers Univ., New Brunswick, NJ; [3]Iowa Inst. of Human Genet., Iowa City, IA

Abstract: The ability to appreciate speech sounds with complex temporal features characteristic of human voices relies on learning to discriminate brief acoustic cues on the order of milliseconds. Learning-induced neurophysiological plasticity in the adult auditory system that operates on this timescale is essential for changes to sound-evoked activity that can enable memory for significant temporal sound features and guide behavior. Lasting neurophysiological changes require *de novo* gene expression within the auditory system. Gene expression is powerfully controlled by epigenetic mechanisms that modify and remodel chromatin to orchestrate a network of activity-dependent genes that dictate central auditory function, neuroplasticity, and—ultimately—successful sound-cued behavior. Blocking histone de-acetylase 3 (HDAC3), one of the most widely studied epigenetic mechanisms in learning and memory processes, typically increases the accessibility of transcriptional regulatory proteins to promoters to enable activity-dependent gene expression during memory consolidation. In rodent models of memory for amplitude-modulated (AM) sound cues, HDAC3-inhibition promotes the formation of highly precise AM-cue memory by facilitating auditory cortical changes in temporal coding (Rotondo & Bieszczad 2021 *J Neurophysiol*). Results will be presented from genome-wide RNA-sequencing on auditory cortical and subcortical samples in trained rats treated with an HDAC3 inhibitor (vs. a group of vehicle-treated trained rats) learning an established AM rate discrimination task. Thus, HDAC3 manipulation provides an opportunity for a molecular-level investigation of activity-dependent genes that are involved in temporal information encoding within the auditory system. Identifying key differentially regulated genes (DEGs) between groups or individuals that formed highly precise temporal acoustic cue memory (vs. those that did not) opens the door to future discovery of the downstream circuit- and systems-level processes regulated by these gene products that are critical to the success of auditory learning and behavioral function.

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Poster

385. Auditory: Plasticity, Multimodality, Modulation

Location: SDCC Halls B-H

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Program #/Poster #: 385.03

Topic: D.05. Auditory & Vestibular Systems

Support: R01 90266555

Title: Spectro-temporal features supporting representation of concurrent natural sounds by single neurons in auditory cortex

Authors: *G. HAMERSKY, L. SHAHEEN, S. DAVID;
OHSU, Portland, OR

Abstract: In everyday hearing, listeners encounter auditory scenes containing complex and spectrally overlapping sound sources. Accurate perception of complex scenes requires streaming, the grouping of sound features into meaningful sources based on statistical regularities in the time and frequency domains. Numerous psychoacoustic studies have described auditory streaming as a perceptual phenomenon, but less is known about its underlying neural basis. The current study recorded single unit activity in the auditory cortex (AC) of awake ferrets. Passively listening ferrets were presented with a series of natural sound excerpts from two broad, ethologically relevant categories: textures (backgrounds, BGs) and transients (foregrounds, FGs). FG and BG stimuli were presented individually and concurrently. Neural responses to overlapping pairs (BG+FG) were modeled as linear weighted combinations of responses to the isolated BG and FG. Model weights showed BG+FG responses were consistently suppressed relative to responses to the individual sounds. Perceptually, FG stimuli typically pop out from BG. Surprisingly, the linear model showed stronger FG-specific suppression compared to BGs in the neural responses. To investigate the mechanism supporting FG suppression, spectral and temporal statistical features of each sound were regressed against the degree to which a sound was suppressed and the degree to which it suppressed other sounds. Sounds with high temporal stationarity, which is more prominent in BG weree more likely to suppress a concurrent sound and less likely to be suppressed. Conversely, a sound with high stationarity on the spectral axis, more common in FG stimuli, was less likely to suppress paired sounds and more likely to be suppressed itself. Ongoing recordings are being collected during presentation of synthetic versions of the natural sounds, in which temporal, spectral, or spectrotemporal statistics are either matched or shuffled relative to the original sounds. These data will directly test the role of these spectrotemporal statistics on neural responses to concurrent stimuli.

Disclosures: G. Hamersky: None. **L. Shaheen:** None. **S. David:** None.

Poster

385. Auditory: Plasticity, Multimodality, Modulation

Location: SDCC Halls B-H

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Program #/Poster #: 385.04

Topic: D.05. Auditory & Vestibular Systems

Support: NIH R01DC018621

Title: The structure of spectro-temporal receptive fields produces noise-invariant spiking neural networks

Authors: *C. FEHRMAN[1], S. MOSELEY[3], J. WEISSMAN[2], C. MELIZA[4];
[2]Computer Sci., [1]Univ. of Virgina, Charlottesville, VA; [3]Psychology, [4]Univ. of Virginia, Charlottesville, VA

Abstract: Animals must be able to recognize salient auditory signals in a noisy environment. This noise is often spectrally and temporally complex and is highly correlated with conspecific vocalizations. Neurons in the avian auditory cortex have been shown to produce noise-invariant firing at relatively low signal-to-noise ratios (SNRs). It is unknown how they are able to filter out background noise while preserving the structure of the signal of interest. One mechanism for this noise-invariance may lie in the structure of spectro-temporal receptive fields (STRFs). STRFs are often modeled as impulse response functions and act as filters on auditory signals. STRFs in the zebra finch (ZF) auditory cortex are known to overlap with the modulation power spectrum of their song vocalizations. We hypothesized this overlap provides a bank of signal filters that allows the system to filter out complex background noise. We tested our hypothesis by constructing a spiking neural network where each neuron was given a STRF parameterized from a known distribution in the ZF auditory cortex. The STRFs used were drawn from either a temporally bandpass, temporally wideband, or mixed distribution. The mixed condition completely covers the modulation power spectrum of ZF song and we hypothesized this condition would produce the strongest noise-invariant responses. We trained the network to reconstruct a noiseless input signal of ZF song. We then varied the input song across three background noise conditions (white, synthetic, and conspecific) and eleven SNRs (from 70 to -10). A noise-invariant network would produce song reconstructions that filtered out the background noise. Consistent with our hypothesis, using the mixed STRFs produced the strongest noise-invariant networks across all three background noise conditions.

Disclosures: **C. Fehrman:** None. **S. Moseley:** None. **J. Weissman:** None. **C. Meliza:** None.

Poster

385. Auditory: Plasticity, Multimodality, Modulation

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Program #/Poster #: 385.05

Topic: D.05. Auditory & Vestibular Systems

Support: Wellcome Trust DBT India Alliance
CSIR
PMRF

Title: Separate functional subnetworks encode regularity and irregularity of sound sequences within the auditory cortex

Authors: M. MEHRA[1], ***A. MUKESH**[2], S. BANDYOPADHYAY[3];
[1]Advanced Technol. Develop. Ctr., Indian Inst. of Technology, Kharagpur, KHARAGPUR, India; [2]Indian Inst. of Technol. Kharagpur, KHARAGPUR, India; [3]Dept. of E&ECE, IIT Kharagpur, Kharagpur, India

Abstract: Adaptation of neural responses to repeated presentation of a tone (Standard) and a higher response being elicited when a different tone is presented (Deviant) is well known as the Stimulus Specific Adaptation or SSA. Computational models based on narrow frequency channel architecture, short-term synaptic depression, and feed-forward inputs can explain SSA for tonal stimuli. Recently, recurrent networks and inhibitory plasticity based models have also shown to replicate deviant detection in the auditory cortex. However, the above concepts do not readily extend to longer time scales, with stimuli being a sequence of tokens as whole. The auditory cortex is known to operate at multiple time scales, with adaptation to stimuli and their statistical structure, occurring in time scales from a few milliseconds at a single neuron level to minutes likely engaging a larger population of the neural circuitry. We use oddball sequences (standards with a deviant) of a fixed number of tokens, consisting of pure frequency tones and broad-band noise to study how excitatory (EXN) and inhibitory neurons (INN) are involved in adaptation to sequences of stimuli. We use two types of stimuli - regular (RG) and irregular (IR). The above two types of sequences are created by keeping the position of the deviant constant and random respectively with respect to the standard tokens. 2-photon Ca imaging in Layer-2/3 of adult mice to study the role of individual EXNs and the modulatory control exercised by two major INNs - Parvalbumin+ (PV) and Somatostatin+ (SOM), in the encoding of the RG or IR sequences of sounds. We found differential encoding of the two types of sequences by the neural population, with two broad groups emerging within them. One group shows a preference for RG stimuli and the other shows a preference for the IR stimuli. We found separate functional subnetworks being formed which encode the temporal statistical features within the stimuli in different ways. The subgroup formation is dictated by connections, particularly with SOM neurons. Overall, these results throw light on the separate computation being performed with the same neuronal population by separate subgroups to encode the differences in the temporal pattern of the stimuli.

Disclosures: **M. Mehra:** None. **A. Mukesh:** None. **S. Bandyopadhyay:** None.

Poster

385. Auditory: Plasticity, Multimodality, Modulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 385.06

Topic: D.05. Auditory & Vestibular Systems

Support: NIH NIDCD R01DC015527
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Title: Mechanism for novelty response modulation by temporal regularity in auditory cortex

Authors: *X. DING[1], M. N. GEFFEN[2];
[1]Neurosci. Grad. Group, [2]Dept. of Otorhinolaryngology, Univ. of Pennsylvania, Philadelphia, PA

Abstract: The statistical structure of sensory stimuli is a key component of how animals process and interact with the external world. In the auditory domain, temporal regularity is a common part of the statistical structure of stimuli, due to physical constraints on naturalistic sound sources. Human subjects exhibit differential responses to regular versus random sound sequences and also exhibit increased behavioral sensitivity to regular sequences of sound. In the mouse brain, temporal regularity modulates the neuronal response in the auditory cortex (AC) to novel tones in an oddball paradigm. Here, we investigated how inhibitory interneurons modulate differential responses to temporally regular and random sequences in sound. We used 2-photon imaging to record calcium traces of AC neurons in head-fixed somatostatin(SST)-cre mice injected with a JAWS inhibitory opsin. The mice were presented with a set of moving ripples presented in a regular or random sequence. In both sequences, a novel ripple occasionally replaced one from the base set and a laser was used to pseudo-randomly inhibit SST interneurons during sound presentation. Neurons in AC exhibited novelty response, as demonstrated by an increased response to the novel ripple as compared to the same ripple presented as part of a random sequence. The novelty response was modulated by the regularity of the sound sequence: more neurons exhibited a novelty response in the regular sequence (36%) as compared to the random sequence (19%). This novelty response modulation by sequence regularity was abolished when SST activity was suppressed optogenetically: on novel ripple presentations with optogenetic inhibition of SST interneurons, the same number of neurons exhibited a significant response in both regular and random sequences. Our results establish that the novelty responses in AC are modulated by sequence regularity, and that this modulation is controlled by a specific type of inhibitory interneuron. This modulation likely supports complex aspects of speech and music perception.

Disclosures: X. Ding: None. **M.N. Geffen:** None.

Poster

385. Auditory: Plasticity, Multimodality, Modulation

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Title: Intra-cortical mechanisms for integration of auditory and olfactory information

Authors: *N. VOGLER, V. TU, A. VIRKLER, R. CHEN, T. LING, J. GOTTFRIED, M. GEFFEN;
Univ. of Pennsylvania, Philadelphia, PA

Abstract: In complex environments, the brain must integrate information from multiple sensory modalities, including the auditory and olfactory systems. However, little is known about how the brain integrates auditory and olfactory stimuli. Here, we investigated the mechanisms underlying auditory-olfactory integration using anatomy, electrophysiology and behavior. We first used viral tracing strategies to investigate the circuits underlying auditory-olfactory integration. Our results demonstrate direct inputs to the auditory cortex (ACx) from the piriform cortex (PCx), mainly from the posterior PCx, suggesting an anatomical substrate for olfactory integration in ACx. We next developed an experimental system for delivering combinations of auditory and olfactory stimuli during *in vivo* electrophysiology, and tested the effect of odor stimuli on auditory cortical responses to sound in awake mice. Odor stimuli modulate the responses of ACx neurons in a stimulus- and sound level-dependent manner, suggesting a neural substrate for olfactory integration in ACx. Finally, we trained mice on a sound detection Go/No-Go task to assess how odor stimuli affect auditory perception and behavior. Odors facilitate auditory perception by lowering sound detection thresholds. Together, our findings reveal novel circuits and mechanisms for auditory-olfactory integration involving the ACx.

Disclosures: N. Vogler: None. **V. Tu:** None. **A. Virkler:** None. **R. Chen:** None. **T. Ling:** None. **J. Gottfried:** None. **M. Geffen:** None.

Poster

385. Auditory: Plasticity, Multimodality, Modulation

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Program #/Poster #: 385.08

Topic: D.05. Auditory & Vestibular Systems

Support: NIH R01 DC016363 to JMG

Title: Visual cues bias single-trial responses in the macaque inferior colliculus to favor a visually-paired sound

Authors: *M. N. SCHMEHL[1], S. T. TOKDAR[2], J. M. GROH[1];
[1]Neurobio., [2]Statistical Sci., Duke Univ., Durham, NC

Abstract: Visual cues can influence brain regions that are sensitive to auditory space (for review, see Schmehl & Groh, *Annual Review of Vision Science* 2021). However, how such visual signals in auditory structures contribute in the perceptual realm is poorly understood. One particularly interesting possibility is that visual inputs help the brain distinguish among different sounds, allowing better localization of behaviorally relevant sounds in a noisy environment (i.e., the cocktail party phenomenon). Our lab previously reported that when two sounds are present, auditory neurons may switch between encoding each individual sound across time (Caruso *et al*.,

Nature Communications 2018). We sought to study how pairing a visual cue with one of two sounds might change these time-varying responses (e.g., Atilgan *et al*., *Neuron* 2018). To test this, we trained one rhesus macaque (*Macaca mulatta*, female, 15 years old) to localize one or two sounds in the presence or absence of accompanying lights. While the monkey performed this task, we recorded extracellularly from single neurons in the inferior colliculus (IC), a critical auditory region that receives visual input and has visual and eye movement-related responses. We found that pairing a visual cue with a sound can change an IC neuron's response to that sound, even if the neuron is unresponsive to visual input alone. Further, when two sounds are present and one sound is paired with a visual cue, neurons are more likely to spend individual trials responding to the visually-paired sound. Together, these results suggest that the IC alters its sound representation in the presence of visual cues, providing insight into how the brain combines visual and auditory information into a single perceptual object.

Disclosures: M.N. Schmehl: None. **S.T. Tokdar:** None. **J.M. Groh:** None.

Poster

385. Auditory: Plasticity, Multimodality, Modulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 385.09

Topic: D.05. Auditory & Vestibular Systems

Support: Leon Levy Foundation
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Title: Motor-related predictions in mouse auditory cortex are context-dependent

Authors: *A. LA CHIOMA, D. M. SCHNEIDER;
Ctr. for Neural Science, New York Univ., New York, NY

Abstract: Auditory perception relies on predicting the acoustic consequences of our actions. Correspondingly, neural circuits in the brain respond differently to expected versus unexpected self-generated sounds. In the real world, the same motor action can produce different sounds depending on the environment in which the behavior is produced - e.g. footsteps sound different when walking on concrete compared to fallen leaves. Yet it remains untested whether the brain can dynamically update predictions about self-generated sounds in a context-dependent manner and on an ethological timescale. To address this question, we developed a visual-acoustic virtual reality (VR), in which a head-fixed mouse on a treadmill repeatedly traverses two different environments, each consisting of a distinct visual corridor accompanied by distinct artificial footstep sounds. Following extensive behavioral acclimation, we made high-density neuronal recordings from auditory cortex as mice traversed VR and experienced either expected or deviant footsteps. We observe overall strong suppression of neural responses to self-generated sounds compared to the same sounds heard passively. When mice hear footstep sounds in the wrong visual context, neural responses are on average larger than when mice hear footstep sounds in the

correct context, consistent with an expectation violation. Expectation violations emerge almost immediately after a mouse enters a new context, suggesting a rapid updating of predictions in parallel with behavior. Neurons with strong context-dependent modulation tend to reside in infragranular cortex. Together, our results suggest that the auditory cortex may combine auditory and motor signals with visual spatial cues for real-time, context-dependent processing of self-generated sounds.

Disclosures: A. La Chioma: None. **D.M. Schneider:** None.

Poster

385. Auditory: Plasticity, Multimodality, Modulation

Location: SDCC Halls B-H

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Program #/Poster #: 385.10

Topic: D.05. Auditory & Vestibular Systems

Support: NIH R21DC018327
Klingenstein-Simons Fellowship Award in Neuroscience
Hearing Health Foundation Emerging Research Grant

Title: Diverse influences of pupil-linked arousal on cell-type specific auditory processing

Authors: *K. J. KAUFMAN[1], R. F. KRALL[1], M. P. ARNOLD[1], T. SUAREZ OMEDAS[2], R. S. WILLIAMSON[1];
[1]Otolaryngology, Univ. of Pittsburgh, Pittsburgh, PA; [2]Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Stimulus-independent nervous activity associated with arousal state, as indexed by pupil diameter, varies continuously and influences membrane potentials, cortical state, and sensory processing. Indeed, previous studies have shown that the response strength, reliability, and tuning selectivity of layer (L) 2/3 neurons within the auditory cortex (ACtx) are modulated by arousal. The processing of acoustic information recruits a diverse set of excitatory neurons that span the entire cortical lamina. These excitatory neurons can be broadly categorized as intratelencephalic (IT), extratelencephalic (ET), or corticothalamic (CT), based on their downstream targets. These distinct neural populations have their own unique anatomy, morphology, and intrinsic and synaptic properties. These marked differences will likely lead to state-dependent changes in sensory coding.
We combined *in vivo* two-photon calcium imaging and full-face videography in awake mice to investigate how arousal regulates the response properties and functional connectivity of L2/3 IT, L5 IT, ET, and CT neurons. We first analyzed both the amplitude and reliability of neural responses to pure tones and found that both measures scaled monotonically with arousal in ET and CT neurons, but not in L2/3 and L5 IT neurons. This indicates that ET and CT cells become more excitable when animals are more alert. We then utilized a measure of lifetime sparseness to investigate whether arousal could explicitly alter the shape of the neural response distributions. The sparsity of L2/3 IT and ET neurons decreased as a function of arousal indicating that, at high

arousal states, these neurons typically respond to an increased number of stimuli. In contrast, the sparsity of L5 IT cells increased monotonically with arousal and the sparsity of CT cells was lowest at intermediate arousal states. Modulating the tuning properties of neurons can result in similar patterns of activity for different stimuli, degrading the precision of sound encoding. We explicitly tested this using a statistical neural decoder, where we could determine how accurately stimulus identity could be predicted at each arousal state using the different neural populations. This analysis revealed an inverse-U function whereby intermediate arousal states had the highest decoding accuracy, suggesting stimulus representations are less faithfully encoded at low and high levels of arousal. Together, these findings provide the building blocks for a detailed description of how sensory coding is differentially altered in specific cortical projection neurons across pupil-linked arousal states.

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Poster

385. Auditory: Plasticity, Multimodality, Modulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 385.11

Topic: D.05. Auditory & Vestibular Systems

Title: 40 hz gamma synchrony: association cortices are more vulnerable than primary auditory cortex to nmda blockade

Authors: *D. GAUTAM, M. U. RAZA, D. R. SIMMONS, S. V. DIGAVALLI;
Dept. of Pharmaceut. Sci., East Tennessee State Univ., Johnson City, TN

Abstract: The 40 Hz auditory steady state response (ASSR) is an EEG measure of local gamma neural synchrony that is evoked by the repeated presentation of a 40 Hz click train. While the principal cortical generators of this response appear to be bilateral primary auditory cortices as they show the largest phase locking and evoked power, other regions across the cortical mantle synchronize too, including the prefrontal cortex (PFC) that receives input from the primary auditory cortex and is involved in higher order cognitive functions. In schizophrenia, PFC function is compromised and it is hypothesized that NMDA-dependent neurotransmission is involved. Indeed, in rodents, NMDA antagonists reliably disrupt set shifting, a working memory task linked to PFC function. It is however not known if NMDA antagonism would disrupt the 40 Hz ASSR in PFC. In the following study, we equipped separate groups of female SD rats with epidural electrode targeting the PFC (1.5 mm anterior and 1 mm lateral to bregma) or the primary auditory cortex (5 mm caudal, 7.25 mm lateral and 3.5 mm ventral to bregma). Two epidural screw electrodes on cerebellum served as ground and reference. After recovery from surgery, and acclimation, rats were pretreated with a modest dose of the NMDA antagonist MK801 (0.05 mg/kg) or saline (2 ml/kg, sc) in a cross-over design, tethered to EEG cables and EEG was amplified and acquired (Signal 7.0; CED1401 Micro 3). Trains of square waves (~ 1

ms duration; 40/s) were generated and played through the house speakers at ~ 50 dB SPL. EEG was acquired as 5 s sweeps while a 40 Hz click train played between 1-2 s, of each sweep; 50 trials were recorded from each subject, 30 min post-treatment. Post-vehicle, robust EEG entrainment was noted in both the temporal cortex as well as the PFC. As expected, the EEG signal and phase-locking from the temporal cortex was notably larger compared to the PFC. Nevertheless, both regions showed clear 40 Hz entrainment to click trains. However, MK801 effect on the 40 Hz ASSR was disparate across the two regions. The temporal lead showed a significant *increase* in intertrial coherence (ITC) of the 40 Hz ASSR ($P=0.03$; paired t-test), while background gamma (35-45 Hz) power also increased ($p=0.027$). Evoked power showed a trend towards an increase ($p<0.1$). In contrast, ITC was strongly *disrupted* in the frontal lead ($p=0.005$) and evoked power showed a trend towards reduction ($p=0.059$), while background gamma was unaffected. These results indicate that gamma neural synchrony in higher order cortices like the PFC is more vulnerable to NMDA antagonist-mediated disruption, as compared to the primary auditory cortex.

Disclosures: D. Gautam: None. **M.U. Raza:** None. **D.R. Simmons:** None. **S.V. Digavalli:** None.

Poster

385. Auditory: Plasticity, Multimodality, Modulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 385.12

Topic: D.05. Auditory & Vestibular Systems

Support: U19NS107464

Title: Neural dynamics for implicit learning of complex sounds in auditory cortex

Authors: *H. KANG[1], P. O. KANOLD[2];
[1]Dept. of Biomed. Engin., [2]Biomed. Engin., Johns Hopkins Univ., Baltimore, MD

Abstract: A key function of the brain is sensory perception in changing and uncertain environments. In audition, fast implicit learning of incoming auditory inputs is a fundamental ingredient of efficient perception and segregation in complex auditory scenes that contain both changing and unchanging elements. However, neural mechanisms for implicit learning have not been clearly identified. We identify changes in neural responses to randomly re-occurring sounds, considered as mnesic trace, in auditory cortex (ACtx) to study neural mechanisms for implicit learning. To do so, we present a series of spectro-temporally complex sounds, dynamic random chords; DRCs. DRCs are artificial sound sequences in which at each time bin (20 ms for 1 sec) multi-frequency tone pips with varying sound levels (50 - 90 dB SPL) in each frequency bin (4-40 kHz separated by 10 frequency bins following a logarithmic scale) are generated. Average sound level of each sound is set at 70dB SPL. We take CBA x Thy1-GCaMP6s F1 mice to trace response changes of excitatory cells. While each DRC is generated afresh (Random

sound; 20 different tokens), one specific sequence (Target sound) will re-appear at random trials for 20 times. We play a series of these DRCs in a random order to passively listening awake young adult mice while conducting two-photon imaging in three different subregions: thalamocortical layer L4 of primary auditory fields (A1), superficial layer L2/3 of A1, and secondary auditory fields (A2). We quantify whether there is any distinctive neural dynamics for the Target sound from its repetitive exposure, compared to Random sounds. We also identify whether the 'learning cells' are more prevalent from certain cell types based on their tuning curve, per subregions. We observed a trend of greater adaptation of neural responses to the re-occurring Target sound compared to other Random sounds in high-order regions. This can be seen as a larger response of the Random sound over the re-occurring Target sound. Our results suggest that the higher-order region in the ACtx is more involved for implicit learning processes.

Disclosures: H. Kang: None. **P.O. Kanold:** None.

Poster

385. Auditory: Plasticity, Multimodality, Modulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 385.13

Title: WITHDRAWN

Poster

385. Auditory: Plasticity, Multimodality, Modulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 385.14

Topic: D.05. Auditory & Vestibular Systems

Support: R01DC014101
R01NS116598

Title: Inconsistency, variability, and fragility in pupillometry measurements: implications for cross-animal comparisons

Authors: *T. R. OLSEN, A. R. HASENSTAUB;
Dept. of Otolaryngology-Head and Neck Surgery, UCSF, San Francisco, CA

Abstract: Even when environmental luminance is held constant, pupil diameters still fluctuate, largely reflecting internal variation in neuromodulatory state and arousal (Reimer et al., 2016). Many studies have shown that variation in pupil diameter explains much of what was previously considered "noise" in neuronal activity, highlighting the close coupling between arousal and

brain function (McGinley et al., 2015). However, both arousal levels and the extent to which sensory stimulation modulates arousal may differ from one recording to the next or from one animal to the next. This variability may confound our attempts to compare results between animals, or particularly between studies: for instance, some studies normalize pupil diameters according to the range of diameters observed during one recording session (e.g., McGinley et al., 2015), while others report raw pupil diameter measurements (e.g., Yang et al., 2021). We sought to quantify the differences in pupil dynamics across recordings, and to determine what these differences imply about the relationship between pupillometry and neuronal data.

We recorded pupillometry, spontaneous activity, and sound-evoked activity from the auditory cortex of 17 male and female mice (101 recordings) while presenting identical sequences of intermittent noise burst stimuli. We find that even under constant light illumination, pupil dynamics vary greatly from one recording to the next, and from one animal to the next, producing qualitatively different relationships between neural firing and pupil size depending on how pupil diameters were normalized. We also found that over the course of an experiment, both baseline pupil diameters and sound-evoked pupil dilations typically grew smaller; however the degree of this adaptation differed greatly from one recording to the next. Finally, we show that variations in pupil dynamics across recordings relate to variations in evoked and spontaneous firing.

In summary, our results highlight the importance of tracking features of pupil dynamics specific to individual mice across recordings, especially when results are averaged across recordings, or compared across studies.

McGinley MJ, et al., (2015) Waking State: Rapid Variations Modulate Neural and Behavioral Responses. Neuron 87:1143-1161.

Reimer J, et al., (2016) Pupil fluctuations track rapid changes in adrenergic and cholinergic activity in cortex. Nat Commun 7.

Yang H, et al., (2021) Locus coeruleus spiking differently correlates with S1 cortex activity and pupil diameter in a tactile detection task. Elife 10.

Disclosures: **T.R. Olsen:** None. **A.R. Hasenstaub:** None.

Poster

385. Auditory: Plasticity, Multimodality, Modulation

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Topic: D.05. Auditory & Vestibular Systems

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R01MH122478
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National Science Foundation
Klingenstein Foundation

Title: Encoding of non-auditory inputs by coordinated neuronal ensembles in mouse auditory cortex

Authors: *J. BIGELOW[1], R. MORILL[2], T. OLSEN[1], J. DEKLOE[3], C. SCHREINER[1], A. R. HASENSTAUB[1];
[1]Otolaryngology-Head and Neck Surgery, UCSF, San Francisco, CA; [2]Zuckerman Inst., Columbia Univ., New York, NY; [3]Stryker Sch. of Med., Western Michigan Univ., Kalamazoo, MI

Abstract: Mounting evidence suggests synchronous activity among multiple neurons may be an essential aspect of cortical information processing and transmission. Such activity can be measured using dimensionality reduction techniques to define coordinated neuronal ensembles (cNE), which reflect groups of neurons with temporally coincident changes in firing. Previous work in auditory cortex (AC) has shown that cNE events contain more information about sound features than individual member neuron spikes on a per spike event basis (encoding efficiency). Moreover, the preferred sound features encoded by single neurons often depend on whether they were spiking with a given cNE ("information multiplexing"). In addition to sound encoding, it is well known that AC neurons are influenced by a wide range of non-auditory inputs reflecting motor activity, arousal, crossmodal sensory events, reward expectation, and attention. Nevertheless, it remains unknown whether these extramodal inputs are processed through population-level encoding in the same way as acoustic signals from the ascending auditory pathway. In the present study, we addressed this question by examining single neurons and cNEs in AC of mice performing an audiovisual attention switching task. As in previous studies, many single units and cNEs responded to sounds and were often modulated by non-auditory variables including movement velocity, pupil size, and modality specific attention. Importantly, we found that cNE representation of non-auditory inputs contained more information about events and states than member neuron spikes, similar to patterns previously described for sounds. Furthermore, modulation of single neuron activity by non-auditory inputs often depended on whether its activity coincided with a cNE. Our findings suggest auditory and extra-modal inputs may be subject to similar processing by cNEs in AC, and support prior work suggesting cNE activity as an information processing motif in cortex.

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Poster

385. Auditory: Plasticity, Multimodality, Modulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 385.16

Topic: D.05. Auditory & Vestibular Systems

Title: Eeg activation patterns of auditory spatial attention in the front vs. back hemispace

Authors: *A. L. BODNAR[1], J. R. MOCK[2], E. J. GOLOB[2];
[1]Johns Hopkins Med. Institutions, Baltimore, MD; [2]Univ. of Texas at San Antonio, San Antonio, TX

Abstract: The ability to detect and attend to sounds in panoramic 3-D space is fundamental, yet most research focuses on the frontal region of auditory space. This study compared auditory spatial when directed to midline locations in front vs. back space within the horizontal (azimuth) plane. The main hypothesis is that crossmodal influences of vision on auditory spatial attention would generate a more concentrated focus of attention in the front vs. back hemispace. Analyses focused on multimodal temporo-parietal cortex, with right involved in spatial orienting, and medial prefrontal cortex involved in attention control. Subjects (n=25) attended to either front or back midline (separate blocks), and distinguished amplitude-modulated (AM) white noise (25 or 75 Hz) from 5 speakers at 45° intervals in the front or back 180° hemispace. Oscillations in EEG frequency bands that are implicated in attention control (theta, 4-8 Hz) and modulation of sensory processing (alpha, 8-12 Hz), were examined using independent component analysis and cortical source localization. Most stimuli were presented from the attended midline location ("standard", p=.84), with occasional stimuli from one of the other 4 speakers ("shift", p=.04/location). Reaction times to shift locations were significantly slower than the standard in the front (p<.004), but not back (p=.11), hemispace. Right temporo-parietal alpha power increased when attending to front vs. back standards (280-400ms, p<.001), and increased in the front hemispace (standard < shifts during 80-200 ms; (p<.001). In the left temporo-parietal cluster, alpha power increased in the front vs. back standard location (F>B; p<.01) during 280-400ms time-window. Alpha power also increased at standard vs. shift locations in the front hemispace (standard<shifts; p<.001), but not in the back hemispace (280-400 ms). The midfrontal cluster had significantly greater theta power at standard vs. shift locations in the front hemispace (standard<shifts; p<.001), during earlier latencies (20-230 ms) that equalized across locations at later latencies. Taken together, the findings suggest that the allocation of auditory spatial attention was more focal in the front vs. back hemispace. Behavioral and EEG activity in the back hemispace were comparable across locations, while in the front behavioral and some neuron oscillations increased as a function of angular distance from the attended location.

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Poster

385. Auditory: Plasticity, Multimodality, Modulation

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Program #/Poster #: 385.17

Topic: D.05. Auditory & Vestibular Systems

Support: NIH-NIDCD 5R01DC014279

Title: Modeling non-linear dynamics of auditory cortical adaptation to changing background noise with deep neural networks

Authors: *G. MISCHLER[1], M. KESHISHIAN[2], S. BICKEL[3], A. D. MEHTA[4], N. MESGARANI[1];
[2]Electrical Engin., [1]Columbia Univ., New York, NY; [3]Neurosurg. / Neurol., Hofstra Northwell Sch. of Med., Manhasset, NY; [4]Neurosurg., Hofstra North Shore LIJ Sch. of Med., Great Neck, NY

Abstract: The human auditory system displays a robust capacity to adapt to sudden changes in background noise, allowing for continuous speech comprehension despite changes in background environments. However, despite comprehensive studies characterizing this ability, the computations that enable the brain to achieve this are not well understood. The first step towards understanding a complex system is to propose a suitable model, but the classical and easily interpreted model for the auditory system, the spectro-temporal receptive field (STRF), cannot match the nonlinear dynamics of noise adaptation. To overcome this, we utilize a deep neural network (DNN) to model neural adaptation to noise, illustrating its effectiveness at reproducing the complex dynamics at the levels of both individual electrodes and the cortical population. By closely inspecting the model's STRF-like computations over time, we find that the model alters both the gain and shape of its receptive field when adapting to a sudden noise change. We show that a DNN model's gain changes allow it to perform contrast gain control, while the shape changes enable noise filtering by altering the model's receptive field. Further, we find that models of electrodes in nonprimary auditory cortex exhibit different filtering changes compared to primary auditory cortex, suggesting differences in noise filtering mechanisms along the cortical hierarchy. These findings demonstrate the capability of deep neural networks to model complex neural adaptation and offer new hypotheses about the computations that the auditory cortex performs to enable noise-robust speech perception in real-world, dynamic environments.

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Poster

386. Subcortical Visual Processing, Including Feedforward and Feedback Pathways

Location: SDCC Halls B-H

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Program #/Poster #: 386.01

Title: WITHDRAWN

Poster

386. Subcortical Visual Processing, Including Feedforward and Feedback Pathways

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 386.02

Topic: D.06. Vision

Support: Google PhD Fellowship
Google Cloud Research Credits

Title: Modeling Natural Visual Stimulus Encoding in the Lateral Geniculate Nucleus using Deep Learning

Authors: *M. GAMAL[1], S. ELDAWLATLY[2,1];
[1]Computer Sci. and Engin., German Univ. in Cairo, Cairo, Egypt; [2]Computer and Systems Engin., Ain Shams Univ., Cairo, Egypt

Abstract: The lateral geniculate nucleus (LGN) role in visual information processing has been found to include nonlinearities in addition to its well-known linear role as a relay nucleus in the thalamus linking the retina with the visual cortex. However, our understanding of the LGN nonlinearities is still limited. Recently, advances in deep learning opened the door to developing complex nonlinear encoding models of neural responses. Such encoding models could offer a better understanding of nonlinear computations in the visual system compared to the commonly used basic statistical models. In this study, we developed a deep convolutional neural network (CNN) encoding model of mouse LGN neural responses to natural stimuli. The developed model is based on a pre-trained CNN model that was originally trained on an object recognition task. Using the Allen Brain Observatory's Visual Coding Neuropixels dataset, we have examined the model's performance in predicting LGN neural responses to natural scenes. The model succeeded in predicting the LGN responses with the highest predictive power at the first convolutional layers compared to the subsequent layers. Using the feature space of the first convolutional pre-trained layer, the model achieved on average, across neurons recorded from 12 animals, a correlation of 0.39 computed between the actual and predicted LGN neural responses. Additionally, an average fraction of explainable variance (FEV) of 28% was achieved. This finding shows that the early layers of the models trained on object recognition better match the LGN processing stage than the intermediate and late layers in contrast to the primary visual cortex which was found to better match the intermediate layers. This is consistent with the location of the LGN along the visual pathway as an early processing site. Accordingly, we have used this model, with the feature space of the first pre-trained layer, in examining and understanding nonlinear computations in the LGN. The LGN was found to encode information about how far the high-intensity pixels are scattered in a natural scene. The results of this study indicate that transfer learning using deep pre-trained CNN models can be successfully used in predicting the LGN responses to natural stimuli and in understanding nonlinear computations in the LGN.

Disclosures: M. Gamal: None. **S. Eldawlatly:** None.

Poster

386. Subcortical Visual Processing, Including Feedforward and Feedback Pathways

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 386.03

Topic: D.06. Vision

Title: Spike-time-dependent plasticity of retinal input to Superior colliculus wide-field neurons

Authors: *F. THOMAS[1], G. J. STUART[1,2];
[1]Eccles Inst. of Neurosci., Australian Natl. Univ., Canberra, Australia; [2]Dept. of Physiology, Biomedicine Discovery Inst., Monash Univ., Melbourne, Australia

Abstract: The rodent superior colliculus (SC) plays a critical role in the generation of innate defensive behaviours, such as freezing and escape responses to threatening visual stimuli. While these behaviours are thought to be hardwired, they show habituation, suggesting a role of synaptic plasticity in the SC. Of the four main cell types in the SC, wide-field (WF) neurons are particularly responsive to small and slow-moving objects, akin to a predator flying overhead. Furthermore, responses in WF neurons have been suggested to habituate to repeated looming stimuli, which typically generate escape responses. To better understand the capacity of retinal input to the SC to undergo synaptic plasticity, and thereby the cellular mechanisms underlying habituation of innate defensive behaviours, here we investigate spike-time-dependent plasticity (STDP) of retinal input to WF neurons. We investigated the retinocollicular pathway in adolescent (6 week old) mice using a combination of optogenetics and electrophysiology *in vitro*. Intravitreal injection of AAV2-Syn-Chronos-GFP was performed to expressed the Channelrhodopsin variant Chronos in retinal ganglion cell (RGC) axons. Whole-cell patch clamp recordings were obtained from SC cells in brain slices, with different SC cell types identified based on their electrophysiological and morphological properties. STDP protocols during brief (2 ms) blue-light (470 nm) activation of RGC axons was used to investigate changes in the strength of the retinocollicular connection to WF neurons. We identified four distinct populations of SC cells based on their electrophysiological and morphological characteristics and found that each of these SC cell types received direct, monosynaptic input from RGCs. Activation of retinal input 10 ms prior to action potential generation in WF cells reliably produced timing-dependent long-term potentiation (t-LTP) at retinocollicular synapses, whereas optogenetic-driven retinocollicular input 15 ms after action potential generation induced timing-dependent long-term depression (t-LTD). t-LTP response was block by bath application of both APV (an NMDA receptor antagonist) and nimodipine (a L-type Ca^{2+} channel blocker), whereas t-LTD was only blocked by nimodipine. These findings indicate that retinal input to the SC can undergo long-term synaptic plasticity, which may play a role in the habituation of behavioural responses to threatening stimuli.

Disclosures: F. Thomas: None. **G.J. Stuart:** None.

Poster

386. Subcortical Visual Processing, Including Feedforward and Feedback Pathways

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Program #/Poster #: 386.04

Topic: D.06. Vision

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CIHR (Project Grant 437007)
Connaught New Researcher Awards

Title: The role of inhibitory neurons in the brainstem circuit mediating optokinetic reflex

Authors: *Y. HE, A. LAVOIE, J. LIU, B. LIU;
Univ. of Toronto, Mississauga, Univ. of Toronto, Mississauga, Mississauga, ON, Canada

Abstract: The role of inhibitory neurons in the brainstem circuit mediating optokinetic reflex
In spite of a minor population, inhibitory neurons play critical roles in encoding and processing information. Although their circuits and functions in the sensory cortices have been extensively studied in the past decades, their contribution to the sensory processing in the brainstem remains unclear. To fill this knowledge gap, we chose to study a brainstem complex consisting of the nucleus of the optic tract and the dorsal terminal nucleus (NOT-DTN). This structure mediates the optokinetic reflex (OKR), an innate ocular movement that stabilizes retinal images, and it has abundant GABAergic inhibitory neurons. We examined the response properties of inhibitory NOT-DTN neurons and their contribution to the feature selectivity of excitatory NOT-DTN neurons. First, with optogenetic assisted identification of cell types, we discovered that inhibitory neurons were more heterogeneous in preferred directions and exhibited less direction selectivity than excitatory neurons. They also preferred lower spatial frequencies, compared to the excitatory neurons. Next, using electrophysiology and anatomical circuit tracing, we found that inhibitory NOT-DTN neurons that project to the superior colliculus did not overlap with those projecting to the MTN, and these two inhibitory populations differed in direction and spatial frequency tuning curves. Lastly, with optogenetic circuit perturbation, we uncovered that inhibitory neurons sharpened the tuning curves of excitatory neurons and enhanced their selectivity to visual features. Our results demonstrate that the inhibitory neurons in the NOT-DTN have distinct visual feature selectivity and play an important role in visual information processing of brainstem OKR circuit.

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Poster

386. Subcortical Visual Processing, Including Feedforward and Feedback Pathways

Location: SDCC Halls B-H

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Program #/Poster #: 386.05

Topic: D.06. Vision

Support: NIH R01EY026286

Title: Mapping visual functions onto molecular cell types in the mouse superior colliculus

Authors: *Y. LIU[1], E. SAVIER[1], V. DEPIERO[1], C. CHEN[2], D. SCHWALBE[1], R.-J. FAN[1], H. CHEN[1], J. CAMPBELL[1], J. CANG[3];
[1]Dept. of Biol., [2]Dept. of Psychology, [3]Dept. of Biol. and Psychology, Univ. of Virginia, Charlottesville, VA

Abstract: The superficial superior colliculus (sSC) manifests diverse visual responses to specific stimuli and plays critical roles in visual processing. Although these visual response properties have been widely studied in the sSC, it remains unclear whether they are specifically encoded by molecularly distinct cell types. To address this question, we performed single-nucleus RNA-sequencing (snRNA-seq) and unsupervised clustering analysis to classify tens of thousands of sSC neurons unbiasedly into 28 types. We then used differential expression analysis to identify marker genes for each population. In situ hybridization of these markers revealed a layer-specific organization in the sSC. Next, we combined in vivo 2-photon calcium imaging and fluorescence in situ hybridization (FISH) to unmask the molecular identities of functional subtypes. Specifically, we sparsely labeled neurons in the sSC as landmarks and used 2-photon imaging to determine neuronal responses to visual stimuli. This was followed by horizontal sectioning, FISH of previously identified markers and confocal imaging. By comparing the landmarks, neuronal morphology, and spatial locations between in vivo functional imaging and ex vivo FISH, we were able to register the functionally defined neurons to their genetic markers. We identified a genetically distinct neuron subtype which accounts for ~50% of the direction selective cells in the sSC. The gene expression profile of this population indicates that it is an inhibitory neuron population. Together, our studies generate a comprehensive molecular atlas of sSC neuron subtypes and identify their differentially enriched genes. This information will allow genetic access to study the function, connection, and development of sSC neuron subtypes. Finally, the registration method we have developed to map visual responses onto molecularly-defined cell types can be widely applied to reveal the molecular identities of other functional cell types in the SC and beyond.

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Poster

386. Subcortical Visual Processing, Including Feedforward and Feedback Pathways

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FONDECYT 1210169

Title: The Chilean brush tailed mouse (Octodon degus): a diurnal precocial rodent as a new model to study visual receptive field properties of superior colliculus neurons.

Authors: *N. I. MÁRQUEZ[1], P. FERNÁNDEZ-ABURTO[2], A. R. DEICHLER[1], I. PERALES[1], J.-C. LETELIER[1], G. J. MARÍN[1], J. MPODOZIS[1], S. L. PALLAS[2];
[1]Biol., Univ. de Chile, Santiago, Chile; [2]Dept. of Biol., Univ. of Massachusetts, Amherst, Amherst, MA

Abstract: The midbrain superior colliculus (SC) is responsible for a variety of visually driven behaviors such as gaze shifts, orientation toward objects of interest, and defensive responses to looming objects. Neurons from the superficial layers of the SC are tuned to specific configurations of visual stimuli. Developmental studies in rodents have shown that the organization of SC receptive fields (RFs) is well established in newborns, and that visual experience is critical to maintain RF properties. However, rodent species studied so far (hamsters, rats, and mice) are nocturnal, altricial, and possess a very simple visual system. As an initial step to establish a viable alternative, we have characterized the RF properties of SC neurons in the Chilean degu, a diurnal, precocial rodent species with a better-elaborated visual system. We characterized isolated neuronal responses from the degu SC using four types of visual stimuli: (1) a moving white square on a dark background, (2) sinusoidal gratings with varying spatial frequencies, (3) a black expanding circle (looming), and (4) a stationary black circle. RF sizes of single units were consistently smaller in the most superficial layers (25-80 deg^2 above 500 µm) and increased in size in deeper layers (30-290 deg^2 below 500 µm). Most neurons displayed spatial frequency tuning ranging from 0.08 to 0.24 cycles/degree. Finally, we found looming-responsive units, all of which increased their firing rate as the looming object increased in size. When tested with a stationary stimulus, looming units showed ON, OFF, or ON-OFF responses. Our study is an important step in understanding the role of visual experience in a rodent species that differs in visual habits and phylogenetic position from more commonly studied species.

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Poster

386. Subcortical Visual Processing, Including Feedforward and Feedback Pathways

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 386.07

Title: WITHDRAWN

Poster

386. Subcortical Visual Processing, Including Feedforward and Feedback Pathways

Location: SDCC Halls B-H

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Program #/Poster #: 386.08

Topic: D.06. Vision

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Title: Functional convergence of on-off direction selective ganglion cells in the visual thalamus

Authors: Q. JIANG[1], E. Y. LITVINA[1,2], H. ACARÓN LEDESMA[1], G. SHU[1], T. SONODA[1], W. WEI[3], *C. CHEN[1];
[1]F.M. Kirby Neurobio. Ctr., Boston Children's Hospital, Harvard Med. Sch., Boston, MA; [2]Natl. Inst. of Neurolog. Disorders and Stroke, Bethesda, MD; [3]Dept. of Neurobio., The Univ. of Chicago, Chicago, IL

Abstract: Understanding how sensory information is parsed in the brain has been key to progress in neuroscience. In the mouse visual system, multiple types of retinal ganglion cells (RGCs) each encode distinct features of the visual space. How this information is parsed in their downstream target, the visual thalamus, is unclear. In the present study, we explored the functional connectivity of directional inputs in the dorsal lateral geniculate nucleus (dLGN) by using mouse genetics to label different subsets of a RGC type: the on-off Direction Selective Ganglion Cells (ooDSGCs). OoDSGCs can be classified into 4 subtypes, each tuned to one of four cardinal axes: dorsal, ventral, nasal or temporal. We made use of *Cart-IRES2-Cre-D* and *BD-CreER2* mice to label and drive ooDSGCs tuned to the vertical directions and to only ventral motion, respectively. Our immunohistochemical, electrophysiological, and optogenetic experiments reveal that only a small fraction (< 15%) of thalamocortical (TC) neurons in the dLGN receive greater than 50 % of their RGC inputs from the labeled ooDSGCs. The majority of the functionally identifiable ooDSGC inputs in the dLGN are weak and converge together with inputs from other RGC types. Yet our modeling indicates that mixing of convergent RGC types is not random: BD-CreER^{+} ooDSGC inputs converge less frequently with ooDSGCs tuned to the opposite direction than with non-CART-Cre^{+} RGC types. Taken together, these results indicate that convergence of distinct information lines in dLGN follow specific rules of organization. While RGC inputs tuned to different features of the visual space can converge onto common TC neurons, this mixing does not apply for subtypes of ooDSGCs tuned to opposite

directions of motion. Overall, our findings point to a greater complexity in the rules governing convergence of different types versus subtypes of RGCs than previously understood.

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Poster

386. Subcortical Visual Processing, Including Feedforward and Feedback Pathways

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Topic: D.06. Vision

Support: NIH Grant EY029703

Title: Duration of labeling by rAAV2retro-hSyn-EGFP and rAAV2retro-hSyn-mCherry of retinal ganglion cells projecting to the superior colliculus

Authors: *M. D. DILBECK, J. R. ECONOMIDES, J. C. HORTON;
Univ. of California, San Francisco, San Francisco, CA

Abstract: Recently we demonstrated that 30-40% of retinal ganglion cells are transduced one month after injection of rAAV2retro-hSyn-EGFP and rAAV2retro-hSyn-mCherry into the superior colliculus of the rat (Nanjappa et al, Exp. Eye Res. 2022). For viral vectors to be effective as a therapeutic tool, they must express the gene of interest for much longer, preferably years. Fluorescently labeled ganglion cells can be visualized easily with a fundus camera, allowing one to compare the number of transduced cells at different time points following virus injection. Here, we have extended our previous study by testing for how long virus labeling persists in retinal ganglion cells. Four rats received bilateral injections of 1 µl containing an equal mixture of the rAAV2 retro virus with the gene for EGFP or mCherry, using electrophysiology to target the visually responsive layer of the superior colliculus. Fundus imaging was performed 14, 45, and 230 days later. The number of labeled cells was compared in photographs that captured the same regions of retina at each date. At 14 days, the EGFP cell counts were: OD 180, OS 166 (Rat A), OD 622, OS 195 (Rat B), OD 777, OS 766 (Rat C), OD 828, OS not imaged (Rat D). By day 45, every cell labeled at day 14 was still visible. 2-12 more cells were visible at day 45, but this increase may have been due to improved photographic technique. At 14 days, the mCherry cell counts were: OD not imaged, OS not imaged (Rat A), OD 1,480, OS 385 (Rat B), OD 240, OS 992 (Rat C), OD 305, OS not imaged (Rat D). By day 45, for fundi imaged at both dates, every cell labeled at day 14 was still visible. 1-23 more cells were visible at day 45. These observations indicate that by 14 days after rAAV2retro injection into the superior colliculus, label has become visible in every retinal ganglion cell that eventually will show label, and this label remains stable. Continued observation with serial fundus imaging will establish the ultimate duration of gene expression in transduced ganglion cells.

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Poster

386. Subcortical Visual Processing, Including Feedforward and Feedback Pathways

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 386.10

Topic: D.06. Vision

Support: NIH Grant EY027888
William M. Wood Foundation

Title: Characterizing extracellular spike shape and chromatic receptive fields in the lateral geniculate nucleus of awake macaques

Authors: *S. SUN[1,2], N. J. KILLIAN[3], J. S. PEZARIS[1,2];
[1]Massachusetts Gen. Hosp., Boston, MA; [2]Neurosurg., Harvard Med. Sch., Boston, MA;
[3]Neurosurg., Albert Einstein Col. of Med., Bronx, NY

Abstract: Have we identified all cell types in the lateral geniculate nucleus of the thalamus (LGN)? Unlike the exhaustive determination of the retina, key populations in LGN may have been missed. Of retinal cells projecting to LGN, perhaps only 80% carry signals associated with classic thalamic responses; if the missing 20% also make 1-to-1 projections with LGN neurons, we might expect to identify the hypothesized target population through rigorous analysis of LGN recordings in awake monkeys.

Extracellular spikes are instrumental in studying neuronal responses in the visual pathway. They are dominated by a negative-voltage excursion followed by a smaller positive-voltage excursion, caused by the action of sodium and potassium channels. With the development of multi-electrodes and sophisticated spike-sorting algorithms, we can now sample the brain with reduced sampling bias, which may reveal previously overlooked signals. These developments have led to an influx of studies reporting more non-traditional waveform shapes such as spikes that are positive-dominant. Using these tools, we have begun to characterize the full range of neuronal responses in the LGN of awake monkeys.

We recorded from 244 single units in the LGN of three macaques using multi-electrodes during presentation of colored noise visual stimuli. Using a nonlinear classification algorithm, all the 244 single units' extracellular spike waveforms were classified into seven distinct classes: four negative-dominant classes (63%) that are consistent with what is commonly reported in the literature; one triphasic class (13%) that are like negative-dominant waveforms but have an initial positive peak; and two positive-dominant classes (24%) that are not often reported in the literature. Eighty percent of the total units (n = 196) had their receptive field (RF) mapped using spike-triggered averaging, and were classified into magnocellular (MC, 51%), parvocellular (PC, 40%) and koniocellular (KC, 9%). Spike classes were then correlated with their mapped RF and response characteristics to identify any relationships between spike shape and neuronal class. In comparison to the common negative-dominant classes, the positive-dominant classes had a higher proportion of MC units, higher levels of activity, shorter response latencies, larger RF sizes and larger eccentricities. This observation indicates that, possibly due to the sampling

biases inherent with traditional single electrodes, the population of LGN cells may be broader than traditionally held. Understanding the full gamut of spike shapes may help identify the previously missing retinothalamic projections.

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Poster

386. Subcortical Visual Processing, Including Feedforward and Feedback Pathways

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Program #/Poster #: 386.11

Topic: D.06. Vision

Support: MOST-111-2636-B-002-021

Title: Retinal Innervation Pattern in Central Clock, Suprachiasmatic Nucleus of Mice

Authors: *C. LIONG, S.-K. CHEN;
Natl. Taiwan Univ., Taipei, Taiwan

Abstract: Circadian rhythms modulate our daily activity patterns and other bodymechanisms, such as metabolic and neuroendocrine rhythms. In mammals, circadian rhythms are controlled by the central clock, suprachiasmatic nuclei (SCN). The external light signal could entrain SCN through intrinsically photosensitive retinal ganglion cells (ipRGCs). SCN contains different neurons, while AVP and VIP neurons are critical for the networking of circadian rhythm. AVP neurons are mainly expressed in shell region of SCN, while VIP neurons are expressed in core region. In a previous study, VIP neurons are suggested as light signal receiving neurons, and AVP neurons are the primary output neurons for the SCN. However, in single ipRGC tracing study suggested that ipRGCs innervation does not limit to VIP neurons specifically but throughout the whole SCN. In addition, the mechanisms of how SCN neurons communicate with each other's remain unknown. To explore the route of SCN neurons, we will use expansion microscopy to observe the connection and retinal innervation of SCN neurons. Revealing the circuit within SCN will advance our understanding of circadian rhythms, thus finding new treatments for circadian arrhythmic diseases.

Disclosures: C. Liong: None. **S. Chen:** None.

Poster

386. Subcortical Visual Processing, Including Feedforward and Feedback Pathways

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Program #/Poster #: 386.12

Topic: D.06. Vision

Support: NIH Grant EY029703

Title: Decussating axons within the core of the macaque optic chiasm shown by injection of a different tracer into each eye

Authors: *J. C. HORTON, M. D. DILBECK, J. R. ECONOMIDES;
Univ. of California, San Francisco, San Francisco, CA

Abstract: In primates the axons of ganglion cells emanating from the nasal retina decussate at the optic chiasm. It is unclear why compressive lesions, such as pituitary tumors, cause more injury to crossing nasal fibers, thereby giving rise to temporal visual field loss in each eye. To address this issue, the course of fibers through the macaque optic chiasm was examined by injection of a different fluorescent tracer into each eye. Under general anesthesia, cholera toxin subunit B — Alexa Fluor 488 was injected into the right eye and cholera toxin subunit B — Alexa Fluor 594 was injected into the left eye of a single normal adult male rhesus monkey. After a week's survival to allow for anterograde transport, serial sections were cut in the coronal plane through the primary optic pathway with a freezing microtome and examined for fluorescence using appropriate filters. Crossing fibers were confined mainly to a core zone within the anterior and mid portions of the optic chiasm. This zone of decussation was characterized by a stack of interwoven sheets of green (right eye) and red (left eye) fibers. At 300 μm behind the junction of the optic nerves, the decussating fibers filled 20% of the chiasm. Moving posteriorly, the size of this central zone of crossing fibers expanded steadily. At 1260 μm from the junction of the optic nerves, corresponding to the mid-chiasm, it occupied 55% of the cross-sectional area. In the posterior third of the chiasm, crossed and uncrossed fibers became so intermingled that one could no longer reliably assign them to separate zones. These data reveal that crossing fibers are concentrated primarily within a distinct, central compartment located within the anterior two thirds of the optic chiasm. Tumors in the sellar region focus their compressive force on this portion of the optic chiasm, explaining in part why they stereotypically produce a bitemporal hemianopia.

Disclosures: J.C. Horton: None. **M.D. Dilbeck:** None. **J.R. Economides:** None.

Poster

386. Subcortical Visual Processing, Including Feedforward and Feedback Pathways

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Topic: D.06. Vision

Support: NIH Grant R01MH122258

Title: Single pulse electrical stimulation produces non-additive modulation of visual event-related potential in ventral temporal cortex

Authors: *H. HUANG[1], N. M. GREGG[2], K. N. KAY[5], G. OJEDA VALENCIA[3], G. A. WORRELL[2], B. N. LUNDSTROM[2], K. J. MILLER[4], D. HERMES[3];
[1]Med. Scientist Training Program, [2]Neurol., [3]Physiol. and Biomed. Engin., [4]Neurologic Surgery, Mayo Clin., Rochester, MN; [5]Radiology, Univ. of Minnesota Twin Cities, Minneapolis, MN

Abstract: Understanding how electrical stimulation modulates excitability in neuronal circuits may contribute toward novel neurostimulation therapies for epilepsy and visual disorders. In 6 human participants, we previously found that single pulse electrical stimulation (SPES) in subcortical areas reduced neuronal activity in ventral temporal cortex (VTC) in the 1-second interval post-stimulation. We now test how SPES specifically affects the VTC's response to a visual stimulus.

One human participant (M, age 18) had stereo EEG electrodes implanted for epilepsy surgery evaluation. Visual stimuli were presented on-screen for 1 second on and 2 seconds off, and single biphasic electrical pulses (200 microseconds duration, 6 mA amplitude) were delivered through electrode pairs in the superior frontal sulcus, medial pulvinar, and lateral pulvinar (~15 trials per stimulation site), between 80 and 120 ms (mean 98 ms) before visual onset. Also conducted were 43 trials with visual stimulus and no SPES (sham), as well as 12 SPES-only trials per stimulation site. 6 VTC electrodes (4 in parahippocampal gyrus and 2 in collateral sulcus) were chosen for analysis. At each electrode, the mean visual event-related potential (ERP) amplitude was quantified in 20 ms bins after visual onset. We tested whether ERP amplitudes differed between each stimulation site and sham, and whether that difference could be explained by the amplitude of evoked potentials induced by SPES alone.

SPES in medial and lateral pulvinar resulted in a significant reduction in ERP amplitude between 280 and 460 ms after visual onset ($P < 0.05$, T-test), across 3 parahippocampal gyrus electrodes and both collateral sulcus electrodes. Only collateral sulcus electrodes showed significant amplitude reduction following SPES in superior frontal sulcus. Compared to sham, SPES in the pulvinar sites also shifted the peak amplitude earlier by ~50 ms. Significant differences in ERP amplitude between the 3 stimulation sites were found across electrodes between 200 and 240 ms after visual onset ($P < 0.05$, ANOVA). Finally, ERPs following pulvinar SPES remained significantly different from sham after subtracting the SPES-only evoked potential ($P < 0.05$, Bootstrapped difference), with peaks shifted even earlier.

In this participant, SPES in pulvinar modulated the ERP amplitude of a subsequent visual stimulus, and this difference was not simply explained by the SPES-only evoked potential. The general reduction in ERP amplitude may be due to an inhibitory effect of subcortical SPES on VTC activity consistent with our previous findings. Our results point to SPES as a potential effector in future neurostimulation therapies.

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Poster

386. Subcortical Visual Processing, Including Feedforward and Feedback Pathways

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Program #/Poster #: 386.14

Topic: D.06. Vision

Title: Look-up and Look-down neurons in mouse visual thalamus during freely moving exploration

Authors: P. ORLOWSKA-FEUER[1], A. EBRAHIMI[2], A. G. ZIPPO[3], R. S. PETERSEN[1], R. J. LUCAS[4], ***R. STORCHI**[1];
[1]Univ. of Manchester, Manchester, United Kingdom; [2]Div. of Neurosci. and Exptl. Psychology, Univ. of Manchester, MANCHESTER, United Kingdom; [3]Consiglio Nazionale Delle Ricerche, Segrate (milan), Italy; [4]Univ. Manchester, Manchester, United Kingdom

Abstract: Visual information reaches cortex via the thalamic dorsal lateral geniculate nucleus (dLGN). dLGN activity is modulated by global sleep/wake states and arousal, indicating that it is not simply a passive relay station. However, its potential for more specific visuomotor integration is largely unexplored. We addressed this question by developing robust 3D video reconstruction of mouse head and body during spontaneous exploration, paired with simultaneous neuronal recordings from dLGN. Unbiased evaluation of a wide range of postures and movements revealed a widespread coupling between neuronal activity and few behavioural parameters. In particular, postures associated with the animal looking up/down correlated with activity in &gt 50% neurons and the extent of this effect was comparable to that induced by full body movements (typically locomotion). By contrast, thalamic activity was minimally correlated with other postures or movements (e.g. left/right head and body torsions). Importantly, up/down postures and full body movements were largely independent and jointly coupled to neuronal activity. Thus, while most units were excited during full body movements, some expressed highest firing when the animal was looking up (“look up” neurons) while others when the animal was looking down (“look-down” neurons). These results were observed in the dark, thus representing a genuine behavioural modulation, and were amplified in a lit arena. Our results demonstrate that the primary visual thalamus, beyond global modulations by sleep/awake states,

is potentially involved in specific visuomotor integration, and reveal two distinct couplings between up/down postures and neuronal activity.

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Poster

386. Subcortical Visual Processing, Including Feedforward and Feedback Pathways

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 386.15

Title: WITHDRAWN

Poster

386. Subcortical Visual Processing, Including Feedforward and Feedback Pathways

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 386.16

Topic: D.06. Vision

Support: NIH Grant EY014924
NIH Grant EY029759

Title: Differential selectivity to Natural Images across Cell Classes in Primate V1

Authors: *W. HU[1], S. ZHU[3], T. MOORE[4], X. CHEN[2];
[1]Dept. of Neurobiology, Physiol. and Behavior, Univ. of California, Davis, Davis, CA; [2]Dept. of Neurobiology, Physiology, and Behavior, Univ. of California, Davis, DAVIS, CA; [3]Howard Hughes Med. Inst., Stanford, CA; [4]Neurobio., Howard Hughes Med. Inst. - Stanford Univ., Stanford, CA

Abstract: The visual system is believed to have adapted to the statistical properties of the natural environment. However, the extent to which visual neurons respond selectively to natural images, and the stage at which that selectivity emerges, remains unclear. We recently found that neuronal activities in the primary visual cortex (V1) exhibited rapid selectivity to natural images across layers of primate V1. In this study, we examined whether different cell types within V1 show such sensitivity in a homogenous manner. To address this question, we first used a non-linear dimensionality reduction method to partition cells into three clusters, axonal neurons, inhibitory neurons, and excitatory neurons. We found that different clusters exhibited distinct laminar distributions. Next, we used the generalized linear model (GLM) to quantify the natural image selectivity across different cell types and their sensitivity to orientation and contrast information

within the images. We observed that all three cell types showed selectivity to natural images compared to statistically matched synthetic images. In addition, we found this selectivity differs among cell types in a dynamic way. Specifically, in the early response epoch (65-85 ms), putative inhibitory neurons show higher selectivity to natural images than putative excitatory neurons, but in the late response epoch (115-200ms), the relative selectivity of these neuron types is reversed. A similar pattern was observed for both orientation and contrast representations. Lastly, we examined whether this differential selectivity to natural images among different cell types is consistent across different cortical layers. To examine this, we divided neurons into different laminar compartments and compared sensitivity between different cell types within different layers. The result shows that the selectivity difference among different types of V1 neurons is not only time-specific, but also layer-specific. Our results reveal the cell-type-specific dynamics of sensitivity to natural images and suggest inhibitory and excitatory neurons play supplementary roles in processing natural images.

Disclosures: **W. Hu:** None. **S. Zhu:** None. **T. Moore:** None. **X. Chen:** None.

Poster

386. Subcortical Visual Processing, Including Feedforward and Feedback Pathways

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Title: An Optogenetic Brain System (OBServ) to Restore Visual Perception in the Blind

Authors: *S. MACKNIK, S. MARTINEZ-CONDE;
SUNY Downstate Hlth. Sci. Univ., Brooklyn, NY

Abstract: Ultra-large mesoscopic imaging advances in the cortex open new pathways to develop neuroprosthetics to restore foveal vision in blind patients. Using targeted optogenetic activation, an optical prosthetic can focally stimulate spatially localized lateral geniculate nucleus (LGN) synaptic boutons within the primary visual cortex (V1). If we localize a cluster within a specific hypercolumn's input layer, we will find that activation of a subset of these boutons is perceptually fungible with the activation of a different subset of boutons from the same hypercolumn input module. By transducing these LGN neurons with light-sensitive proteins, they are now sensitive to light and we can optogenetically stimulate them in a pattern mimicking naturalistic visual input. Optogenetic targeting of these purely glutamatergic inputs is free from

unwanted co-activation of inhibitory neurons (a common problem in electrode-based prosthetic devices, which result in diminished contrast perception). We must prosthetically account for rapidly changing cortical activity and gain control, so our system integrates a real-time cortical read-out mechanism to continually assess and provide feedback to modify stimulation levels, just as the natural visual system does. We we accomplish this by reading-out a multi-colored array of genetically-encoded and transduced bioluminescent calcium responses in V1 neurons. This hyperspectral array of colors can achieve single-cell resolution. By tracking eye movements in the blind patients, we will account for oculomotor effects by adjusting the contemporaneous stimulation of the LGN boutons to mimic the effects of natural vision, including those from eye movements. This system, called the Optogenetic Brain System (OBServ), is designed to function by optimally activating visual responses in V1 from a fully-implantable coplanar emitter array coupled with a video camera bioluminescent read-out system. It follows that if we stimulate the LGN input modules in the same pattern as natural vision, the recipient should perceive naturalistic prosthetic vision. As such, the system holds the promise of restoring vision in the blind at the highest attainable acuity, with maximal contrast sensitivity, using an integrated nanophotonic implantable device that receives eye-tracked video input from a head-mounted video camera, using relatively non-invasive prosthetic technology that does not cross the pia mater of the brain.

Disclosures: **S. Macknik:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); I am an inventor on several patents related to the presented technology. **S. Martinez-Conde:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); I am an inventor on several patents related to the presented technology.

Poster

386. Subcortical Visual Processing, Including Feedforward and Feedback Pathways

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

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Title: Impact of multiple competitors on neural signatures of stimulus competition in the optic tectum

Authors: *S. MARTIN[1], G. T ANANDAN[1], S. P. MYSORE[1,2,3];
[1]Dept. of Psychological and Brain Sci., [2]Dept. of Neurosci., [3]Kavli Neurosci. Discovery Inst., Johns Hopkins Univ., Baltimore, MD

Abstract: The midbrain superior colliculus (SC; called optic tectum, OT, in non-mammals) is a key sensorimotor hub that is conserved across vertebrates. It is known to be critically involved in the competitive selection of the most salient (or highest priority) stimulus across space. To date, the bulk of work elucidating the neural representations of competing stimuli in the SC/OT has been performed using two stimuli. In particular, recent studies of the avian midbrain have shed light on how competitive stimulus interactions are represented in OT neurons as a function of the relative spatial locations, relative strengths, and sensory modalities of the competing stimuli. However, little is known about the rules that govern multistimulus competition in the SC/OT (i.e., when there are more than two competing stimuli). Here, we investigate this question with electrophysiological recordings in the intermediate and deep layers of the OT (OTid) in barn owls. We first show that, consistent with prior work in primates, the responses of OTid neurons decrease as the number of competing stimuli is increased systematically. Next, we investigate how the previously discovered rule of divisive inhibitory interactions among two competing stimuli is modulated when a third stimulus is introduced. Finally, we investigate the signaling of the strongest stimulus by OTid responses. Previous work has shown that responses of individual OTid neurons to a stimulus inside the receptive field (RF) are modulated by the increasing strength of a distant competitor, with a qualitative change in responses occurring, on average, exactly when the competitor's strength exceeds that of the RF stimulus. Here, we find that when multiple competitors are present, this signaling by individual OTid neurons is altered such that the qualitative change in responses no longer occurs when the RF stimulus is equal in strength to the strongest competitor(s); it occurs consistently when the competitor is weaker than the RF stimulus. Together, these results provide an essential complement of data that, together with modeling, can uncover the rules that underlie multistimulus competition.

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Poster

386. Subcortical Visual Processing, Including Feedforward and Feedback Pathways

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Program #/Poster #: 386.19

Topic: D.06. Vision

Support: NIH R01EY027718
JHU Start-up Funds

Title: Circuit Mechanism For The Control of The Flexibility of Neural Selection Boundaries

Authors: *G. T ANANDAN[1], S. P. MYSORE[1,2,3];
[1]Psychological and Brain Sci., [2]Neurosci., [3]Kavli Neurosci. Discovery Inst., Johns Hopkins Univ., Baltimore, MD

Abstract: Selection of the most salient stimulus in an animal's spatial environment is an essential component of adaptive behavior. This requires that the neural selection 'boundary' that

separates the representation of the strongest stimulus from those of the others be flexible, changing dynamically as the strengths of the stimuli in the environment change. The circuit mechanism underlying such flexible neural selection boundaries is unknown. Previous work in the barn owl has shown that such a flexible selection boundary is encoded explicitly in the neural responses to competing stimuli in the optic tectum (OT). The OT (called superior colliculus, SC, in mammals) is a midbrain sensorimotor hub, whose intermediate and deep layers (SCid/OTid) are critically involved in the competitive selection of the most salient stimulus. Specifically, responses of OTid neurons to a stimulus inside their receptive field (RF) are typically increasingly suppressed by the increasing strength of a distant competitor, with a qualitative transition in responses occurring, on average, when the competitor's strength just exceeds that of the RF stimulus. This qualitative transition (or "selection boundary") shifts flexibly if the strength of the RF stimulus is changed. Here, motivated by previous computational modeling, we test experimentally in barn owls the hypothesis that reciprocal neural inhibition between the representations of the two competing stimuli controls the flexibility of selection. To this end, we measure the flexibility of the selection boundary in OTid, without and with selective silencing of reciprocal inhibition between neurons in a midbrain inhibitory nucleus called Imc, known to be the primary source of inhibition among OT representations of competing stimuli. OTid responses are measured using extracellular electrophysiology, and selective silencing of reciprocal inhibition is accomplished by the reversible iontophoretic release of bicuculline (GABA antagonist) onto Imc neurons. Our results support the modeling prediction and show reciprocal inhibition to be a regulator of flexible selection boundaries in OTid. Additionally, they show that upon loss of reciprocal inhibition, the 'fixed' selection boundary in OTid is largely specified by the single-stimulus response properties of distant Imc neurons. Together, these results reveal reciprocal inhibition as a novel circuit mechanism for the control of flexible neural selection boundaries.

Disclosures: G. T Anandan: None. **S.P. Mysore:** None.

Poster

386. Subcortical Visual Processing, Including Feedforward and Feedback Pathways

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Title: Neural correlates of stimulus competition across space in the mouse superior colliculus

Authors: *A. BANERJEE[1], N. B. KOTHARI[1], S. P. MYSORE[1,2,3];
[1]Dept. of Psychological and Brain Sci., [2]Dept. of Neurosci., [3]Kavli Neurosci. Discovery Inst., Johns Hopkins Univ., Baltimore, MD

Abstract: Selective spatial attention requires competition among the representations of stimuli occurring at different spatial locations, leading eventually to the selection of the most salient stimulus and the ignoring of competing distracters. The midbrain superior colliculus (SC in mammals, optic tectum in birds) is known to be involved critically in the control of selective spatial attention. However, a systematic understanding of how competitive interactions across spatial locations are represented in the mammalian SC is currently missing. Here, we addressed this question with electrophysiological recordings in the intermediate and deep layers of the SC (SCid) in head-fixed, passive mice. Specifically, we examined how the responses of SCid neurons to visual stimuli inside their receptive fields (RFs) are modulated when a second, competing stimulus is presented at various locations across the 2-D space map. We found that SCid responses to the RF stimulus are powerfully suppressed by a competitor outside the RF. Notably, the strength of response suppression was largely constant, no matter where the competing stimulus was presented. We characterized the nature of this response suppression and found that it reflected a divisive inhibitory influence exerted by the competitor stimulus on the neuron's responses. Together, these results reveal a global, competitive (inhibitory) surround operating divisively in SC, one that can serve as a functional substrate for spatial stimulus selection and selective attention.

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Poster

386. Subcortical Visual Processing, Including Feedforward and Feedback Pathways

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Support: NIH Grant 5R01EY009593

Title: Modeling feature-specific inhibition with convergent input from local interneurons in mouse visual thalamus

Authors: *Y. MIAO[1], A. GORIN[1], S. AHN[1], F. T. SOMMER[3], J. A. HIRSCH[2];
[1]Neurosci. Grad. Program, [2]Neurobio., USC, Los Angeles, CA; [3]Univ. California, Helen Wills Neurosci Inst., Helen Wills Neurosci. Inst., Berkeley, CA

Abstract: Before reaching the visual cortex, retinal information is processed by intrinsic circuits in the visual thalamus. Specifically, relay cells in the dorsal lateral geniculate nucleus (dLGN) receive powerful feedforward inhibition from local interneurons. In carnivore and primate, the arrangement of inhibition and excitation in the relay cell's receptive field has a stereotyped, center-surround structure with a push-pull profile (i.e., in subregions where a bright stimulus excites, a dark stimulus inhibits and vice versa); interneurons have receptive fields with similar structures. The situation in rodent differs. While 40-50% of murine relay cells have center-surround receptive fields, others have single On or Off regions, or overlapping On and Off

responses with varied arrangements of inhibition. We thus asked how local interneurons might contribute to diverse forms of inhibition in murine relay cells and tested competing hypotheses about whether interneurons provide a one-size-fits-all form of inhibition (as is primarily the case in mouse cortex) or if the inhibition they provide is feature-specific. Our approach combined physiological and computational methods. First, we made multielectrode recordings from optogenetically labeled local interneurons in dLGN and mapped their receptive fields with sparse-noise stimuli (bright and dark squares of various sizes flashed separately at randomized positions in the stimulus grid). We found that interneurons had diverse receptive-fields, similar to relay cells, including center-surround, On, Off, and On-Off varieties. To quantify these receptive field structures and overcome the empirical constraint of limited data, we used a linear-nonlinear Poisson model (with two linear components in the case of On-Off cells). We then used these models to explore how input from different types of local interneurons might generate patterns of visually-evoked inhibitory currents recorded from relay cells. Towards this end, we separated net suppressive currents from the raw recordings from relay cells by subtracting EPSCs (sorted using a support vector machine and fitted by a linear-rise-exponential-decay function) and spikes, followed by rectification to remove any uncaptured excitation. Then we utilized metaheuristic methods to find an ensemble of interneuron models that best resembled the relay cell's suppressive field. This method gives a lower bound for ensemble performance. Our models provide proof of concept that diverse types of interneurons can provide feature-specific inhibition to relay cells.

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Poster

386. Subcortical Visual Processing, Including Feedforward and Feedback Pathways

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Topic: D.06. Vision

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Title: Interneurons in the lateral geniculate nucleus of the thalamus form dense and complex dendrodendritic networks

Authors: *S. AHN[1,2], R. MELTZER[2], A. KUMAR[2], A. GORIN[1,2], D. ALSTON[3], V. SURESH[2], M. A. FOX[4], J. A. HIRSCH[1,2], M. E. BICKFORD[3];
[1]Neurosci., [2]Biol. Sci., USC, Los Angeles, CA; [3]Anatom. Sci. & Neurobio., Univ. of Louisville, Louisville, KY; [4]Neurosci., Virginia Tech., Blacksburg, VA

Abstract: Comparative perspectives reveal strategies brains use to process information. We applied this approach to explore inhibitory circuits in the dorsal lateral geniculate nucleus. Previously, we showed that recordings from relay cells in carnivore are dominated by serial EPSCs while those from interneurons comprise trains of IPSCs, each preceded by a depolarizing notch (this profile suggested involvement of dendrodendritic synapses between interneurons). By contrast, recordings from both cell types in mouse feature trains of EPSCs, consistent with a dominance of retinal vs intrinsic inhibitory input. Thus, we were motivated to use serial block-face electron microscopy to study inhibitory networks across species. We first explored glomeruli--synaptic clusters in which dendrodendritic synapses are often embedded and participate in triads. Classical triads involve one retinal bouton that synapses on a dendritic appendage of a relay cell and another of an interneuron that contacts the relay cell in turn. In principle, the feedforward inhibition triads generate could explain the notch+IPSC recorded from carnivore, should triads with two interneurons exist. We observed that glomeruli in carnivore comprised a large retinal bouton encircled by dendritic appendages of interneurons and relay cells and a few other inputs. The pattern in mouse was almost the inverse; glomeruli included many retinal boutons, consistent with reports of far greater retinal convergence in mouse than carnivore. Triads involving two interneurons were present in both species but situated in different synaptic milieux. In carnivore, triads involved only one (rarely two) retinal bouton(s). In mouse, by contrast, interneuron appendages received input from more retinal boutons than the one driving the triad, potentially diluting triadic impact. Further, we found that one interneuron often synapsed on the shaft of another; this finding was more common in carnivore, where we also observed lengths of dendrite that lacked retinal input but synapsed onto ≤ ten other interneuron dendrites. Moreover, dendrodendritic inputs usually targeted thicker dendrites for carnivore vs mouse interneurons, suggesting a longer-range influence. Thus, our findings are consistent with the idea that the notch+IPSC profile arises from triadic connections between interneurons as these appear to have a stronger impact in carnivore than mouse, and/or with schemes in which one retinal afferent forms dispersed contacts with two interneurons connected via shafts. All told, interneurons in the geniculate are richly interconnected and their mutual influence is particularly powerful in highly visual animals.

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Poster

386. Subcortical Visual Processing, Including Feedforward and Feedback Pathways

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Title: Retinal direction tuning predicts optokinetic eye movements across stimulus conditions

Authors: *S. C. HARRIS[1,2], F. A. DUNN[2];
[1]Neurosci. Grad. Program, [2]Dept. of Ophthalmology, Univ. of California, San Francisco, San Francisco, CA

Abstract: Introduction: The optokinetic reflex (OKR) is a highly conserved, visually evoked behavior that stabilizes vision during self-motion. OKR occurs when ON direction-selective retinal ganglion cells (oDSGCs) detect slow global image motion associated with retinal slip. However, little is known about how oDSGC signals are centrally processed to evoke OKR. Here, we test the hypothesis that vertical (dorsal-ventral) OKR is predicted by the difference in firing rate between oDSGCs that prefer upward and downward motion.
Methods: oDSGCs encoding vertical motion were targeted for electrophysiology experiments in adult mice via injection of a retrograde tracer into their central projection nucleus. The spike outputs of oDSGCs that prefer upward (n=35) and downward (n=45) motion were subsequently recorded in *ex vivo* retina in response to a drifting bar stimulus that moved in multiple directions at multiple contrasts. The gain (eye velocity/stimulus velocity) of OKR across stimulus directions and contrasts was predicted from these data by computing the difference in spike output between upward- and downward-preferring oDSGCs under each stimulus condition. These linear predictions were then compared to empirically measured vertical OKR gain in a separate cohort of head-fixed animals (N=5). All statistics are Mann-Whitney U (m.w.u.) or Wilcoxon signed-rank (w.s.r) tests.
Results: oDSGCs that prefer upward motion spike more ($p=2.12x10^{-5}$ m.w.u) and have broader tuning curves ($p=1.7x10^{-6}$ m.w.u) than oDSGCs that prefer downward motion. However, this asymmetry decreases with stimulus contrast. Further, we found that the tuning curves of both types of oDSGCs reduced in both magnitude ($p=1.15x10^{-15}$ w.s.r) and width ($p=1.7x10^{-11}$ w.s.r) with decreasing stimulus contrast. Given a central subtraction of oDSGC outputs, these physiological results led to six linear predictions about the relative magnitude of vertical OKR gain across stimulus directions and contrasts. Key among these predictions was that OKR gain is greater in response to upward than to downward moving stimuli, but that this asymmetry decreases with stimulus contrast. Behavioral experiments revealed that this prediction ($p=2.04x10^{-10}$ m.w.u), along with each of the other 5, held true in head-fixed mice performing vertical OKR ($p<0.01$ for all predictions).
Conclusion: The difference in firing rate between oDSGCs that prefer upward and downward motion accurately predicts vertical OKR gain. Moreover, changes in OKR gain across stimulus conditions, including direction and contrast, are explained by features of motion detecting circuits in the retina.

Disclosures: S.C. Harris: None. **F.A. Dunn:** None.

Poster

386. Subcortical Visual Processing, Including Feedforward and Feedback Pathways

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Support: MOST 111-2636-B-002-021

Title: Intrinsically photosensitive retinal ganglion cells inhibit socio-sexual recognition memory through supraoptic oxytocin neurons

Authors: *Y.-F. HUANG[1], S.-K. CHEN[2];
[1]Natl. Taiwan Univ., Natl. Taiwan Univ., Taipei, Taiwan; [2]Natl. Taiwan Univ., Natl. Taiwan Univ., Not Hispanic or Latino or Spanish Origin, Taiwan

Abstract: Social memory between the same gender or even different gender is a complex and heavily modulated process in the nervous system. The regulation of memory formation could be influenced by both the internal status of the animal and the external environmental condition. Among many external stimulations, luminance exerts a profound influence on physiology, behavior, and cognitive functions in humans and rodents. It has been shown that various forms of recognition memory, such as odor and object memory, are downregulated by acute light exposure in rodents. However, the neuronal circuitry involved in light-dependent social recognition memory modulation remains unclear. Here, we show that acute light exposure could impair the socio-sexual recognition memory (SSRM) in male mice. Activation of oxytocin neurons in the SON (SON^{OT} neurons) using channelrhodopsin is sufficient to enhance the SSRM performance in male mice. On the contrary, light exposure could inhibit SON^{OT} neurons through M1 SON-projecting ipRGCs and GABAergic neurons ($pSON^{GABA}$) in the pSON. Together, these results show that sensory input such as light could modulate SSRM through a minimal ipRGC-$pSON^{GABA}$-SON^{OT} neuronal circuitry. Our findings demonstrate the neural basis of how luminance affects cognitive functions through the oxytocin system, which is a powerful modulatory neurohormone in the central nervous system.

Disclosures: Y. Huang: None. **S. Chen:** None.

Poster

387. Plasticity in the Visual System

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Topic: D.06. Vision

Support: R21 EY031052

Title: Retinal excitotoxic lesions differentially impact neurons in the ferret dorsal lateral geniculate nucleus

Authors: *J. YANG[1], K. R. HUXLIN[2], F. BRIGGS[1];
[1]Neurosci., [2]Flaum Eye Inst., Univ. of Rochester, Rochester, NY

Abstract: The dorsal lateral geniculate nucleus (LGN) of the thalamus relays the majority of inputs from the retina to the visual cortex. However, little is known about the neurophysiological changes that occur among LGN neurons after injury, as in retinal diseases such as glaucoma. In cats, acute lesions to the retina completely erased responses of LGN neurons whose receptive fields were within the retinal scotoma. Yet, thirty days after lesion, LGN neurons in the deafferented region shifted their receptive fields to the immediate surround of the retinal scotoma (Eysel et al., 1980). Other than shifts in receptive fields and changes in basic spontaneous light-evoked activity, little is known about how physiological response properties of LGN neurons change following retinal damage. Thus, we sought to address whether specific changes in response properties of surviving LGN neurons depend on their receptive field position relative to the scotoma. We also aimed to test the hypothesis that retinal excitotoxic lesions differentially impact LGN neurons in the X and Y parallel processing streams. To test these hypotheses, we made retinal lesions in one eye of ferrets by injecting 5 µL of 2mM kainic acid (KA) into the eye. Before and after making retinal lesions, we measured the thickness of the retinal nerve fiber and retinal ganglion cell (RGC) layers of the injected eye using Optical Coherence Tomography (OCT). We also verified RGC loss in the lesioned eye using Electroretinography (ERG). We then recorded the responses of LGN neurons both contralateral and ipsilateral to the lesion. We displayed a battery of visual stimuli and recorded LGN neuronal activity using multi-electrode arrays in anesthetized and paralyzed ferrets. We computed the area and eccentricities of RGC loss in the retina using RBPMS staining, then determined the relative eccentricities of recorded LGN neurons based on electrode tracts. Our preliminary data suggest that light-responsive LGN neurons were those with receptive fields near the scotoma boundary, while LGN neurons whose receptive fields were within the scotoma were not responsive to any stimuli presented. Additionally, we observed normal transient responses but altered sustained responses to flashing stimuli among responsive OFF LGN neurons contralateral to the lesioned eye. We also found out that response latency, and tuning to spatial frequency and temporal frequency were altered in responsive LGN neurons. Together, these findings support the notion that KA lesions may differentially impact X and Y parallel processing streams.

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Poster

387. Plasticity in the Visual System

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Davidson Research Initiative

Title: Distribution and development of ipsilateral projecting retinal ganglion cells labeled in the Sert-Cre reporter mouse

Authors: *L. BYER[1], J. SU[2], Y. LIANG[2], B. LOM[3], M. A. FOX[4];
[1]Davidson Col. Neurosci. Program, Davidson, NC; [2]Virginia Tech. Carilion Res. Inst., Roanoke, VA; [3]Biol. Dept. and Program in Neurosci., Davidson Col., Davidson, NC; [4]Sch. of Neurosci., Virginia Tech., Blacksburg, VA

Abstract: The mouse retina includes over forty different types of retinal ganglion cells (RGCs) that are typically classified by morphology, function, and/or expression of specific genes. RGCs can also be classified by where their axons target the brain's ipsilateral (ipsiRGCs) or contralateral (contraRGCs) hemisphere. ContraRGCs are abundant and widespread throughout most regions of the mouse retina, while ipsiRGCs represent only 5% of RGCs with their cell bodies localized in the ventrotemporal (VT) crescent region. In this study, we examined how several subtypes of ipsiRGCs were spatially distributed across the VT retina at developmental stages before (P0, P3) and after (P14, Ad) eye opening. We labeled ipsiRGCs by crossing *Sert-Cre* (a.k.a. ET33-Cre) mice with a *Rosa-Stop-tdT* reporter line so that serotonin-expressing ipsiRGCs were fluorescently labeled. We then immunostained to label ipsiRGC subtypes. Examining ipsiRGCs in retinal whole-mounts revealed both ipsi- and contraRGCs in overlapping domains of the VT crescent as expected. Interestingly, we also observed that *Sert-Cre::Rosa-Stop-tdT*+ ipsiRGCs were scattered across over 20% of the mouse retina after eye-opening (P14, Ad). Our immunostaining data further revealed that *Sert-Cre::Rosa-Stop-tdT*+ ipsiRGCs consisted of intrinsically photosensitive RGCs that express the photopigment melanopsin (immunostained by Opn4) and subtypes of αRGCs, identified by their large cell bodies and high levels of neurofilament protein (immunostained by SMI-32 antibodies). Moreover, to assess the function of ipsiRGCs in vision, we crossed *Sert-Cre* mice to a *Rosa-Stop-DTA* line, in which diphtheria toxin (DTA) is expressed in a Cre-dependent manner. This approach led to the ablation of Cre+ RGCs in the retina. To our surprise, however, a subset of ipsiRGC projections persisted in the brains of *Sert-Cre::Rosa-Stop-DTA* mice. Thus, either *Sert-Cre* is not expressed in all mouse ipsiRGCs or non-Cre+ RGCs are rewired to project to the ipsilateral hemisphere following the early developmental ablation of *Sert-Cre*+ RGCs. Taken together, these results provide a more detailed characterization of a genetic tool frequently used to label ipsiRGCs and reveal novel features of the distribution of ipsiRGCs in the developing mouse retina.

Disclosures: L. Byer: None. **J. Su:** None. **Y. Liang:** None. **B. Lom:** None. **M.A. Fox:** None.

Poster

387. Plasticity in the Visual System

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 387.03

Topic: D.06. Vision

Support: SERB(SR/SO/AS-27/2012,TCN)

Title: The GABAergic pathway of the inner retinal neurons and its influence on ambient light.

Authors: *S. GUPTA, T. C. NAG;
ANATOMY, ALL INDIA INSTITUTE OF MED SCIENCE, NEW DELHI, India

Abstract: Retinal degenerative diseases like AMD and retinitis pigmentosa are characterized by photoreceptor cell loss. Light-induced retinal damage additionally causes alterations in the inner retinal neurons, like horizontal cells (HC) and amacrine cells (AC). Our objective was to see how the HC and AC respond in light damage to maintain the retinal circuitry. We exposed Sprague-Dawley rats to light of variable photoperiods and intensities and created an experimental situation that mimics retinal degenerative diseases where photoreceptor cell death is common. Adult rats (N= 30) were acclimated in 300 lux at 12 hour light: 12 hour dark (12L: 12D) photoperiod for 7 days and then exposed to 3000 lux at 12L: 12D for 2 days, followed by exposure to constant light (24L: 0D) for 2 days. Rats were then brought back to 300 lux and reared for 15 days in 12L: 12D cycles. They were sacrificed at different days to see retinal HC and AC remodeling. Immunoreactivity (IR) of GABA, calbindin D-28k (Calb), GABA A receptor alpha1 subunit (GR) was examined. $Calb^+$ HC decreased in number under constant light but increased after light intensity was reversed (from 6 to 8/ 280 µm length of INL; $p<0.05$), whereas abnormal $Calb^+$ HC increased in number with constant light and decreased with light reversal. There was an increase in total number of $Calb^+$ HC under constant light (13/ 280 µm length of INL; $p<0.05$). Total number of $Calb^+$ AC was increased in recovery phase at day 15, as compared to day 5 of recovery phase (8 vs 22 in 280 µm length of INL; $p<0.05$). GABA IR was localized in AC, with a significant increase in their number in day 15 of recovery phase in comparison to 3000 lux 12L:12D group (34 vs 62/ 280 µm length of INL; $p<0.05$). IR of HC for GABA and GR was seen in control group, disappeared under constant light and reverted back in the recovery phase. GR IR was localized in INL and IPL sublaminae, with the number of AC (5/280 µm length of INL in control group) and IR in IPL increased under constant light and persisted even in the recovery phase at day 15 (9/280 µm length of INL; $p<0.05$). Colocalization of $Calb^+$ and GR showed similar results, supporting the above-mentioned results. Our data show that continuous light damages the HC and AC, and remodeling happens with the reversal of the photic insult, by re-establishing connectivity with the extant healthy photoreceptors via GABA pathway.

Disclosures: S. Gupta: None. **T.C. Nag:** None.

Poster

387. Plasticity in the Visual System

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Topic: D.06. Vision

Support: NIH/NEI U01EY025858-04
-NIH/NEI R01EY031589-01

Title: Defining compensation after central vision loss, and relating it to functional connectivity in the visual cortex.

Authors: *E. J. CUTTS[1], M. MANIGLIA[2], L. L. FLEMING[1], K. M. VISSCHER[1];
[1]Dept. of Neurobio., Univ. of Alabama, Birmingham, Birmingham, AL; [2]Dept. of Psychology, Univ. of California, Riverside, Riverside, CA

Abstract: Expected to affect 288 million people worldwide by 2040, central vision loss caused by macular degeneration is an increasingly prevalent visual health problem as the global population ages. Because these patients have lost their central vision, they must learn to use peripheral vision to perform their daily activities. This ability to compensate varies from patient to patient, such that two patients with similar retinal damage may show widely different performance on complex visual tasks. While this difference in compensation has been difficult to quantify, quantification is a necessary step to understanding the neural mechanisms underlying compensatory visual strategies. To validate a measure to quantify visual compensation following central vision loss, we performed a factor analysis on low-level vision assessments (measuring visual acuity and contrast sensitivity) and high-level vision assessments (measuring attention, episodic memory, emotion recognition, and general visual function) to identify high-level vision assessments that were statistically more separable from the low-level assessments. A linear regression compared composite scores of the low-level assessments to the high-level assessments. An individual patient's distance from the regression line represents how much better (or worse) they performed on high level vision tasks than was expected based on their low level vision performance. We refer to this concept as that patient's "compensation." Because our hypothesis is that the mechanism of compensation is through changes in neural connections, individual differences in these compensation scores were compared to individual differences in cortical connections hypothesized to be important for using peripheral vision. Using fMRI resting-state functional connectivity, we examined functional connections between the frontal eye fields (FEF), a region involved in directing spatial attention and saccadic eye movements, to parts of primary visual cortex representing preferentially used portions of the peripheral retina, non-preferentially used portions of the peripheral retina, and portions of the retina affected by the lesion. We found that FEF had a retinotopically specific relationship to V1 that depended on the level of compensation. These results suggest that we can quantify the level of compensation in response to central vision loss, and that these compensation scores relate meaningfully to functional connections. Applying these same approaches to future work will allow investigation of other behavioral and neural mechanisms of compensation in response to poor vision caused by central vision loss.

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Poster

387. Plasticity in the Visual System

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Unrestricted and career development award from RPB

Title: Specific effects of glaucoma on optic radiation tissue properties measured in the UK Biobank

Authors: *J. KRUPER[1,2], A. RICHIE-HALFORD[1,2], N. C. BENSON[2], S. CAFFARRA[4,5], J. P. OWEN[3,6], Y. WU[3,6], A. LEE[3,6], C. LEE[3,6], J. YEATMAN[4], A. ROKEM[1,2];
[1]Psychology, [2]eScience Inst., [3]Dept. of Ophthalmology, Univ. of Washington, Seattle, WA; [4]Grad. Sch. of Educ. and Div. of Developmental Behavioral Pediatrics, Stanford Univ., Stanford, CA; [5]Univ. of Modena and Reggio Emilia, Modena, Italy; [6]Roger and Angie Karalis Johnson Retina Ctr., Seattle, WA

Abstract: Glaucoma is a prevalent condition affecting the visual system. It does not directly affect the optic radiations (OR), a structure which carries visual information from the thalamus to the primary visual cortex (V1). However, as people age, changes in visual input due to disease or other factors may indirectly affect the tissue properties of the white matter. To study this, we performed white matter tractometry using the software package pyAFQ (https://yeatmanlab.github.io/pyAFQ) on a large sample of diffusion MRI (dMRI) data from the UK Biobank dataset (UKBB), in both healthy controls (N=5,292; age 45-80) and participants with glaucoma (N=905; age 49-80). We characterized white matter tissue properties in parts of the OR that transmit information about the foveal ($<3^o$ eccentricity), macular ($>=3^o$,$<7^o$), and peripheral ($>7^o$) visual fields. We further analyzed two non-visual control bundles: the corticospinal tract and the uncinate fasciculus. To reduce bias from confounders, we used statistical matching to construct a matched dataset. We matched on age, sex, ethnicity, and the Townsend deprivation index, a measure of socioeconomic status. We used a residual convolutional neural network (CNN) modified for 1-dimensional data on this matched dataset to classify participants' glaucoma diagnosis from their white matter tissue properties. The CNN achieved significantly more accurate performance (area under the ROC curve (AUC) of 0.68) using tissue properties from the foveal and macular bundles than when using tissue properties from the peripheral bundle or from the non-visual control bundles (periphery AUC of 0.59, best control AUC of 0.56. all comparisons $p<0.05$ using DeLong's test). We also tried using regularized linear regression to predict participants' glaucoma diagnosis, and it did not achieve the same performance as the CNN (best OR AUC is 0.59, controls not significantly different). Taken together, these findings in the largest dataset of dMRI measured in glaucoma to date demonstrate a subtle, non-linear relationship between glaucoma and the visual white matter, in particular the foveal and macular bundles.

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Poster

387. Plasticity in the Visual System

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Topic: D.06. Vision

Support: DFG Grant: GR988-27/1
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BaCaTec: Bavaria California Technology Center

Title: Training-induced modulatory effects on population receptive field properties in early visual cortex

Authors: *M. W. GREENLEE[1], M. MALANIA[1], C. HÖRMANDINGER[1], Y.-S. LIN[1], J. S. WERNER[2], T. PLANK[1];
[1]Univ. Regensburg, Univ. Regensburg, Regensburg, Germany; [2]Univ. California Davis, Univ. California Davis, Davis, CA

Abstract: Neural circuits in the brain are subject to change driven by sensory inputs causing cells to modify their properties. Practice-driven changes that occur at the cellular level can be studied by means of population receptive field (pRF) measurements. This study aims to investigate the effect of perceptual learning on pRF estimates. Fifteen healthy volunteers (10 females and 5 males, average age of 25.9 years) were tested to estimate pRF parameters. The study design consisted of three experiments: One behavioral experiment, and two fMRI measurements, one before and another after behavioral training. In the behavioral experiment, participants were trained on crowding task over 4 consecutive days, about 1.5 hours per day. Drifting bar stimuli moving along 8 different directions were used for pRF mapping during fMRI measurements. pRF sizes were estimated before and after behavioral training in the brain area that corresponded to the trained retinal location. Additionally, multiple regions of interest (V1, V2_dorsal, V2_ventral, in the ipsilateral hemisphere) were examined. Results of statistical analyses indicate significant improvement on the crowding task ($t(14)=3.05$, $p=0.009$) in all participants. We observed a significant reduction of pRF sizes within voxels in an ROI that corresponded to the trained retinal location ($t(14)=3.36$, $p=0.005$). No significant changes of pRF estimates were found in the voxels in V1 ($t(14)= -1.58$, $p=1.36$), V2_dorsal ($t(14)=1.25$, $p=2.33$), and V2_ventral ($t(14)= -0.64$, $p=0.53$) for other (untrained) retinotopic locations. The results

suggest that training on a visual crowding task leads to measurable changes in early visual cortex for that retinotopic locus.

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Poster

387. Plasticity in the Visual System

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Program #/Poster #: 387.07

Topic: D.06. Vision

Support: Seed Fund for Basic Research, The University of Hong Kong, #202011159092

Title: Tms-induced plasticity of the adult stereoscopic system

Authors: *K. OR, D. H. F. CHANG;
Dept. of Psychology, Hong Kong Univ., Hong Kong, Hong Kong

Abstract: Continuous theta burst stimulation (cTBS) over the primary visual cortex (V1) has been shown to improve a range of visual capacities in individuals with visual impairment. Particularly, stereoacuity and contrast sensitivity of amblyopic patients can be transiently improved following cTBS over V1. Whether cTBS can bring about reorganizational benefits in the healthy brain is largely unknown. Here, we tested the effects of cTBS over two visual areas on stereoscopic vision in visually-normal individuals: V1 and the lateral occipital complex (LOC), the latter of which has been shown to play a significant role in serving the ability to discriminate small depth differences. A total of 52 subjects (mean age 21.8 years, 20 male, 32 female) was tested. Subjects were randomly assigned to receive cTBS over V1, LOC, or vertex (control region). Subjects in the V1 and LOC stimulation groups were randomly assigned to receive stimulation over one hemisphere only. A total of 600 pulses were applied continuously (5 bursts of 3 pulses at 50 Hz per second), and participants were tested on depth and contrast discrimination tasks before and after stimulation. In the depth task, subjects were required to judge the depth position of the stimuli. In the contrast task, subjects compared the contrast of the central target relative to the surround. Task difficulty was adjusted according to a staircase in order to attain thresholds at the 82%-correct level. Results indicated that depth thresholds of those who received LOC (but not V1 or vertex) stimulation improved by 20.5%. Contrast thresholds did not change following stimulation across all stimulation sites. Our findings suggest that neuroplasticity of the adult stereoscopic system can be driven by cTBS over the extrastriate (but not primary) visual cortex. Moreover, cTBS acts on the stereoscopic system through different neural mechanisms in amblyopic versus normal vision.

Disclosures: K. Or: None. **D.H.F. Chang:** None.

Poster

387. Plasticity in the Visual System

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Program #/Poster #: 387.08

Topic: D.06. Vision

Support: General Research Fund, Research Grants Council 21/22, Hong Kong (#17612621)

Title: Perceptual learning improves depth sensitivity in aging brains

Authors: *L. NG[1], D. CHANG[1,2];
[1]Dept. of Psychology, [2]The State Key Lab. of Brain and Cognitive Sci., the Univ. of Hong Kong, Hong Kong, Hong Kong

Abstract: A wide range of visual capacities has been shown to decline with age. Many of the visual deficits observed with aging have been associated with changes occurring centrally (i.e., in the brain) rather than in the peripheral organ (i.e., the eye). Here, we investigated the effectiveness of perceptual training in improving depth sensitivity in aging brains. Older (n=36, mean age = 68.5, 24 Female: 12 Male) and younger (n=36, mean age = 25.5, 24 Female : 12 Male) human observers were tested on both signal-in-nose segregation (SNR) and disparity-difference (fine) depth discrimination tasks before and after 3 days of training. Participants received training on either the SNR task, fine task, or received no training (i.e., control). In all tasks, static random-dot stereograms in a center-surround configuration were presented to observers. In the SNR task, a proportion of dots (i.e., signal-dots) appeared at a fixed disparity of ± 6 arcmin, and noise dots were assigned a random disparity (between ± 0-6 arcmin). In the fine task, stimuli were 100% coherent, with signal dots positioned ± 0-4 arcmin from the surround, which was assigned a fixed disparity of ± 12 arcmins. For both tasks, participants were asked to judge whether the center was in front (“near”) or behind (“far”) the surround annulus. Task difficulty was manipulated by changing the signal-to-noise ratio (SNR task) or the relative disparity between the center and surround (fine task), yielding thresholds at the 82% correct level. We observed a significant improvement in SNR task performance after dedicated SNR task training and in fine task performance after dedicated fine task training. There was a comparably smaller improvement in SNR task performance after fine task training, suggesting a transfer of learning. No transfer of learning was observed for those receiving SNR task training. Our findings suggest that perceptual learning is a powerful tool that can improve depth sensitivity in aging brains, and the benefits gained mirror those obtainable in younger brains.

Disclosures: L. Ng: None. **D. Chang:** None.

Poster

387. Plasticity in the Visual System

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Program #/Poster #: 387.09

Topic: D.06. Vision

Support: RGC General Research Fund 17612920

Title: Sensory eye dominance plasticity as driven by dichoptic perceptual training in the human adult visual cortex

Authors: *K. KAM, D. H. F. CHANG;
Dept. of Psychology, The Univ. of Hong Kong, Hong Kong, Hong Kong

Abstract: Sensory eye dominance is a form of functional asymmetry of the two eyes resulting from the visual cortex weighing one eye's input more heavily than that of the other. Visual training protocols using dichoptically presented signal-in-noise motion stimuli have been shown to successfully reduce eye dominance in both the clinical and healthy populations; however, the neural mechanisms underlying these learning-driven improvements are not well understood. Here, we sought to identify the neural mechanisms of adult sensory eye dominance plasticity by measuring fMRI responses concurrently with behaviour, before and after a five-day visual training protocol. Fifty visually normal observers (mean age 22.2 years; SD 3.4 years; 27 males) were randomly assigned to receive training on either a dichoptic or binocular variant of a signal-in-noise (left-right) motion discrimination task over five consecutive days (6000 trials). In the dichoptic variant, signal dots carrying a coherent motion direction (left or right) and noise dots carrying a random motion direction were presented to different eyes. In the binocular variant, signal and noise dots were presented to both eyes. Results showed that sensory eye dominance shifted following dichoptic (but not binocular) visual training. Pattern analyses of fMRI responses using a linear support vector machine revealed that the primary visual cortex (V1) and the human motion complex (hMT+) are implicated in eye dominance for both groups, but only before training. Notably, after dichoptic (but not binocular) visual training, the fMRI pattern responses of these regions no longer predicted eye dominance. Our data suggest that visual training involving a dichoptic presentation of signal-in-noise motion stimuli may drive a reweighting of the data from the two eyes in both the primary and task-related extrastriate visual areas.

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Poster

387. Plasticity in the Visual System

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Program #/Poster #: 387.10

Topic: D.06. Vision

Title: High-level stimulus-selective response potentiation: late ERP modulation following prolonged 6hz presentation of faces and houses

Authors: *N. H. HELLER[1], K. ORTEGO[2], B. DUCHAINE[1], P. U. TSE[1], V. S. STÖRMER[1]; [1]Psychology and Brain Sci., [2]Dartmouth Col., Hanover, NH

Abstract: For nearly 50 years, high-frequency electrical stimulation has been the main neurophysiological method used to investigate the cellular mechanisms responsible for long-term potentiation and depression. More recently, visual analogs of this paradigm have been developed, such as visual tetanic stimulation or stimulus-selective response potentiation. In these paradigms, prolonged, repeated presentation of low-level visual stimuli, such as checkerboards and gratings, induce long-lasting modulation of visually evoked responses that can be stimulus specific. In-vivo studies have measured this effect directly in V1 and it has been shown to depend on the same NMDA-mediated mechanisms as long-term potentiation. Here, we test whether it is possible to obtain stimulus-specific modulation of evoked responses using high-level visual stimuli. In a within-subjects design (N=11), across two sessions, we compared the effect that rapid, prolonged presentation of faces vs houses had on subsequent stimulus specific ERPs. During each session, baseline ERPs were first obtained. In this baseline ERP block, 100 face images and 100 house images were presented for 500ms each, randomly interleaved at a rate of ~2hz. Participants reported the image category by button press. Then, during the potentiation block, participants were presented with either faces or houses for ~10 minutes at a rate of ~6hz, for a total of 3500 separate presentations. Some of the images were tilted slightly to the right or left and participants had to detect and report the direction. Finally, two post-potentiation ERP blocks, one ~2 minutes after potentiation and the other ~20 minutes after potentiation, were performed that were identical to the baseline test. For each ERP block (baseline, post 1, and post 2), ERP difference waves (face minus house) were obtained to examine category-specific effects. We found that, starting at ~200ms, the difference waves measured over occipital cortex diverged in opposite directions for each condition: Following face-potentiation, the difference waves were significantly more negative, while following house-potentiation, the difference waves were significantly more positive. This result suggests that stimulus-selective response potentiation is not limited to low-level vision. Rather, this method of neuroplastic modulation may be able to target high-level, category specific visual processes.

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Poster

387. Plasticity in the Visual System

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Topic: D.06. Vision

Support: Early Career Scheme, Research Grants Council, Hong Kong (27612119)

Title: Context-dependent stereoscopic depth perception in visual area V4

Authors: *Z. LI, D. H. F. CHANG;
Dept. of Psychology, The Univ. of Hong Kong, Hong Kong, China

Abstract: We have previously demonstrated that visual sensitivity to depth position is curiously modulated by object context, and that depth sensitivity can be enhanced with perceptual training that attaches meaning to otherwise meaningless objects. Here, we used fMRI to investigate the neural substrates associated with changes in stereosensitivity before and after object-label ("Greeble") classification training. Participants (N = 30) were scanned before and after 2-5 training sessions (depending on performance attainment). In the pre-test and post-test sessions, participants were presented with stereoscopic Greebles and performed a signal-to-noise (SNR) depth position (near versus far) task. Stimulus difficulty was adjusted through a 1-up/2-down staircase procedure in order to attain performance thresholds. Blood oxygenation level-dependent (BOLD) signals were then measured while participants completed the task in-bore using at-threshold stimulus values. Participants were subdivided into two groups that differed in terms of the training type received: one group (N = 20) was trained to classify the nonsensical objects into Greeble genders and individuated identities; a second group (N = 10) was trained instead on an orientation-discrimination task (using identical stimuli). Behaviourally, depth discrimination thresholds were significantly lower post- versus pre-training, but for the Greeble-classification training group only. Thresholds did not change for the orientation-trained group. Using the fMRI data, we trained linear support vector machines (SVM) to predict whether the data were from the pre or post-training sessions. Results showed that prediction accuracies in V4 were higher for the Greeble-classification group as compared to the orientation group for which accuracies were at chance level. In addition, prediction accuracies in V4 were negatively correlated with response times for Greeble identification. Our data suggest that V4 is implicated in an expertise-dependent tuning manner that goes on to exert influence on stereoscopic depth retrieval.

Disclosures: Z. Li: None. **D.H.F. Chang:** None.

Poster

387. Plasticity in the Visual System

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Topic: D.06. Vision

Support: BioTechMed-Graz Young Researcher Group Grant to NZ

Title: Fast and functionally specific cortical thickness changes induced by visual stimulation

Authors: *N. ZARETSKAYA[1,2], E. FINK[1], A. ARSENOVIC[1,2], A. ISCHEBECK[1,2];
[1]Univ. of Graz, Graz, Austria; [2]BioTechMed-Graz, Graz, Austria

Abstract: Structural characteristics of the human brain serve as important markers of brain development, aging, disease progression, and neural plasticity. They are considered stable properties, changing slowly over time. Multiple recent studies reported that structural brain changes measured with magnetic resonance imaging (MRI) may occur much faster than previously thought, within hours or even minutes. The mechanisms behind such fast changes remain unclear, with hemodynamics as one possible explanation. Here we investigated the functional specificity of cortical thickness changes induced by a flickering checkerboard and compared them to blood oxygenation level-dependent (BOLD) functional MRI activity. We found that checkerboard stimulation led to a significant thickness increase, which was driven by an expansion at the gray-white matter boundary, functionally specific to V1, confined to the retinotopic representation of the checkerboard stimulus, and amounted to 1.3% or 0.022 mm. Although functional specificity and the effect size of these changes were comparable to those of the BOLD signal in V1, thickness effects were substantially weaker in V3. Furthermore, a comparison of predicted and measured thickness changes for different stimulus timings suggested a slow increase of thickness over time, speaking against a hemodynamic explanation. Altogether, our findings suggest that visual stimulation can induce structural gray matter enlargement measurable with MRI [1].
[1] Zaretskaya, Fink, Arsenovic & Ischebeck (2022). bioRxiv. https://www.biorxiv.org/content/10.1101/2022.02.25.482013v2

Disclosures: **N. Zaretskaya:** None. **E. Fink:** None. **A. Arsenovic:** None. **A. Ischebeck:** None.

Poster

387. Plasticity in the Visual System

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Program #/Poster #: 387.13

Topic: D.06. Vision

Title: Development of Face perception processing after visual deprivation in early infancy

Authors: *S. GUPTA[1], T. K. GANDHI[1], P. SINHA[2];
[1]Indian Inst. of Technol., New Delhi, India; [2]MIT, USA, MA

Abstract: One of the most salient abilities that humans possess is face processing. The speed and accuracy with which it occurs in the brain signify that there may be some specialized circuit to process it. But it is still unknown whether one owns this ability since birth, or it develops over time with experience. This debate of nature and nurture can be resolved by conducting developmental studies on sensory deprivation. The visual deprivation due to congenital cataract can be used as a basis to understand brain functioning during development. In addition, the concept of sensitive period for neuro-cognitive development in children can be understood through such study. We have a unique opportunity to work with Project Prakash Charitable Trust, where the subjects have experienced extended early-onset blindness (beginning before 1 year of age and lasting 8-17 years) before the removal of bilateral cataracts. They are patients

with treatable congenital blindness. By following the visual development of these children immediately after sight onset, we can gain unique insights into fundamental questions regarding visual learning and brain plasticity. The present study investigates the development of face perception ability in a sample of five visually deprived children (age range 7-20 years) after the visual restoration surgery. The Electroencephalography(EEG) data was collected for these subjects while presenting the face object paradigm. The EEG data were also collected from age-matched five healthy controls having the same age and socio-economic background. The event-related potentials (ERP) were evaluated at occipito-temporal electrodes in the left and right hemispheres separately before sight onset and after sight-restoring surgery. We have compared the ERP's of experimental and healthy control subjects. It is observed that the latency of N170 (i.e. biological facemarker) for newly sighted individuals before the surgery was away from the healthy subjects and not close to 170 milliseconds. The ERP's were compared again after 1 year of sight restoration for both the age groups and it was observed that the N170 latency for newly sighted individuals getting improved and is quite close to healthy subjects. The results were consistent for both the hemispheres. The improvement in visual acuity was also observed over time (approx. 1 year) after the sight restoration for both the age groups. Thus, the ERP results reflect that the face perception ability may develop with experience and may not depend on early visual input.

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Poster

387. Plasticity in the Visual System

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 387.14

Topic: D.06. Vision

Support: Natural Science and Engineering Research Council of Canada (NSERC) #327588
Canada Foundation for Innovation (CFI)
Canada First Research Excellence Fund (CFREF), Vision Science to Application (VISTA) #2015-00013

Title: Decreased volume in the pulvinar thalamic nucleus in people who have had one eye removed early in life

Authors: S. S. MORO, D. J. GORBET, *J. K. E. STEEVES;
York Univ., Toronto, ON, Canada

Abstract: The pulvinar is the largest thalamic nucleus and is a key structure for information processing and communication across cortical areas. It is considered critical for active vision and plays a role in regulating visual cortical processing. Functionally different sub-regions within the pulvinar have specific pulvino-cortical connections, including the presence of topographic visual field maps. People who have had one eye removed early in life have both enhanced (contrast

sensitivity and foveal acuity) and impaired (face processing and motion in depth) visual processing abilities. Additional differences in audiovisual processing including the reduced ability to perceive audiovisual illusions such as the McGurk Effect and Double Flash Illusion indicate the presence of cross-modal plasticity. Structurally, compared to controls, people with one eye have decreased lateral geniculate nuclei (LGN) volume resulting from the 50% deafferentation of the visual system. However, LGN volume is larger than predicted contralateral to the remaining eye, indicating altered structural development likely through recruitment of deafferented LGN cells. Furthermore, people with one eye have an asymmetrical medial geniculate body (MGB) volume compared to controls indicating subcortical reorganization. *Purpose:* The current study investigated whether structural pulvinar changes are also present in this group given the observed changes in visual and auditory processing. *Methods:* Pulvinar volumes were measured in adults who had undergone early unilateral eye enucleation (mean age: 23 months) and were compared to binocularly intact controls with FreeSurfer software's Thalamic Segmentation tool. *Results:* Compared to controls, people with one eye have a decreased volume of the right pulvinar, driven by a decreased volume in the right inferior pulvinar sub-region. *Conclusions:* The decreased volume observed in the right pulvinar in people with one eye may be directly related to their decreased visual input as the right hemisphere pulvinar contains a topographic map of the visual environment. Additionally, the ventral pulvinar is implicated in temporal sensitivity potentially accounting for this groups inability to perceive audiovisual illusions.

Disclosures: **S.S. Moro:** None. **D.J. Gorbet:** None. **J.K.E. Steeves:** None.

Poster

387. Plasticity in the Visual System

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Program #/Poster #: 387.15

Topic: D.06. Vision

Title: Neural mechanisms underlying responses to short term monocular deprivation in primary visual cortex

Authors: *D. Y. TS'O, R. A. MILLER, III;
SUNY - Upstate Med. Univ., Upstate Med. Univ., Syracuse, NY

Abstract: Normal interocular balance, the relative strengths of left and right eye inputs to the central visual pathways, is an important prerequisite for normal binocular vision. Human psychophysical studies have demonstrated an interocular imbalance following a period of short-term (1-3 hours) monocular deprivation (STMD). The unexpected outcome of STMD is a relative increase, not decrease, in the deprived eye (DE) gain post-deprivation, which may last from 10 up to 90 minutes. This example of adult neural plasticity is non-Hebbian and is opposite in sign to that expected by traditional monocular eye-patching. The neural mechanisms underlying this STMD response are largely unknown. We have previously conducted functional

optical imaging STMD studies in V1 of adult macaque monkeys, which demonstrated an increased V1 response to stimuli presented in the DE relative to the non-deprived eye (NDE) after an STMD period of ~2 hours.
To further reveal the neural mechanisms underlying the STMD response, we have recorded and analyzed single-unit responses in V1, before, during and after a 1-2 hour STMD in adult macaque monkeys. In a sample cell population (N=155) recorded from a 32-channel linear multi-electrode array, the single-unit responses revealed an average relative gain of the DE responses over the NDE responses of 29% post-deprivation, as compared with their respective pre-STMD levels. The DE responses remained elevated for about 25 minutes on average, after which they returned to their pre-STMD levels. In contrast, the post-STMD NDE responses were depressed as compared with their pre-STMD levels, even beyond 25 minutes, thus suggesting possible differences in the time courses of the DE vs NDE STMD effects. Although the supragranular cell population exhibited more robust STMD effects, cells impacted by STMD were found in all V1 cortical layers and constituted 46% of the sample. Preliminary results indicated that extending the duration of the STMD period to 7.5 hours significantly enhanced the STMD effect. Overall, these results from single-unit recordings in primate V1 under the STMD protocol match several key features of the STMD effect reported in the psychophysical literature, and indicate an important contribution to the STMD effect within the V1 circuitry.

Disclosures: D.Y. Ts'o: None. **R.A. Miller:** None.

Poster

387. Plasticity in the Visual System

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Support: CIHR PJT-159597
NSERC CGSM

Title: Visually evoked potentials (VEPs) elicited by motion-onset stimuli are amplified in the deaf

Authors: *S. ZHU, X. BAO, P. BARNES, S. G. LOMBER;
Physiol., McGill Univ., Montreal, QC, Canada

Abstract: When deprived of a sensory modality, the brain often compensates with supranormal performance in other intact sensory systems. This phenomenon is known as cross-modal plasticity, where areas of the brain responsible for a certain sensory modality are reorganized and repurposed because of the sensory loss. Approximately 1.33 billion (18.5%) people in the world are affected by hearing impairment, identifying it as one of the most prevalent neurological disorders. Deaf humans and cats have superior visual motion detection abilities, and this advantage has been causally demonstrated to be mediated by reorganized auditory cortex. The

present study sought to determine the electrophysiological response of hearing and deaf cats to motion-onset stimuli of different velocities. We hypothesized that VEPs would be larger in adult cats that are perinatally deaf compared to adult, hearing cats. Deafness was induced in the first postnatal month by systemic administration of ototoxic drugs. In maturity, we examined VEPs in both hearing and deaf cats generated from electroencephalogram (EEG) recordings in lightly anesthetized subjects. VEPs are an averaged and amplified record of the gross electrical action potentials generated by the brain in response to visual stimulation, and examination of VEPs is a commonly used non-invasive ophthalmological technique to assess the functional state of the visual system. The stimulus consisted of 200-ms long coherently leftward-moving dots with randomly generated positions at 10 speeds between 2 to 64 deg/sec. VEP waveforms were produced from the average of 160 trials for each speed. In both groups, peak amplitudes increased with increasing stimulus speeds, and significantly larger peak amplitudes were observed in deaf subjects at higher speeds (8 deg/s and above, Mann-Whitney U test $p < 0.05$). Cross-modal reorganization in auditory cortex underlying the significant improvement of motion detection found in deaf subjects could be contributed by the increase in neuronal discharge to visual motion stimuli, and this can lead to increased measurable VEP amplitudes. This study furthers current understanding of cortical plasticity during hearing loss and can establish the assessment of VEPs as an additional tool in the evaluation of cross-modal plasticity following hearing loss. This work was supported by grants from the Canadian Institutes of Health Research and the Natural Sciences and Engineering Research Council of Canada.

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Poster

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Title: Visual experience specifies primary dendrites in cortical interneurons for visual perception

Authors: *X. HOU[1], E. KITAYAMA[1], K. SAKIMURA[2], S. SUGIYAMA[1];
[1]Div. of Developmental Physiol., Niigata Univ., Niigata, Japan; [2]Dept of Animal Model Develop., Brain Res. Ins Niigata Univ., Niigata, Japan

Abstract: Individual experience shapes cerebral cortical circuits during postnatal development. Actin cytoskeleton, as a fundamental constituent of dendrites is increased in the juvenile cortex through sensory experience. There is scarce information whether and how actin organization modifies dendritic morphology in turn to influence local circuits of the primary visual cortex. We have previously identified actin depolymerization factor homology (ADF-H) proteins, that are essential for actin polymerization/depolymerization and treadmilling. The cell type specific expression of ADF-H proteins prompted us to assess the function of ADF-H protein, and among them coactosin, was localized around the necks of primary dendrites within parvalbumin-positive cells (PV-cells). Here, we found that genetic deletion of coactosin transdifferentiated multipolar properties of PV-cells into bipolar-like properties in the juvenile visual cortex. Thus, experience-dependent expression of coactosin was involved in specifying dendritic properties depending on cell type via sensory experience. Moreover, recording of visual-evoked potentials (VEPs) revealed coactosin deletion facilitated visual responses and discrimination of behaving animals at a specific spatial frequency. Simultaneously, coactosin deletion declined cortical plasticity for binocular vision. Thus, the changes of dendritic properties in inhibitory neurons may influence activation pattern of inhibition for visual perception. Interestingly, such contradictory results that improved visual discrimination and impaired plasticity occur simultaneously in the same animal is reminiscent of uneven development in autism spectrum disorders. Hence, experience-dependent actin remodeling offers a deeper understanding of a link between primary dendritic properties and sensory processing, and of a heterogeneity of intracortical circuits to provide functional individuality.

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Poster

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Topic: D.06. Vision

Support: R01EY024678

Title: Alignment of cortical binocular input proceeds in the absence of ErbB4-mediated maturation of parvalbumin inhibitory neurons

Authors: T. FUCHS, B. D. FEESE, A. SWAIN, B. JEON, M. ZHU, ***S. J. KUHLMAN**; Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Maturation of acuity, ocular dominance plasticity, and the alignment of binocular input between the eye pathways represent three prominent milestones of visual system development. The time course of refinement for ocular dominance plasticity and binocular alignment overlaps, indicating that their development may rely on similar circuit mechanisms. To determine whether similar to ocular dominance plasticity, binocular alignment requires the

maturation of parvalbumin (PV) inhibitory neurons, we examined the development of binocular alignment in mutant mice in which the maturation of PV neurons is halted due to a lack of ErbB4 signaling specifically in PV neurons. In primary visual cortex (V1), the evoked firing rate of PV neurons was reduced by 33% in mutants compared to age-matched wildtype counterparts. We found that perceptual acuity and neural discriminability of natural scenes in V1 was compromised in mutant mice. However, in contrast to ocular dominance plasticity, binocular matching proceeded in the absence of PV-ErbB4 signaling. Thus, experience-dependent refinement of orientation preference appeared normal in mutant mice. Consistent with the latter observation, neural discriminability of simple grating stimuli was indistinguishable between mutant and wildtype mice. Our results reveal that cortical alignment of binocular input is robust to perturbations that disrupt ocular dominance plasticity as well as acuity, and demonstrate that the development of simple stimulus representation is dissociable from ocular dominance plasticity and complex scene processing.

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Poster

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Support: NIH Grant EY021580
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Title: Perineuronal nets are not required to close the critical period for ocular dominance plasticity

Authors: E. CROUSE[1], *A. W. MCGEE[2];
[1]Anatom. Sci. and Neurobio., [2]Univ. of Louisville, Louisville, KY

Abstract: In the developing visual system, a transient critical period demarcates when cortical circuits are most sensitive to visual experience. The maturation of perineuronal nets (PNNs) is proposed to close the critical period. These extracellular structures contain chondroitin sulfate proteoglycans (CSPGs) and ensheath parvalbumin-positive (PV) interneurons in visual cortex. The gene for the principal neuronal CSPG aggrecan (*acan*) is essential for the formation of PNNs. Constitutive loss of *acan* expression (knock-out) is lethal. In this study, we investigated the role and source of *acan* in limiting ocular dominance (OD) plasticity with a conditional allele. We selectively deleted the *acan* gene from all PV interneurons with *PV-Cre*, most inhibitory interneurons with *Dlx5/6-Cre*, all inhibitory neurons with *Gad2-Cre*, and all neurons with the pan-neuronal Cre driver *BAF53b-Cre*. PNNs surrounded nearly all PV interneurons in visual cortex of *acan flx/flx* mice lacking Cre, but PNNs were nearly absent in mice with PV-Cre,

and completely absent in mice with *Dlx5/6-Cre*, *Gad2-Cre*, and *BAF53b-Cre*. Interestingly, only mice lacking *acan* expression in all neurons with *BAF53b-Cre* displayed OD plasticity in response to brief (4-day) monocular deprivation as adults. OD was measured with multi-unit electrophysiologic recordings from mice anesthetized with isoflurane. Thus, loss of PNNs is not sufficient to sustain critical-period plasticity in adult mice. We propose that aggrecan operates outside of PNNs to close the critical period for OD plasticity.

Disclosures: E. Crouse: None. **A.W. McGee:** None.

Poster

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Support: Rutgers-Newark Chancellor's seed grant
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Title: Neuropilin 2 role in maintaining the functional neuronal connectivity in the mouse primary visual cortex

Authors: *H. KHDOUR[1], T. S. TRAN[2], P.-O. POLACK[3];
[1]Ctr. of Mol. and Behavioral Neurosci., [2]Biol. Sci., Rutgers Univ., Newark, NJ; [3]Ctr. for Mol. and Behavioral Neurosci., Rutgers Univ. - Newark, Newark, NJ

Abstract: Semaphorin 3F (Sema3F) signaling has been shown to play an important role in establishing cortical connectivity during development. The expression of the Sema3F secreted ligand and the Neuropilin2 (Nrp2) receptor proteins is highest in the embryonic and early postnatal neocortex and gradually declines after birth. However, Sema3F and Nrp2 remain expressed in the adult neocortex, but the function of Sema3F-Nrp2 in the adult brain is unclear. It is possible that Sema3F signaling is still necessary after development to maintain the cortical connectivity and functions, and/or may even be involved in the mechanisms of cortical plasticity. Since the advent of two-photon imaging and the ability to follow the morphology of the same dendritic spines over days, it is established that many synapses in the adult cortex of the mouse are dynamic. The stabilization of new spines in cortical areas that display functional changes suggests that synapse formation and elimination likely contribute to the experience-dependent rewiring of cortical circuits. To assess the role of Nrp2 during adulthood, we compared the morphology and the spine densities of the dendritic arborization of neurons located in the primary visual cortex (V1) before and after the acute, local knock-out of Nrp2 in V1 layer 5 (L5) neurons. We found a significant increase in the spine density on the proximal and mid-section of the primary apical dendrite of V1 adult Nrp2 KO L5 neurons compared to WT L5 neurons. Using calcium imaging, we then compare the functional connectivity between WT V1 and KO

V1 L5 neurons. Our results suggest that Nrp2 is necessary for the maintenance of the functional connectivity in the adult cortex.

Disclosures: H. Khdour: None. **T.S. Tran:** None. **P. Polack:** None.

Poster

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Support: CRC 889 “Cellular Mechanisms of Sensory Processing” (to S.L. [B5], O.M.S. [B3])

Title: Compromised binocular integration and reduced direction selectivity in the visual cortex of PSD-95 knock-out mice

Authors: *S. LÖWEL[1,2,3], S. BHATTACHARYA[1,2,3], M. KARGAR[1,2], O. M. SCHLÜTER[4,5,3], C. SCHÖNE[1,2], N. C. AGGELOPOULOS[1,2,3];
[1]Goettingen Univ., Goettingen, Germany; [2]CIDBN, Goettingen, Germany; [3]CRC 889, Goettingen, Germany; [4]Univ. of Pittsburgh, Pittsburgh, PA; [5]Univ. Med. Ctr., Goettingen, Germany

Abstract: Postsynaptic density protein 95 (PSD-95) is a signalling scaffold of the PSD in excitatory synapses that regulates AMPA receptor trafficking. We have previously shown that PSD-95 dependent silent synapse maturation closes the critical period (CP) for ocular dominance plasticity in mouse V1: PSD-95 KO mice display both functional and structural hallmarks of CP plasticity into adulthood, and synapses do not properly mature. Since the development of binocularity happens during the CP, we hypothesized that PSD-95 KOs should display compromised binocular integration. To test this hypothesis, we performed both behavioural experiments and multi-electrode electrophysiological recordings in primary visual cortex (V1) of awake mice. In fact, PSD-95 KO mice are better in an orientation discrimination task (visual water task) under monocular compared to binocular conditions, unlike WT, who get worse. PSD-95 KOs are worse compared to WT in catching crickets, a behaviourally relevant task, shown to exploit binocular vision. Finally, our electrophysiolgical data show that in PSD-95 KOs - unlike in WT - i) monocular inputs do not summate and binocular responses are dominated by contralateral eye inputs, indicative of disturbed binocular interactions. In addition, PSD-95 KOs display iii) reduced direction selectivity and iv) highly selective units are less well tuned for direction. Since direction selectivity requires early visual experience this finding can likely be explained by the non-stabilized V1-synapses of PSD-95 KOs. Together, our data support a role of experience-dependent silent synapse maturation for the refinement of cortical circuitry for proper binocular signal processing.

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Poster

387. Plasticity in the Visual System

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Title: WITHDRAWN

Poster

387. Plasticity in the Visual System

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Topic: D.06. Vision

Support: NIH Grant EY016431
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Title: Asymmetric modifications of synaptic ultrastructure by prolonged DE and brief light reintroduction

Authors: *S. MURASE[1], L. DYE[2], E. M. QUINLAN[3];
[1]Univ. of Maryland at Col. Park Dept. of Biol., College Park, MD; [2]NICHD Microscopy & Imaging Core, NIH, Bethesda, MD; [3]Dept. of Biol., Univ. of Maryalnd, College Park, MD

Abstract: Structural and functional synaptic plasticity are significantly constrained over the course of postnatal development in the mammalian cortex underlies the resistant to reversal of amblyopia with age. However, binocular visual deprivation through dark exposure (DE) followed by light re-introduction (LRx) rejuvenates synaptic plasticity in the primary visual cortex (V1) of adult rodents, promoting the structural and functional recovery from severe amblyopia. We previously demonstrated that DE lowers the threshold for light-induced activation of peri-synaptic proteolysis by matrix metalloproteinase9, and that MMP9 activity is preferentially increased at thalamo-cortical (TC) synapses, which are particularly aplastic in adulthood (Murase (17) eLife). LRx induces cleavage of several synaptic cell adhesion molecules including Neuroligin-1 and beta-dystroglycan, suggesting regulation of synaptic structure.Here we use transmission electron microscopy to quantify changes in synaptic ultrastructure known to impact synaptic efficacy: We analyzed the 2D profiles of n=264, 219, 320 excitatory synapses onto layer 4 neurons from n=5, 4, 6 subjects for control (Con), DE (10 d), LRx (2 h) adult (>P90) mouse V1b. The width of the synaptic cleft decreased following 10

days of DE (Con: 20.0±0.1 nm, DE: 19.0±0.2 nm, KS-Test, *p<0.001), and the decrease was unchanged by 2 hours of LRx (19.2±0.1 nm, KS-Test, p=0.46). The density of synaptic vesicles that contact the active zone membrane, presumptive docked vesicles, increased following DE (Con: 0.86±0.02, DE: 1.2±0.03 per 100 nm, KS-Test, *p<0.001), and the increase was unchanged by 2 hours of LRx (1.1±0.03, KS-Test, p=0.09). In contrast, the area occupied by synaptic vesicles, the synaptic vesicle zone (SVZ), was increased by DE (Con: 3.5±0.1 x 10^5 nm^2, DE: 3.9±0.2 x 10^5 nm^2, KS-Test, *p<0.001), and reversed by LRx (3.6±0.1 x 10^5 nm^2, KS-Test, *p<0.001). Similarly, distance of centroid of the SVZ to active zone was increased by DE (Con: 1.8±0.06 x 10^5 nm^2, DE: 2.3±0.16 x 10^5 nm^2, KS-Test, *p=0.002), and reversed by LRx (1.9±0.07 x 10^5 nm^2, KS-Test, *p=0.028). These results reveal dynamic, asymmetric modifications of synaptic ultrastructure by prolonged DE and brief light reintroduction, and suggest that reorganization of presynaptic ultrastructure contribute to the rejuvenation of plasticity in the adult visual cortex.

Disclosures: **S. Murase:** None. **L. Dye:** None. **E.M. Quinlan:** None.

Poster

387. Plasticity in the Visual System

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P41GM103533 JRY
R01MH067880 JRY

Title: Cell type-specific proteomic analysis on visual activity-induced dynamics in critical period and adult mouse visual cortex

Authors: *Y. XIE, Y. MA, D. MCCLATCHY, J. R. YATES, III, H. T. CLINE;
The Scripps Res. Inst., Scripps Res. Inst., San Diego, CA

Abstract: Visual cortex development and function rely heavily on visual experience, but the protein dynamics induced by visual activity are not well understood in a cell-type specific context. After eye opening, during the critical period (P20-P35), visual cortex shows potent plasticity in visual system rewiring, contributing to the fine tuning of visual system development. In adults, plasticity is less prominent, but manifested in a more specific and sophisticated manner, potentially contributing to more complex vision-dependent learning behavior. In this project, we aim to compare the visual activity-induced protein dynamics in the critical period and adult, utilizing a metabolic protein labeling strategy that allows high-fidelity cell type specific proteomic interrogation. We applied an established mutant Methionine tRNA Synthetase (L274G) transgenic mouse line and generated vGat-mMetRS and EMX-mMetRS mouse lines to

target inhibitory and excitatory neurons in the visual cortex. By combining the Direct Detection of Biotinylated Tag (DiDBiT) method for sample processing and stringent MS/MS data filtering, we were able to detect 2092 proteins on average in each condition. We quantified 364, 278, 253, 231 significantly regulated proteins in EMX-P56, EMX-P28, vGat-P56 and vGat-P28 respectively, with both unique and shared candidates amongst the tested conditions. Through bioinformatics comparison to published translatomics analysis under similar conditions, we identified groups of proteins that correlated with their translating mRNA, as well as those that didn't, indicating more than one layer of regulation of cellular protein content, potentially through protein degradation and translational regulation. Pathway analysis revealed protein clusters that participated in synaptogenesis, cytoskeleton remodeling, cell adhesion and neuronal excitability regulation, from which we selected 11 candidates for subsequent in vitro semi-high throughput screening. We used synaptic marker, Calcium sensor and immediate early gene expression to profile the effect of these candidates on synapses, neuronal spontaneous and evoked activity. In summary, we have systematically compared the activity- induced proteomic dynamics in different cell types and developmental stages, and showed that our top candidates have an effect on several aspects of neuronal characteristics in vitro. Moving forward, we are going to further study the plasticity mechanism of our candidate proteins in vivo.

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Poster

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Support: NIH Grant 5F31EY031602
R01EY025613

Title: Cholinergic modulation of homeostatic plasticity in visual cortex

Authors: *J. BOTTORFF, S. PADGETT, G. TURRIGIANO;
Biol., Brandeis Univ., Waltham, MA

Abstract: Homeostatic plasticity mechanisms are essential to keep neuronal circuits stable in a constantly changing environment. Previous visual manipulation paradigms in rodents have shown that upward firing rate (FR) homeostasis and downward FR homeostasis in monocular visual cortex (V1m) are both gated by behavioral states, but in opposite ways: upward FR homeostasis after monocular deprivation (MD) is driven by active wake, while downward FR homeostasis after eye reopening is driven by sleep (Torrado-Pacheco et al., 2021). Since neuromodulatory tone differs drastically during different behavioral states and is a key regulator of experience dependent plasticity, we hypothesized that neuromodulators were responsible for

this homeostatic regulation of FR. Acetylcholine (ACh) is a key signature of active wake, so we first tested whether ACh gates the homeostatic rebound in FR in V1m after activity deprivation via MD. We found that chemogenetically inhibiting ACh neurons in the basal forebrain (BF), the major source of ACh to V1m in rats, indeed prevented the homeostatic rebound in FR after MD (mean FR on MD4 is 54% of baseline, p=0.002 n = 21 cells, wilcoxon sign rank test). We then asked if BF ACh inhibition affects the induction of synaptic scaling or intrinsic excitability, since these are two of the main cellular mechanisms thought to underlie the homeostatic rebound in FR. Surprisingly, BF ACH inhibition did prevent the induction of synaptic scaling in V1m after activity deprivation, but it also increased the baseline amplitude of spontaneous excitatory post synaptic currents (mEPSC), suggesting that further synaptic scaling may simply be occluded (mean mEPSC CNO only = 10.44pA; Hm4di in V1 + CNO = 11.17pA; Hm4di in BF ACh neurons + CNO = 11.11pA; Hm4di in BF ACh and V1 + CNO = 11.20pA; n>15 cells). BF ACh inhibition alone did not affect intrinsic excitability of V1m pyramidal neurons, but the combination of V1m activity deprivation and BF ACh inhibition caused a significant decrease in intrinsic excitability (area under FR-current injection curve with Hm4di in BF ACh neurons and V1 + CNO is 55% of that with CNO only, p = 0.008, n>13 cells, 1 way ANOVA). This specific effect on V1m intrinsic excitability could explain why BF ACh inhibition prevented the expression of FR homeostasis *in vivo*. We are now working to knock down specific ACh receptors in V1m to ask if these neuromodulatory effects on homeostasis are due to local ACh transmission, and if so, through what receptors. These results will provide important insight into how behavioral states, through neuromodulation, are able to gate plasticity to enable efficient flexibility and stability in complex neocortical circuits.

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Poster

387. Plasticity in the Visual System

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Topic: D.06. Vision

Title: Temporal sequence learning in the primary visual cortex

Authors: *P. PARK, M. J. BERRY II;
Neurosci., Princeton Univ., Princeton, NJ

Abstract: Central to predictive coding is the idea that brain circuits learn statistical regularities in the environment that can predict later events. To test these ideas, we designed a *temporal sequence learning* experiment, in which we presented natural movie clips (2-sec duration, 40 frames/movie) repeatedly in the same order over 4 successive days to 10 transgenic mice expressing GCaMP6f. On the first and the last day of the experiment, the same movies were presented in random order, as a control. Animals were awake and free to move, but performed no task. Calcium fluorescence in layer 2/3 of the primary visual cortex (V1) was recorded using a

two-photon microscope.
After learning, we found that many neurons responded significantly earlier to individual movies. We quantified this ***temporal advance*** in three ways: peak time, cross-correlation, and onset time. Over all the responsive neurons (n = 177), the average temporal advance was 73 ms. Moreover, a large fraction (33%) had an advance greater than 100 ms, which is beyond the range of latencies observed in V1. Such large advances are unlikely to result from neurons responding more rapidly to the same visual features, and instead suggest that neurons developed tuning for different features that occurred earlier in the natural movies.
We also compared neural responses during the repeated movie sequence to responses from the same movie clips presented in random order. We found that the "larger temporal context" of the repeated sequence ***delayed*** and ***suppressed*** the responses of many neurons, in line with ideas about predictive coding. Another subset of neurons had responses that were ***enhanced*** by the temporal context.
Altogether, we found that multi-day temporal sequence learning reorganized the neural code to emphasize sequence elements that occurred earlier, while modulating activity during the entire sequence in a compensatory fashion.

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Poster

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Support: NIH Grant R01EY029245

Title: Using the visual cliff assay to detect and study recovery from amblyopia in mice

Authors: *H. DE JESÚS-CORTÉS[1], F. REILLY-ANDÚJAR[1], E. D. GAIER[2], D. BOWEN[2], M. F. BEAR[1];
[1]Picower Inst. for Learning and Memory, MIT, Boston, MA; [2]Dept. of Ophthalmology and Picower Inst. for Learning and Memory, Boston Children's Hosp. and MIT, Cambridge, MA

Abstract: Amblyopia is a neurodevelopmental disorder of the visual system caused by uncorrected refractive error, cataract, strabismus, or ptosis during infancy and early childhood. The current standard of care is to strengthen the weak eye by depriving, or patching, the fellow eye. While this treatment can improve acuity in the amblyopic eye, these gains often come at the expense of acuity in the fellow eye, are not associated with improvements in stereoscopic depth perception, and are typically only achieved if treatment is initiated in early childhood (within the critical period). In our lab we use a mouse model of amblyopia induced by monocular deprivation (MD) to study the pathophysiology of this disorder and test novel therapeutics. The visual cliff assay (VCA) has been used in multiple animal models, including humans, to assess

stereopsis and depth perception, two key features of binocular vision that are disrupted in amblyopia. However, to date, no study has used the VCA to assess the effects of MD and characterize the recovery from amblyopia in mice. The VCA is advantageous in that we can compare functional and behavioral deficits within animals to determine the severity of amblyopia and track subsequent recovery, and it does not require operant conditioning. We first characterized and validated multiple aspects of the VCA including contribution of binocular vision and the test-retest reliability. We demonstrate that after long-term MD (3 weeks), mice exhibit reduced preference for the safe side, more crosses to the cliff side and take less time to cross into the cliff side compared to their "sham" (no MD) littermate controls. When mice perform the VCA with their fellow eye closed, they spend less time in the safe side, have more crosses between sides, and take less time to cross into the cliff side compared to animals with their amblyopic eye closed. We conclude that the VCA is sensitive to detect amblyopia and therefore can be leveraged to study the efficacy of various treatments targeted at curing amblyopia in mice.

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Poster

387. Plasticity in the Visual System

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 387.28

Topic: D.06. Vision

Support: NIH Grant EY025102
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Title: Can the mouse be used as a model system for strabismus?

Authors: *J. M. SAMONDS[1], C. BARR[1], S. POEKLER-WELLS[1], H. C. BOONE[2], A. W. MCGEE[2], N. J. PRIEBE[1];
[1]Univ. of Texas at Austin, Austin, TX; [2]Univ. of Louisville, Louisville, KY

Abstract: Strabismus is a disorder where people are unable to align their eyes properly on a target. Typically, they direct one eye inward or outward relative to the other eye, and overall, there is increase in variability in binocular alignment. This has consequences in binocular vision in general, and specifically for binocular depth perception. The mouse has widely been used as a model system for the development of binocular vision, but their eyes are directed laterally outward and their binocular eye movements differ from primates. Perturbations of visual experience during development do produce changes in binocularity and acuity that are consistent with amblyopia, but questions remain on whether they could also be a model for strabismus. Following typical development, the eyes of wild type (WT) mice do not re-align on objects at varying depths (Samonds et al. 2019, J Neurosci), their binocular alignment is highly variable

(Samonds et al. 2019; Meyer et al. 2020, Curr Biol) (±3 degrees), and mice have a temporal-to-nasal (or convergent) bias in their saccadic (Meyer et al, 2020) (2 degrees) and ocular kinetic reflex eye movements (Kodama and du Lac 2016, J Neurosci) (50% higher gain). These are all signatures of what is observed in primates during early development before the cortex is fully developed or in adults with strabismus. Nonetheless, WT mice do sense depth based on binocular cues (Samonds et al. 2019; Boone et al. 2021, Curr Biol). We examined whether their depth sensation is altered by monocular deprivation. Deprivation during cortical development increases binocular alignment variability (±3 to ±6 degrees) and reduces binocular depth discrimination performance by 50% compared to WT mice. We also examine whether depth sensation is altered in Fmr1 knockout (KO) mice, a model of Fragile-X syndrome, which is a genetic condition with a higher prevalence of strabismus compared to typical development. These mice also had increased variability in binocular alignment (±3 to ±5 degrees), increased the bias from 2 to 5 degrees for convergent eye movements, and also had 30-40% worse binocular depth discrimination compared to WT mice. Overall, this suggests that mice could provide insight as a model system for understanding the underlying circuitry of strabismus, the influence of strabismus versus cortical changes on binocular vision deficits, and the validity of potential treatments for strabismus and related conditions.

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Poster

388. Sensorimotor Transformations: Neural Circuits

Location: SDCC Halls B-H

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Program #/Poster #: 388.01

Topic: D.07. Visual Sensory-Motor Processing

Support: ANR-18-CE37-0011 – DECORE
ANR-20-CE37-0018-04- Shooting Star
ANR-18-IAHU-01
EU Horizon 2020 No 861423

Title: Context-dependent selectivity to natural scenes in the retina

Authors: *S. VIRGILI[1], M. GOLDIN[1], B. LEFEBVRE[1], M. PHAM VAN CANG[2], A. ECKER[3], T. MORA[4], U. FERRARI[1], O. MARRE[1];
[1]Sorbonne Univ., Inst. de la Vision, Paris, France; [2]Inst. Pasteur, Inst. de l'Audition, Paris, France; [3]Univ. of Göttingen, Inst. of Computer Sci. and Campus Inst. Data Sci., Göttingen, Germany; [4]Sorbonne Univ. and Univ. of Paris, Lab. de physique de l'Ecole normale supérieure, Paris, France

Abstract: Retina ganglion cells extract specific features from natural scenes and send this information to the brain. In particular, they respond to local light increase (ON responses), and/or

decrease (OFF). However, it is unclear if this ON-OFF selectivity, characterized with synthetic stimuli, is maintained when they are stimulated with natural scenes. Here we recorded the responses of ganglion cells of mice and axolotls to stimuli composed of natural images slightly perturbed by patterns of random noise to determine their selectivity during natural stimulation. The ON-OFF selectivity strongly depended on the natural image. A single ganglion cell can signal luminance increase for one natural image, and luminance decrease for another. Modeling and experiments showed that this was due to the non-linear combination of different pathways of the retinal circuit. Despite the versatility of the ON-OFF selectivity, a systematic analysis demonstrated that contrast was reliably encoded in these responses. Our perturbative approach thus uncovers the selectivity of retinal ganglion cells to more complex features than initially thought during natural scene stimulation.

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Poster

388. Sensorimotor Transformations: Neural Circuits

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Topic: D.07. Visual Sensory-Motor Processing

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NRF2022R1A2C2007599
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Title: A visuomotor circuit for evasive flight turns in Drosophila

Authors: *H. KIM[1], A. J. KIM[2];
[1]Artificial Intelligence, [2]Artificial Intelligence;Electronic Engineering;Electrical and Biomed. Engin., Hanyang Univ., Seoul, Korea, Republic of

Abstract: Visual systems extract multiple features from a scene using parallel neural circuits. Ultimately, the separate neural signals must come together to coherently influence action. In this study, we delineate a neural circuit underlying evasive flight turns in flying *Drosophila*. Using an optogenetic activation technique, we first screened various types of visual projection neurons (VPNs) and descending neurons (DNs) by unilaterally activating their dendrites. We found five types of VPNs and one type of DN that caused flies to turn away from the activated side. To test whether these neurons are indeed required for the small object avoidance, we reversibly blocked synaptic transmission of these neurons using thermogenetics while presenting a translating or looming visual object pattern. We found that the amplitude of wing responses reduced significantly when LPLC2 and DNp06 neurons were silenced, for both visual patterns. From the

hemibrain connectome, we found that LPLC2 and DNp06 form strong synaptic connections, likely to mediate the small object avoidance. To further corroborate the role of these neurons in the visual avoidance behavior, we measured physiological responses of LPLC2 neurons with calcium imaging and DNp06 with electrophysiological means. We found that both neurons respond to both visual patterns (i.e., translating or looming objects). How does the LPLC2-DNp06 pathway attain visual selectivity to a looming disc as well as to laterally moving spots? We noted that LPLC2 neurons have two dendritic ramifications, one in the lobula and the other in the lobula plate. When we blocked the majority of visual inputs to the lobula plate by silencing elementary motion detectors (T4/T5), calcium responses of LPLC2 neurons decreased selectively for a looming visual object and fast-moving spot. Furthermore, direction-selective visual responses to a laterally moving spot almost completely disappeared when T4/T5 neurons were silenced. Combining these results, we concluded that LPLC2 neurons' visual properties emerge by integrating multiple visual features - speed, size, direction, and shape - that are represented in two separate, early visual structures, and DNp06 neurons inherit most of these visual properties. This study highlights a clear example of how distinct visual signals converge on a single class of premotor neurons to drive action, revealing a concise pathway for evasive flight maneuvers in *Drosophila.*

Disclosures: H. Kim: None. **A.J. Kim:** None.

Poster

388. Sensorimotor Transformations: Neural Circuits

Location: SDCC Halls B-H

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Program #/Poster #: 388.03

Topic: D.07. Visual Sensory-Motor Processing

Support: National Science Foundation Grant No. IOS-1921065
National Institutes of Health, NINDS R01NS118562

Title: A circuit for a looming responsive descending neuron, DNp03, in Drosophila melanogaster

Authors: *H. CROKE[1], H. JANG[1], A. N. VASSERMAN[1], B. W. HINA[1], K. EICHLER[2], T. STUERNER[2], M. COSTA[2], J. S. PHELPS[3], B. MARK[4], J. C. TUTHILL[4], W.-C. A. LEE[5], J. AUSBORN[6], C. R. VON REYN[1,6];
[1]Sch. of Biomed. Engineering, Sci. and Hlth. Systems, Drexel Univ., Philadelphia, PA; [2]Dept. of Zoology, Univ. of Cambridge, Cambridge, United Kingdom; [3]Dept. of Neurobio., Harvard Med. Sch., Cambridge, MA; [4]Dept. of Physiol. and Biophysics, Univ. of Washington, Seattle, WA; [5]F.M. Kirby Neurobio. Ctr., BCH / Harvard Med. Sch., Boston, MA; [6]Dept. of Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Responding to sensory stimuli with appropriate motor action is necessary across species. However, relatively little is understood about how the brain transforms sensory

information into motor commands. Understanding how sensory information is integrated requires knowledge of the connectivity between neurons within sensorimotor circuits and the role neurons play in sensorimotor computations. This poses a challenge since we rarely have accessible circuits with known connectivity. Recent advancements in electron microscopy in *Drosophila melanogaster* are now enabling us to circumvent these limitations. Here, we investigate a sensorimotor circuit centered around the descending neuron, DNp03, which receives information from the visual field and sends transformed information to the ventral nerve cord (VNC). We identified major pre- and postsynaptic partners to DNp03 within the hemibrain (Scheffer et al., 2020), full adult fly brain (Zheng et al., 2018) and FANC (Phelps et al., 2021) datasets using R (Bates et al., 2020), automatic tracing and synapse detection (Buhmann et al., 2021) provided for Neuprint (Clements et al., 2020), FlyWire (Dorkenwald et al., 2021, Flywire AI), and FANC. Manual tracing of pre- and postsynaptic neurons to DNp03 was performed when required in FlyWire and FANC. Our analysis revealed DNp03 receives synapses from visual projection neurons (VPNs), including Lobula Plate Lobula Columnar Type 1 (LPLC1), Lobula Plate Lobula Columnar Type 4 (LPLC4), Lobular Columnar Type 4 (LC4), and Lobula Columnar Type 22 (LC22). Both LPLC1 and LC4 encode visual features of looming stimuli (Klapoetke et al., 2022), the 2D projection of an object approaching on a direct collision course. Using whole-cell electrophysiology in tethered flies, we found DNp03 robustly responds to looming stimuli as predicted. We next investigated the postsynaptic partners of DNp03 in the VNC. We found DNp03 synapses onto wing motor neurons including the dorsal longitudinal motor neurons, which are responsible for the downstroke (Sun et al., 1997), the second basilar motor neuron, which is active during saccades (Lindsay et al., 2017), the contralaterally projecting wing haltere interneurons, proposed to be involved in flight steering (Trimarchi et al., 1997), and other interneurons that synapse onto neck motor neurons. Together, these results suggest that DNp03 may be involved in flight maneuvers evoked by a visual looming stimulus, which will be further investigated using optogenetics and behavioral assays. Our work aids in the collaborative effort to map out the *Drosophila* connectome and extract general principles for sensorimotor transformations.

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Poster

388. Sensorimotor Transformations: Neural Circuits

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Topic: D.07. Visual Sensory-Motor Processing

Support: National Science Foundation Grant No. IOS-1921065
National Institutes of Health, NINDS R01NS118562

Title: Bilateral integration of looming responses in Drosophila descending neurons

Authors: *H. JANG[1], D. P. GOODMAN[1], B. W. MCFARLAND[1], C. E. MCKELLAR[2], J. AUSBORN[3], C. R. VON REYN[1,3];
[1]Sch. of Biomed. Engineering, Science, and Hlth. Systems, Drexel Univ., Philadelphia, PA; [2]Princeton Neurosci. Inst. and Computer Sci., Princeton Univ., Princeton, NJ; [3]Neurobio. & Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: The ability to generate appropriate behaviors in response to a rapidly changing environment is essential for the survival of all animals. To accomplish this goal, an animal's brain must acquire and process sensory input from the environment and then integrate this information to select and drive an appropriate behavior. However, the underlying mechanisms behind these sensory to motor transformations are not well understood. Visually-evoked escape behaviors serve as conserved, experimentally tractable models for understanding sensorimotor transformations. Neural responses to looming stimuli mimicking an approaching predator are similar across species and these stimuli trigger a wide variety of escape behaviors fruitful to investigating action selection processes. However, the composition, connectivity, and integration mechanisms within the circuits that drive these behaviors are still unclear. In *Drosophila melanogaster*, looming integration is hypothesized to occur within a population of descending neurons (DNs) that receive visual feature information from visual projection neurons (VPNs) of the central brain and terminate in the ventral nerve cord (VNC). The dynamics of the DN responses - which DN are active and when - are predicted to establish the mode and directionality of an escape behavior. Using whole-cell electrophysiology in behaving animals, we screened candidate looming responsive DNs and found a subset are tuned to looming stimuli but have distinct spike-timing characteristics. We next investigated directional dependencies of looming responses within these DN by presenting looming stimuli to the eye ipsilateral or contralateral to the DN cell body, or center and bilateral looming stimuli that expand across both eyes. Surprisingly, we found multiple DNs that respond to contralateral looming stimuli even though their dendrites were only ipsilateral. DNp01 (also called the giant fiber), in particular, showed response invariance across all stimulus locations. Contralateral eye painting demonstrated that visual information is being provided to DNp01 from the contralateral eye, through a yet to be investigated commissural pathway. Future work will utilize available EM datasets, electrophysiology, and modeling to investigate the source of contralateral looming information and how it contributes to DNp01 directional invariance. This work will extend our knowledge on how complex visual information is dynamically integrated across eyes to generate behavioral responses.

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Poster

388. Sensorimotor Transformations: Neural Circuits

Location: SDCC Halls B-H

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Program #/Poster #: 388.05

Title: WITHDRAWN

Poster

388. Sensorimotor Transformations: Neural Circuits

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 388.06

Topic: D.07. Visual Sensory-Motor Processing

Support: Rosa-Laura und Harmut Wekerle Foundation
Max Planck Society

Title: A multi-input optic glomerulus guides diverse visual behaviors

Authors: *I. RIBEIRO, S. PRECH, M. SAUTER, A. BORST;
Max Planck Inst. Biol. Intelligence i.f., Munich, Germany

Abstract: Processing of visual input transforms an initial 2-dimensional array of light intensities fluctuating over time into behaviorally relevant features. In insects, visual projection neurons relay such features to several regions in the central brain, including synapse-dense optic glomeruli. The largest optic glomerulus, the anterior optic tubercle (AOTu), receives input from different neuronal types belonging to the lobula columnar 10 (LC10) group (LC10a, b, c, and d) and retains a topographic organization that reflects the visual field. LC10a neurons sense moving objects and are essential for tracking the female during courtship [1], a complex social interaction involving a persistent courtship internal state triggered by female chemosensory cues, that modulates LC10a gain [1, 2]. Surprisingly, other LC10-group neuronal types are dispensable for female tracking or directed courtship [1], raising the possibility that the AOTu might participate in different visual behaviors. To address this question, we blocked neurotransmission of the different LC10-group neurons and exposed male flies walking on an air-suspended ball to very small (8º) and fly-sized (30º) objects, as well as long bars, akin to environmental landmarks, and gratings, that elicit an optomotor response. These results revealed that LC10d neurons are required for avoiding very small objects, whereas LC10a neurons mediate tracking of fly-sized objects. Despite distinct genetic access, the arborizations of LC10a and LC10d neurons are remarkably similar, with LC10a cells reaching more distal layers in the lobula. Functional imaging of neuronal calcium responses to a barrage of visual stimuli revealed that LC10d detect discrete objects, with broader tuning properties than LC10a neurons. Lastly, blocking LC10d neurons in naïve males leads to orienting maneuvers and female tracking before courtship is initiated. Together these results suggest that LC10d neurons mediate avoidance of visual objects devoid of a chemosensory signature, and implicate the AOTu as a hub for processing diverse visual cues that subserve different behavioral responses to discrete objects. 1. Ribeiro, Drews, Machacek, Borst, Dickson. 2018. PMID: 300333672. Hindmarsh Sten, Li, Otopalik, Ruta. 2021. PMID: 34234348

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Poster

388. Sensorimotor Transformations: Neural Circuits

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Program #/Poster #: 388.07

Topic: D.07. Visual Sensory-Motor Processing

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Zukunftskolleg Konstanz
Centre for the Advanced Study of Collective Behaviour
University of Konstanz
International Max Planck Research School for Quantitative Behavior, Ecology and Evolution from lab to field

Title: Neural basis of visual information integration and decision-making in larval zebrafish

Authors: *K. SLANGEWAL, M. CAPELLE, F. KÄMPF, A. BAHL;
Univ. of Konstanz, Konstanz, Germany

Abstract: Decision-making is a long-studied topic in neuroscience. We have an increasingly good mechanistic understanding of the neural circuits that allow animals to temporarily integrate specific decision variables. However, it remains unclear how these circuits combine, often conflicting, information from multiple sensory channels to form a single decision. Recently, we have described how the larval zebrafish anterior hindbrain integrates visual motion to decide about swimming direction. Other studies, focusing on different sensory stimuli, have identified the same brain area as a central processing structure for sensory-motor control. This raises the hypothesis that the anterior hindbrain forms a general integration hub for decision-making. Here, we employ a combination of behavioral experiments, computer simulations, and two-photon functional imaging to algorithmically and mechanistically describe how larvae integrate motion and luminance cues. Our behavior experiments argue for a parallel arrangement with inhibitory crosstalk, in which separate modules temporally integrate information from distinct visual processing streams. Our imaging experiments support these findings, revealing distinct and partially overlapping activation patterns with slow temporal dynamics. Together, these results allow us to build detailed neural networks that will help us to describe in mechanistic detail how brains combine and evaluate information from multiple sensory sources.

Disclosures: K. Slangewal: None. **M. Capelle:** None. **F. Kämpf:** None. **A. Bahl:** None.

Poster

388. Sensorimotor Transformations: Neural Circuits

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Topic: D.07. Visual Sensory-Motor Processing

Support: International Max Planck Research School for Quantitative Behavior, Ecology and Evolution from lab to field
Centre for the Advanced Study of Collective Behaviour
University of Konstanz
Zukunftskolleg Konstanz
Boehringer-Ingelheim Fonds

Title: The various timescales in larval zebrafish phototactic behavior

Authors: *M. CAPELLE[1], K. SLANGEWAL[2], M. FUCHSLOCH[2], A. BAHL[2];
[1]Univ. of Konstanz, [2]Univ. of Konstanz, Konstanz, Germany

Abstract: To navigate in dynamic natural environments, animals need to constantly integrate and evaluate sensory cues during decision-making. Such behavior needs to be modulated by the internal perceptual state of the animal on timescales across multiple orders of magnitude. However, the mechanistic implementation of these underlying processes in the brain remains poorly understood. Recently, it has been shown that larval zebrafish behavior is more flexible than previously thought, making it a suitable system to address these questions. Here, we employ closed-loop high-throughput behavioral assays to explore this problem in the context of larval zebrafish phototaxis. We demonstrate that phototaxis is regulated by fixed innate preferences and sensory experience on timescales of multiple tens of seconds. We develop a simple computational model combined with multi-objective fitting strategies to capture the dynamics of our behavioral datasets. From this optimized model, we infer the underlying cognitive algorithms, allowing us to make specific predictions about the respective decision-making mechanisms in the nervous system. By using whole-brain two-photon functional imaging, we identify cells and brain regions that may implement our proposed model. Based on our findings, we aim to develop biologically plausible network models for adaptive behaviors and to systemically dissect the underlying neural circuitry.

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Poster

388. Sensorimotor Transformations: Neural Circuits

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Topic: D.07. Visual Sensory-Motor Processing

Support: DFG Grant NI 618/11-1 to A.N.

Title: Crow NCL neurons translate a perceived number into a matching number of self-generated actions

Authors: *M. E. KIRSCHHOCK, A. NIEDER;
Univ. of Tuebingen, Tuebingen, Germany

Abstract: Humans and animals share a primordial and non-symbolic number estimation system. It allows them to not only to perceive numerosity, i.e., the number of objects in stimuli, but also produce a specific number of self-generated actions. While the brain mechanisms representing perceived number have been studied intensively, the neuronal processes of transforming number stimuli into a matching number of self-generated actions are unknown. To explore this sensorimotor transformation, we trained two carrion crows (*Corvus corone*) to judge numerical values in displays and to flexibly plan and perform a matching number of pecks. While the crows performed this task, we recorded single-cell activity within the telencephalic brain area *nidopallium caudolaterale* (NCL), a putative homologue to the mammalian PFC. Here, we report sensorimotor number neurons in the crow NCL that signaled the impending number of self-generated actions. Neuronal population activity during the sensorimotor transformation period predicted whether the crows planned the instructed number of pecks, or mistakenly planned for fewer or more pecks than instructed. During sensorimotor transformation, both a static neuronal code characterized by persistently number-selective neurons and a dynamic code originating from neurons carrying rapidly changing numerical information emerged. The findings indicate that there are distinct functions of abstract neuronal codes supporting the sensorimotor number system in the crow brain.

Disclosures: M.E. Kirschhock: None. **A. Nieder:** None.

Poster

388. Sensorimotor Transformations: Neural Circuits

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Program #/Poster #: 388.10

Topic: D.07. Visual Sensory-Motor Processing

Support: ERC Project Avian Mind

Title: Anatomical characterization of the medial nidopallium/mesopallium of pigeons (Columba livia)

Authors: *K. HASELHUHN, J. TUFF, O. GUNTURKUN, N. ROOK;
Biopsychology, Ruhr Univ. Bochum, Bochum, Germany

Abstract: Higher cognitive functions such as working memory, categorization, extinction learning, sequential behavior and many more have typically been associated with the nidopallium caudolaterale (NCL) in birds, which is considered a functional analogue to the mammalian prefrontal cortex (PFC). However, more recently, studies have found that sequential behavior, fast sensorimotor learning and response inhibition also heavily rely on areas within the medial nidopallium/mesopallium (MNM) in the avian anterior forebrain. To better understand how the NCL and MNM interact and therefore contribute to the processing of these higher

cognitive abilities it is essential to understand the underlying anatomy and connectivity of these areas. While the connectivity and neurochemistry of the avian NCL is well known, these features have so far not been investigated in much detail for MNM.Here we performed in vivo tracings, viral tracings and in vitro tracings to gain a more in depth understanding of the intrinsic and long range connectivity of the MNM subdivisions, including the medial mesopallium (MM), the nidopallium intermedium medialis pars laterale (NIML) and the nidopallium mediale pars medialis (NMm). Furthermore, we complemented our tracings with stainings against different markers such as Calbindin-D28k, Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), tyrosine hydroxylase (TH) and γ-aminobutyric acid (GABA) to gain insights into the neurochemistry of MNM.We found that MNM receives multimodal input and reciprocates with NCL and the (pre)motor pallium. Our in vitro tracings suggested that the nidopallial and mesopallial structures are reciprocally interconnected in a strong way, while the nidopallial subdivisions show only weak connections to each other. Moreover, all three subdivisions of MNM projected heavily to the medial striatum (MSt).Our data suggests that MNM could be part of a cognitive avian pallial network relevant for the acquisition and initiation of complex movements. The NCL might be responsible for evaluating MNM's input and complement it with contextual information and dopaminergic feedback.

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Poster

388. Sensorimotor Transformations: Neural Circuits

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Topic: D.07. Visual Sensory-Motor Processing

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Title: Cortico-tectal circuits involved in motor preparation and navigation

Authors: *T. LUPASHINA[1,2], R. BERGMANN[3], K. SEHARA[3], S. DOMINIAK[4], J. SIBILLE[1,2], K. TEH[1,2], M. E. LARKUM[3], R. N. SACHDEV[3], J. KREMKOW[1,2];
[1]Neurosci. Res. Ctr., Charite Universtätsmedizin Berlin, Berlin, Germany; [2]Bernstein Ctr. for

Computat. Neurosci., Berlin, Germany; [3]Inst. of Biology, Humboldt Univ. of Berlin, Berlin, Germany; [4]Univ. of Sussex, Sch. of Life Sci., Brighton, United Kingdom

Abstract: Cortico-tectal interactions are thought to mediate a range of multimodal behaviors, including navigation, attention, orientation and decision making. Layer 5 pyramidal neurons distributed in widespread cortical areas connect cortex to superior colliculus (SC) and collicular output targets higher order thalamic nuclei. The thalamo-cortical neurons then generate feedback in cortex. How and when these circuits interact to generate complex behavior is not known. When head-fixed mice navigate a plus-maze on an Airtrack system, they orient their body to the maze and they coordinate the movement of their torso and limbs with the movement of their whiskers and eyes (Dominiak et al, 2019; Bergmann et al, 2021). The whiskers and eyes move in a look ahead fashion and independently predict the turn direction that mice impose on the maze. Here we begin to test the hypothesis that the cortico-collicular loop is causal in planning and execution of these behaviors. Our first aim was to look for activity in motor cortical areas (secondary motor cortex (M2), MOs) and SC that was related to these behaviors. We targeted and recorded neuronal activity from across SC layers and in M2 with the use of Neuropixels probes which can record simultaneously from a large population of neurons in several brain areas of an awake animal (Sibille et al, 2021). In the analysis of these recordings, we observed distinct and diverse neuronal activity in both M2 and SC linked to movement of the platform, and position of the eyes. We are currently assessing the diversity of neuronal activity using Uniform Manifold Approximation and Projection (UMAP). With the aim of understanding the behavioral task, we are further looking into whether the coordinated eye and whisker movement is innate or learned. In the future, we would like to use a specialised behavioral algorithm to identify and characterize the unique behavioral sequences which are exhibited by the animal on the Airtrack system and to further understand how activity in the superior colliculus and cortex contributes to generating the entire sequence of movements associated with navigation.

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Poster

388. Sensorimotor Transformations: Neural Circuits

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 388.12

Topic: D.07. Visual Sensory-Motor Processing

Title: Brain wide dynamics of evidence integration preceding a decision and action

Authors: *A. KHILKEVICH[1], M. LOHSE[1], I. ORSOLIC[1], T. BOZIC[1], P. WINDMILL[1], T. D. MRSIC-FLOGEL[2];
[1]Sainsbury Wellcome Ctr., London, United Kingdom; [2]Sainsbury Wellcome Ctr. (UCL), London,, United Kingdom

Abstract: Decisions are often guided by detecting subtle signals in a dynamic sensory environment. Although the brain must track such decision-relevant signals, how they are represented across the brain and transformed into the decision and action remains poorly understood. Here, we recorded neural activity with Neuropixels probes across dozens of brain regions (15 mice, 116 sessions, 128 brain regions, 14493 stable single units) while mice performed a visual change-detection task. We trained mice to detect a sustained increase in temporal-frequency (TF) of a drifting grating stimulus, whose speed fluctuates stochastically around the mean of 1Hz. The task requires mice to remain stationary while continuously monitoring the grating with noisy speed which could increase at any moment, thereby allowing us to study the processing of dynamically changing task-relevant sensory evidence (i.e. TF) in the absence of overt movement and prior to the reporting of choice (lick). We find that even transient fluctuations (50 ms) in TF recruit activity in 10-20% neurons across a large number of distributed brain regions in the absence of choice and other movements. Beyond the visual system, we find such representations in posterior parietal cortex, premotor cortex, higher-order thalamus, midbrain, cerebellum and basal ganglia. Strikingly, only brainstem nuclei driving orofacial movements appear to be devoid of such sensory evidence representations. Interestingly, momentary increases in TF caused transient responses in neurons in visual areas (dLGN, V1 and superior colliculus), but more sustained responses in downstream areas previously associated with sensorimotor learning, working memory and motor planning (frontal and premotor cortex, basal ganglia, cerebellum). These sustained responses allow for integration of multiple samples of stimulus speed, which could provide a robust neural substrate for detecting sustained changes in noisy sensory evidence. Furthermore, we find that the preparatory activity precedes the lick onset for over a second in most brain regions with sustained responses to transient increases in TF (frontal and premotor cortex, basal ganglia, cerebellum). Strikingly, the population dynamics of the TF-responsive subpopulation in these regions contributed to the majority of preparatory activity, consistent with the integration of sensory evidence leading to the onset of the learned action. These results suggest that circuits distributed across several brain regions act to transform sensory evidence into the decision and action.
A. Khilkevich and M. Lohse contributed equally to this work.

Disclosures: **A. Khilkevich:** None. **M. Lohse:** None. **I. Orsolic:** None. **T. Bozic:** None. **P. Windmill:** None. **T.D. Mrsic-Flogel:** None.

Poster

388. Sensorimotor Transformations: Neural Circuits

Location: SDCC Halls B-H

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Program #/Poster #: 388.13

Topic: D.07. Visual Sensory-Motor Processing

Support: Research Foundation of Korea funded by the Ministry of Science and ICT (2021R1A2C3012159, 2021R1A4A2001803)
KAIST Global Singularity Program for 2020

Title: Projection-specific roles of the anterior cingulate cortex in sensory-to-motor transformation

Authors: *H.-Y. PARK, D.-H. MA, S.-H. LEE;
Biol. Sci., KAIST, Daejeon, Korea, Republic of

Abstract: The anterior cingulate cortex (ACC) is implicated in transforming sensory information into motor actions in mice performing perceptual tasks. However, it is still poorly understood how the ACC neurons projecting to different brain areas contribute to the sensory-to-motor transformation in animals performing goal-directed actions. Here, we examined the anatomical features of the V1-projecting ACC (ACC→V1) and the DMS-projecting ACC (ACC→DMS) neurons by dual-retrograde tracing and found that these two populations of neurons are segregated in the ACC at different layers. To figure out their functions, we labeled the ACC→V1 and the ACC→DMS neurons with GCaMP6 by injecting retrograde AAV-Cre in the V1 or the DMS of Ai148 mice. We then trained the mice to perform the visual detection task and measured task-related activities of the labeled neurons before and after the learning. We found that the ratio of the sensory(visual)-responsive neurons increased in both populations after learning. Interestingly, the ACC→DMS neurons showed a higher sensory response in hit trials compared to miss trials. In contrast, the ACC→V1 neurons showed consistent visual responses to the visual stimuli regardless of learning or licking after the stimuli. In both populations of neurons, the ratio of motor(lick)-responsive neurons decreased, but their activity increased after learning. To clarify how the task-relevant activities of the ACC neurons are modulated across the learning, we measured the release of dopamine (DA) and acetylcholine (ACh), well-known neuromodulators in the cortex, using the fiber photometry and imaging GRAB sensors in the ACC. The DA release was higher in hit trials than in miss trials, and the ACh release significantly increased during licking. Together, these results demonstrate that the ACC→V1 and the ACC→DMS neurons show distinct modulation properties during the task, which might be caused by the neuromodulators, such as the DA and ACh, released in the ACC.

Disclosures: **H. Park:** None. **D. Ma:** None. **S. Lee:** None.

Poster

388. Sensorimotor Transformations: Neural Circuits

Location: SDCC Halls B-H

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Topic: D.07. Visual Sensory-Motor Processing

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Wellcome Trust PhD Studentship

Title: Visuomotor learning promotes visually evoked activity in the medial prefrontal cortex

Authors: A.-M. MARICA, J. M. J. FABRE, K. D. HARRIS, M. CARANDINI, ***A. J. PETERS**;
Univ. Col. London, London, United Kingdom

Abstract: The medial prefrontal cortex is necessary for executing learned associations between visual stimuli and movement. This function is supported by the presence of stimulus-driven activity after learning, but it is unknown how this stimulus-driven activity develops across learning and where exactly it appears.
We performed longitudinal widefield calcium imaging of excitatory neurons across days while mice learned a visuomotor detection task. The task consisted of turning a steering wheel with the forelimbs in response to a single visual stimulus to receive a sucrose reward. It was successfully learned over the course of about one week. We monitored cortical responses to the task stimulus in both behavioral and passive contexts. We found that stimulus responses in the medial prefrontal cortex developed over the same time course as task learning. These stimulus responses were present during both the task and quiescent passive viewing, indicating that they were not tied to subsequent movement. Furthermore, both behavioral performance and medial prefrontal stimulus responses increased between - rather than within - training sessions, suggesting that training induces delayed plasticity. Neuropixels recordings revealed that the medial prefrontal stimulus response originates from both the secondary motor and anterior cingulate cortex. The stimulus response was eliminated by visual cortical inactivation. These results show that visuomotor learning establishes a route for visual information to the medial prefrontal cortex.

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Poster

388. Sensorimotor Transformations: Neural Circuits

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 388.15

Topic: D.07. Visual Sensory-Motor Processing

Support: Pritzker Fellowship for Neurosciences
Whitehall Foundation
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NSF-NCS 1835390
NIH RO1 NS121535
University of Chicago

Title: Task-relevant information is enriched in mouse PPC but not selectively propagated to M1

Authors: *P. RAVISHANKAR[1], H. GRIER[2], M. T. KAUFMAN[3];
[1]Committee on Computat. Neurosci., Univesity of Chicago, Chicago, IL; [2]Committee on

Computat. Neurosci., The Univ. of Chicago, Chicago, IL; [3]Organismal Biol. and Anat., Univ. of Chicago, Chicago, IL

Abstract: Mice can readily use complex visual stimuli to inform movement, extracting relevant visual features from among irrelevant information to do so. The computations needed for this process are likely highly distributed across the brain. We investigated the extraction of relevant information in Posterior Parietal Cortex (PPC), an area implicated in the vision-to-movement pathway. We trained head-fixed mice in a novel visuomotor task, in which they moved a joystick to cancel out the drift of a visual stimulus. Crucially, although the stimulus drifted diagonally, only the forward-back axis of the joystick was coupled to the (vertical) visual stimulus drift. Thus, the horizontal visual drift was task-irrelevant. Mice learned to use only the relevant visual information to inform push-pull movements of the 2D joystick (~2-3 months, n=2 mice). After achieving expert performance (correct initial direction on >70% of trials), we performed two-photon calcium imaging in contralateral PPC with GCaMP6f expressed in all pyramidal neurons (6 sessions, 2725 neurons). We then built a cross-validated linear encoding model to identify what task features modulated each neuron's activity. We found that 15% of PPC neurons encoded at least one task variable (>1% cross-validated variance explained): stimulus onset, visual drift velocity, joystick velocity, reward or trial history. PPC neurons were on average most strongly modulated by stimulus onset and joystick movement. Interestingly, most visually-responsive neurons were more strongly modulated by task-relevant than task-irrelevant visual drift (67%), and a slight majority for relevant vs. irrelevant joystick movement (58%). These results argue that PPC activity more strongly includes task-relevant than -irrelevant information. Further, we identified the neurons that project directly from PPC to forelimb M1 (Caudal Forelimb Area, CFA) via retrograde tracing (AAVretro-tdTomato injected into CFA; 109 neurons). Surprisingly, the modulation of these labeled CFA-projecting neurons was highly similar to unlabeled neurons. This argues that the full representation present in PPC is sent downstream to M1, constraining our models of how signals are transformed as they propagate across cortex.

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Poster

388. Sensorimotor Transformations: Neural Circuits

Location: SDCC Halls B-H

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Program #/Poster #: 388.16

Topic: D.07. Visual Sensory-Motor Processing

Support: NIH Intramural program ZIAMH002956
NIH BRAIN Initiative Grant U19NS107464
NIH BRAIN Initiative Grant U01NS108683

Title: Amplified cortical neural responses as animals learn to use novel optogenetic activity patterns

Authors: *B. AKITAKE[1], **H. M. DOUGLAS**[1], P. K. LAFOSSE[1], C. E. DEVEAU[1], A. J. LI[1], L. N. RYAN[2], S. P. DUFFY[1], Z. ZHOU[1], Y. DENG[1], M. H. HISTED[3];
[1]NIH/NIMH, Bethesda, MD; [2]Neurosci., New York Univ., BROOKLYN, NY; [3]Natl. Inst. of Mental Hlth., NIH / NIMH, Bethesda, MD

Abstract: Cerebral cortex supports representations of the world in patterns of neural activity, used by the brain to make decisions and guide behavior. Past work has found diverse changes in the cortex in response to learning, suggesting key computations in sensory learning might occur in areas downstream of the primary sensory cortex, with cortical representations undergoing only small changes in the adult. Alternatively, local cortical circuitry may have substantial capacity to change to improve behavior and process new types of inputs. To examine this, we trained mice to recognize entirely novel, non-sensory patterns of cortical activity created by direct optogenetic stimulation in the primary visual cortex (V1) while simultaneously measuring GCaMP responses using 2-photon imaging. As animals learned to use these 'off-manifold' patterns over a few days, we found large increases in cortical responses to fixed optogenetic input (positive change in response at perceptual threshold: □□F/F = 17.3 ± 5.5%) and animals' detection performance improved in concert with the increased cortical amplification (negative change in normalized optogenetic perceptual threshold: -62 ± 10%, mean ± SEM, N = 3). The neural changes were dependent on animals using the new patterns for behavior, and the increased responses for novel optogenetic inputs had little effect on existing visual sensory responses. Increased amplification would seem to be optimal for improving decision-making in the detection task we used, and thus, we find adult cortical plasticity can act over a few days to optimize processing of novel inputs.

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Poster

388. Sensorimotor Transformations: Neural Circuits

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 388.17

Title: WITHDRAWN

Poster

388. Sensorimotor Transformations: Neural Circuits

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 388.18

Topic: D.07. Visual Sensory-Motor Processing

Support: Doctoral ANID grant 21200230
FONDECYT REGULAR 1190318

Title: Self-initiated stimuli improve visual processing in the primary visual cortex of the rat

Authors: *C. B. LINDSAY[1], S. MADARIAGA[1,2], C. MURÚA[1,2], J. L. VALDÉS[1], P. E. MALDONADO[1,2];
[1]Dept. of Neurosci. and BNI Institute, Fac. of Med., Univ. de Chile, Santiago, Chile; [2]Natl. Ctr. for Artificial Intelligence (CENIA), Santiago, Chile

Abstract: Our brain builds what we perceive. Our perception depends not only on external stimuli but also on our internal states. Thus far, most of what we know about how sensory processing has been obtained with a paradigm where a sudden stimulus is presented to the animal. Nevertheless, our motor actions are primarily responsible for triggering new sensory stimuli (as eye movements generate a new visual stimulation). This motor-triggered perceptual process is also known as Active Sensing. The primary visual cortex (V1) is the first cortical area to represent information about sensory stimuli and process behavioral states. Several studies indicate that motor acts are a crucial behavioral state that modulates neural changes across V1, favoring an optimal visual stimuli processing. However, how active sensing affects the neural dynamics in V1 is still unknown. We hypothesize that under active sensing conditions, the evoked response to a visual stimulus triggered by a motor act would be precisely modulated in timing, improving visual input detection. To elucidate the neural mechanisms underlying the motor modulation of visual perception, we performed behavioral and electrophysiological analysis on rats performing a visuomotor task in both active and passive sensing conditions. Our behavioral tests showed significantly better performance on the visual detection task when the visual stimulus onset was self-triggered by a motor act (nose poke) than passive sensing. Using multisite recording probes that span along with all cortical layers, we seek to characterize V1 physiological patterns of freely behaving rats performing the task. We observed different amplitude and timing characteristics of the V1 light-evoked potentials (EP) at each sensing condition. Interestingly, when analyzing the early component of the EP (<250ms), we observed that the first peak starts a few milliseconds before the stimuli onset under active conditions. In contrast, passive conditions exhibited a longer latency. When analyzing the late component of the EP (>250ms), an N300-like peak followed by a slow oscillatory activity was observed, but only in active sensing conditions. Furthermore, the precise timing of the N300-like peak is associated with shorter reaction times in the visual task. This study indicates a clear motor-dependent impact underlying active visual perception, triggering precise timing mechanisms in the neural response of V1, which enables better visual detection.

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Poster

389. Neurophysiology: Decoding and Neural Processing I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 389.01

Topic: E.05. Brain-Machine Interface

Support: NSF EEC-1028725

Title: Rapid offline identification of neural triggers for a virtual reality brain computer interface (VR-BCI) with intracranial electrodes in humans

Authors: *C. PASCHALL[1], E. TANUMIHARDJA[2], K. E. WEAVER[3], B. L. GRANNAN[4], A. KO[5], J. S. HAUPTMAN[4], J. G. OJEMANN[4], R. P. RAO[2], J. A. HERRON[4];
[1]Bioengineering Dept., [2]Paul G. Allen Sch. for Computer Sci. and Engin., [3]Radiology, [4]Dept. of Neurolog. Surgery, [5]Univ. of Washington, Seattle, WA

Abstract: Virtual Reality (VR) offers a potent new platform for development of brain computer interface (BCI) strategies. Complex behaviors can be initiated in VR by neural triggers that each yield half-dimensional control of a VR-BCI. Triggers, like mouse-clicks, can be layered to achieve nuanced control over a virtual scene. We recently demonstrated a behavioral collection protocol for the identification of multiple VR-BCI triggers, a quick and straightforward approach to offline spectral analysis, and the integration of identified neural triggers in a real-time spectral decoder. Our behavioral collection protocol relies on stereo-electroencephalography (sEEG) and macro-electrocorticography (ECOG) electrode localization over somatosensory and motor cortices. The electrode localization results were used to predict actions likely to elicit cortical activity from recorded cortex which were then pseudo-randomly presented to the patient to build a dataset composed of continuous neural activity during sequential cue-and-action epochs. Behavioral neural signals collection was preceded and followed by 2 minutes of resting state data. We demonstrate that 30-second epochs of cued motor or sensory activity is sufficient for the offline identification of candidate neural triggers associated with each behavior. Offline neural signals analysis included straightforward common average referencing and windowed fourier decomposition for time-frequency analysis. Average power comparisons between cued activity and resting state were used to identify spectral bands of interest and a candidate trigger was identified as any behavioral activity which elicited a 20% or greater average power increase on any one electrode during a 30-second cued epoch. In one human subject, this yielded four candidate triggers, ranked by the percentage and duration of power increase during the activity. We also detail the real-time neural signals processing built to decode neural triggers in real time and communicate trigger identification to update variables in a VR-BCI task. This work facilitates the development of VR-BCI in humans and presents an approachable protocol and circuit design for BCI implementation by providing a means of identifying candidate BCI triggers for further evaluation.

Disclosures: C. Paschall: None. **E. Tanumihardja:** None. **K.E. Weaver:** None. **B.L. Grannan:** None. **A. Ko:** None. **J.S. Hauptman:** None. **J.G. Ojemann:** None. **R.P. Rao:** None. **J.A. Herron:** None.

Poster

389. Neurophysiology: Decoding and Neural Processing I

Location: SDCC Halls B-H

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Program #/Poster #: 389.02

Topic: E.05. Brain-Machine Interface

Support: This work was supported by the U.S. NIH National Institute on Deafness and Other Communication (R01DC008358, R01DC018446, R01DC018055)
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Title: Generalized Decoding of Vocal Elements in Zebra Finch

Authors: *D. BROWN[1], A. SRINIVASAN[1], P. TOSTADO MARCOS[2], J. CHAVEZ[1], D. NGUYEN[1], A. KADWORY[1], E. M. ARNEODO[3], V. GILJA[1], T. GENTNER[4];
[1]Electrical and Computer Engin., UCSD, La Jolla, CA; [2]Bioengineering, UC San Diego, San Diego, CA; [3]UC San Diego, La Jolla, CA; [4]Psycholgy, Univ. Of California San Diego Neurosciences Grad. Program, La Jolla, CA

Abstract: Songbirds are a powerful model for studying the acquisition and production of complex learned vocal behavior. Zebra Finch in particular have been heavily studied for their stereotyped motif which is a major component of their song. Our recent work has leveraged features of local field potential that precede behavior to predict the onset of syllable production (Brown et al., PLoS CompBio, 2021). This work predicted syllables only within the structured motif, a small subset of the zebra finches' vocal repertoire. Thus, we now broaden our analyses to examine the potential to predict vocal events over the longer timescale of the bout. In addition to the stereotyped motif and the introductory notes that often precede the motif, the majority of zebra finch vocal behavior consists of vocalizations collectively referred to as 'calls'. Some calls are learned and others are not. While previous work has found that neural activity in pre-motor nuclei (i.e., HVC and RA) encodes information regarding which syllable the bird will sing, few studies focus on elucidating what activity, if any, relates to call production. Here we report novel spectral features of neural activity that correlate with call identity in a premotor nuclei. We applied unsupervised dimensionality reduction techniques to map spectrograms of manually segmented 'calls' to a lower dimensional representation. The spectrograms of the calls were manually clustered based upon this representation. The neural activity recorded during each call was then analyzed using methods previously used to study motif syllable production in our previous study. Using band power within multiple frequency bins of the local field potential (LFP) in HVC as a feature, and a linear discriminant analysis (LDA) classifier, we identified

separability in neural space for five distinct syllables, the introductory notes, intra-motif silence, and two distinct call clusters recorded during free vocal activity. By applying 4-fold cross validation, we computed classification performance across all vocal units including two call clusters selected for having the highest number of instances, and measure a classification accuracy of 76.31 ±3% (mean +/- s.e.m; binomial chance level is 19.04% $p<0.05$). Binary classification of two calls that were selected for having a high degree of LFP phase locking yielded a peak call classification accuracy of 75 ±0% (mean +/- s.e.m; binomial chance level is 66.66% $p<0.05$). Our results give preliminary evidence that LFP in HVC may be dependent on upcoming vocal units beyond the syllables of the motif. These vocal units may include calls which is unexpected given prior literature and thus merits further exploration.

Disclosures: D. Brown: None. **A. Srinivasan:** None. **P. Tostado Marcos:** None. **J. Chavez:** None. **D. Nguyen:** None. **A. Kadwory:** None. **E.M. Arneodo:** None. **V. Gilja:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); V.G. holds shares in Neuralink, Corp., and Paradromics, Inc. These organizations had no role in study design, data collection and analysis, decision to publish, or preparation of the abstract.. F. Consulting Fees (e.g., advisory boards); V.G. currently consults for Paradromics, Inc. They had no role in study design, data collection and analysis, decision to publish, or preparation of the abstract.. **T. Gentner:** None.

Poster

389. Neurophysiology: Decoding and Neural Processing I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 389.03

Topic: E.05. Brain-Machine Interface

Support: NIH NINDS 1R01NS124222 - 01

Title: Characterization of sensory and motor action potentials recorded by regenerative multielectrode interfaces

Authors: *K. R. HUSSEIN, M. I. ROMERO-ORTEGA;
Biomed. Engin. and Biomed. Sci., Univ. of Houston, Houston, TX

Abstract: Advanced robotic limbs have human-like degrees of freedom and are sensorized. However, providing amputees with naturalistic control and ‘feeling’ of this advanced prosthesis remains a formidable challenge, requiring it to connect to the user’s nervous system. Several strategies have been proposed to accomplish this including targeted muscle reinnervation, and muscle and neural interfacing. The latter can be accomplished with extraneural cuff or FLAT electrodes, indwelling tLIFE, TIME or with regenerative sieve or Regenerative Multielectrode Interface (REMI) electrodes. While much progress has been done by electrical stimulation to convey sensory information through these interfaces, pulsed stimulation at several frequencies often evokes ‘electrical’ sensations, in part since stimulation parameters differ from those evoked

by motor intent of selective cutaneous stimulation. In fact, effectively distinguishing single action potentials of sensory sub-modalities at the interface is challenging, as principal component analysis shows overlapping unit clusters due to the biophysical similarity of myelinated B-fibers. Injury, regeneration and remyelination affects nerve fibers, further complicating the task in regenerating peripheral interfaces. We reasoned that understanding the type of motor and sensory units recorded by the REMI weeks after implanting will reveal changes in the single-unit waveforms, evoked by selective afferent and efferent stimuli, that can inform about the modality and status of axon fibers. This work aims to improve the accuracy of identifying evoked units in the peripheral nervous system. Here, we analyzed neural spike data recorded by indwelling and REMI electrodes implanted in adult Lewis rat sural and tibial nerves and evoked by von Frey filaments or soft touch. Neural signals were recorded acutely using a Plexon Inc system at 40kHz sampling rate and analyzed using the spike sorting software Offline Sorter (Plexon Inc). Complete waveforms were detected using dual thresholding. We observed that mechanoceptive units have similar waveform characteristics such as amplitude and latency despite the touch modality or stimuli strength, thus revealing a degree of consistency in some cutaneous afferent signals. Analysis of motor and thermally evoked units will be evaluated to test if such characteristics are unique to mechanoceptive cutaneous signals. Together, this data suggests signal recognition by REMI electrodes can be used to inform biologically inspired stimulation parameters, which may offer more naturalistic sensations to amputees using sensorized prosthetic devices. Funding: NIH NINDS 1R01NS124222-01

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Poster

389. Neurophysiology: Decoding and Neural Processing I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 389.04

Topic: E.05. Brain-Machine Interface

Title: Decoding Motor Imagery of three-dimensional Random Movements Using Electrocorticographic Signals in Acute Training Setting

Authors: *Y. YANG[1], S. RYUN[2], J. KIM[2], C. CHUNG[3];
[1]Seoul Natl. Univ., [2]Seoul Natl. Univ., Seoul, Korea, Republic of; [3]Seoul Natl. Univ. Hosp., Seoul Natl. Univesity, Seoul, Korea, Republic of

Abstract: Background: Decoding movement-related signals from widespread brain areas may be advantageous given that diverse brain areas which related to movement are activated for complex movements and imagined movements [1]. Electrocorticography (ECoG) is a beneficial option in that it covers wide brain areas with a high signal-to-noise ratio [2]. Here, we used low (0.5-8Hz) and high-gamma (70-100Hz) frequency band to decode human reach-and-grasp movements and imagined movements. Method: Six-teen epileptic patients with intracranial electrodes were asked to execute and imagine reach-and-grasp movements to random targets

with the training of 8.2±3.4 days. A multiple linear regression algorithm was used to estimate the offline prediction of movement and imagined movement trajectories. We used temporal neural siganls which showed high performance when using low-frequency data for decoding. Also, when motor execution (ME) signal decoding, we also used spectral data. The spectral data of high-gamma band showed high performance for the two subjects. Result: The three-dimensional reaching trajectories were decoded in ME and motor imagery (MI) tasks when using temporal low-frequency signal. The mean correlation coefficients of low-frequency filtered data for the x-axis, y-axis, and z-axis were correspondingly 0.57, 0.51, and 0.57 for the ME and 0.53, 0.25, and 0.37 for the MI. Moreover, the decoding performance of spectral data showed higher performance than the decoding performance of temporal data for two subjects (S7, S10). The decoding perforamance was 0.24, 0.49, and 0.56 for ME signals. Thus, we can conclude that high-gamma activity has information related to movement decoding. Conclusion: MI could be decoded in subjects who were acutely trained with acceptable performance when given a random target presentation task. Also, spectral activity of high-gamma frequency showed high decoding perforamnce. Wide covering ECoG BMI might be practical in decoding movements with multiple degrees of freedom in humans.
Reference[1] Nicolelis, M. A. & Lebedev, M. A. Principles of neural ensemble physiology underlying the operation of brain-machine interfaces. *Nat. Rev. Neurosci.* **10**, 530-540. [2] Agrita Dubey et al, Cortical Electrocorticogram (ECoG) is a local signal: J. Neurosci 2019; 10.1523/JNEUROSCI.2917-18.2019

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Poster

389. Neurophysiology: Decoding and Neural Processing I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 389.05

Topic: E.05. Brain-Machine Interface

Support: This study was conducted as part of Global Singularity Research Program for 2022 financially supported by KAIST.

Title: Enhancing EEG-based hand movement trajectory decoding by using an ECoG-informed EEG feature extraction strategy

Authors: *S. JANG[1], J. JEONG[2];
[1]Korea Advanced Inst. in Sci. and Technol., Daejeon, Korea, Republic of; [2]KAIST, Daejeon, Korea, Republic of

Abstract: Hand movement trajectory decoding using human neural signals is one of the key issues in brain-machine interface (BMI). The performance of BMIs largely depends on the choice of neural recording techniques, which include invasive methods such as the electrocorticogram (ECoG), and non-invasive methods such as the electroencephalogram (EEG).

The relatively poor spatial resolution of EEG is often pointed out as the culprit of lower decoding accuracies in trajectory decoding compared to when using ECoG. Considering the similar representation of temporal neural dynamics in EEG and ECoG however, we sought to understand the degree of spatiotemporal congruence between EEG and ECoG feature distribution obtained during hand movement. We hypothesized that the lead field model of the head tissue layer behaves as a linear operator to map a set of local ECoG activities to a composite signal that manifests in the form of EEG. To this end, we conducted an EEG experiment which was designed identical to a preceding ECoG experiment that instructed subjects to perform both real and imagined hand movement trajectories in three-dimensional space. We found that spatial distribution of movement-related neural features was different between the EEG and ECoG. More than half (≈53.3%) of the significant features for real movement originated from the sensorimotor cortex in ECoG, whereas for the EEG the proportion was lower (≈44%). A substantial proportion of movement-related neural features also originated from the superior temporal gyrus (≈32%) for the EEG, whereas a lower involvement of the same area was observed in ECoG (≈13.3%). Average amount of movement-related information was also significantly different between the two signal types (EEG: 0.1794±0.0011, ECoG: 0.1877±0.0014; student's t-test $p<1e-5$). In addition, these features were used for trajectory decoding, which resulted in correlation coefficients of ≈0.3855 and ≈0.4996 for the EEG- and ECoG-based decoding respectively. Finally, we selected features that were highly preserved across the two techniques for developing an EEG-based BMI for robotic arm end-point velocity control. Quadrant hit success rates ranged from 30 to 40%, which was significantly above both the chance level (≈25%) and when feature selection was not informed by cross-comparing EEG features to those of ECoG. These findings suggest that non-invasive trajectory decoding can be enhanced by extending feature extraction strategy to select for neural features that are consistent across recording techniques rather than just extracting only those that are specific to non-invasive recording.

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

Poster

389. Neurophysiology: Decoding and Neural Processing I

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 389.06

Topic: E.05. Brain-Machine Interface

Support: NS053603

Title: Transferring intracortical brain-computer interface decoders across users

Authors: *F. RIZZOGLIO[1], E. L. ALTAN[1], B. DEKLEVA[4], E. J. PERRAULT[2,3,5], A. KENNEDY[1], L. E. MILLER[1,2,3,5];
[1]Neurosci., Northwestern Univ., Chicago, IL; [2]Biomed. Engin., Northwestern Univ., Evanston, IL; [3]Physical Med. and Rehabil., Northwestern Univ., Chicago, IL; [4]Rehab Neural Engin. Labs,

Dept. of Physical Med. and Rehabil., Univ. of Pittsburgh, Pittsburgh, PA; [5]Shirley Ryan Ability Lab., Chicago, IL

Abstract: Intracortical brain-computer interfaces (iBCIs) are a promising technology now being used to restore voluntary movement to paralyzed persons by converting neural signals into control commands to operate external devices or to reanimate limbs. In a typical iBCI, the brain-to-behavior mapping (i.e., the iBCI decoder) is initialized by a supervised calibration procedure which creates a map based on intended actions and corresponding neural activities. While calibrating a decoder for closed-loop cursor control may take only few minutes, the calibration process inevitably becomes more time consuming for more complicated tasks such as those that aim at directly reanimating paralyzed limbs via functional electrical stimulation. Here we propose to facilitate the decoder calibrations for a clinically viable iBCI by exploiting neural representations of motor behaviors that may be preserved across individuals in the form of low-dimensional manifolds. If the latent neural representations across individuals are similar, it may be possible to transfer the iBCI decoder from one monkey to another, or even from a monkey, whose data are easy to obtain, to a paralyzed person. The objective of this study was to develop a framework for cross-subject transfer learning. Building on recent studies showing that the temporal patterns within the neural manifolds can be "aligned" to reveal similar structure over time, our approach was to align the latent neural dynamics across subjects and to evaluate the performance of this cross-subject decoding. We trained three monkeys to perform a center-out task requiring isometric torques to be produced about the wrist. We recorded neural signals from the hand area of the primary motor cortex (M1) together with intramuscular electromyogram (EMG) signals from several forearm and hand muscles. We used Canonical Correlation Analysis (CCA) to find the linear transformations to make the M1 latent dynamics of one monkey maximally correlated to those of another. After training an iBCI decoder to predict the EMG activity for one monkey, we showed that we can retain up to 85% of the EMG decoding accuracy by feeding such decoder with the CCA-aligned latent dynamics of a different monkey. Remarkably, we even were able to feed the monkey decoder with aligned latent dynamics of a paralyzed person attempting to perform the same task and retain up to 60% of the original EMG decoding accuracy. Our findings suggest that consistent representations of motor activity exist across animals and even across species. Discovering this common representation is a crucial first step in designing generalizable iBCI decoders that perform well without subject-specific tuning.

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Poster

389. Neurophysiology: Decoding and Neural Processing I

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Title: Different neural features between the initial and corrective phases of precise reaching identified with AutoLFADS

Authors: *W. LEE[1], B. M. KARPOWICZ[2], C. PANDARINATH[2], A. G. ROUSE[1];
[1]Univ. of Kansas Med. Ctr., Univ. of Kansas Med. Ctr., kansas city, KS; [2]Emory Univ., Atlanta, GA

Abstract: During precision reaching to small targets, initial movements often fail to acquire a target, and subjects make corrective submovements after a low-speed period. In a traditional velocity-encoding model of neural activity, one would expect activity to return to a baseline state during the low-speed period and then encode the smaller corrective submovements with similar tuning but smaller amplitude changes in firing rate. However, the temporal dynamics of the neural population may instead be dependent on the initial reach and not return to a common baseline state. Additionally, given the high dimensionality of the neural population, corrective movements may not be encoded as smaller initial movements but instead have distinct neural features.To examine neural encoding of submovements, we trained two monkeys to perform a precision reaching task with a joystick and recorded multiple sessions using floating microelectrode arrays in primary motor cortex (M1). The small reaching targets induced self-generated errors and unique per-trial corrections, making traditional trial averaging difficult. We used latent factor analysis via dynamical systems with automated hyperparameter tuning (AutoLFADS) with “stitching” to infer a common latent factor space across sessions. This approach allowed us to aggregate data across sessions while providing denoised single trials for analysis.We decoded velocities via linear regression and found that LFADS-inferred latent factors were substantially more informative than smoothed spikes; however, prediction of corrective submovements was worse than for initial. Additionally, decoders trained to predict initial submovements failed to generalize to corrective submovements, suggesting differences in neural encoding between these submovement types.To examine similarities in neural encoding between submovements, we clustered the LFADS-inferred neural factors. For both initial or corrective submovements, neural activity was most similar for movements in similar reach directions. However, corrective submovements occupied a different domain of the latent neural space than initial. Additionally, corrective submovements did not have a single origin point in neural space, but instead had a small number of separate groupings based on hand position.Our results highlight the distinct properties of neural population activity across corrective and initial submovements, which provide a challenge for models of neural tuning and for decoders that generalize across movement types.

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Poster

389. Neurophysiology: Decoding and Neural Processing I

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Program #/Poster #: 389.08

Topic: E.05. Brain-Machine Interface

Support: H2020-EIC-FETPROACT-2019-951910-MAIA

Title: Robust decoding of neural activity from macaque parietal areas during arm reaching

Authors: *F. E. VACCARI[1], S. DIOMEDI[1], M. FILIPPINI[1,2], P. FATTORI[1,2];
[1]Dept. Biomed. and Neuromotor Sci., [2]Alma Mater Res. Inst. for Human-Centered Artificial Intelligence, Univ. of Bologna, Bologna, Italy

Abstract: In the BMI field, the parietal region has been proposed as an alternative source of neural signals to the more studied motor cortices (Andersen et al., 2019), but it has been much less studied for this purpose. Thus, considering also the great heterogeneity of the areas that it comprises, to find the area most suited for real applications is not a trivial problem. Moreover, given the wide range of algorithms available to solve decoding problems (Glaser et al., 2020), comparative results can help researchers to achieve the required performance. For these reasons, we enriched our previous findings about neural states and decoding in parietal cortex (Diomedi et al., 2021; Vaccari et al., 2021). Specifically, we compared the performance of the Hidden Markov Model (HMM) with two more common classifiers, Support Vector Machine (SVM) and LSTM neural network, that represent the state-of-art respectively among classic machine learning methods and deep learning. We analyzed the activity recorded from medial parietal areas PE (monkey1: 42 units, monkey2: 88), PEc (monkey1: 93, monkey2: 83) and V6A (monkey1: 104, monkey2: 105) of 2 macaques while the animals performed a foveated delayed reaching task. Task phase could be decoded from V6A and PEc with similar, high accuracy, whereas this information was much less available in PE (-20% with respect to V6A/PEc). The performance for target decoding showed a decreasing trend (V6A > PEc > PE, overall accuracy: 83, 71 and 38% respectively). Comparing the various algorithms, we found that SVM performed as well as LSTM for task phase, overcoming HMM (SVM ≈ LSTM > HMM; accuracy: 91, 91 and 78%, respectively), and SVM outperformed both LSTM and HMM in target decoding (SVM > LSTM ≈ HMM; accuracy: 69, 62 and 62%, respectively). HMM resulted significantly more robust than SVM and LSTM when decoding neural data after removing of units without re-training (neuron loss). Indeed, when 32 units were removed from each initial population, overall HMM outperformed SVM of ≈9% and LSTM by ≈2%. In conclusion, we found that the differences in decoding performance between areas were invariant to the algorithm used, thus V6A/PEc areas emerged to be a preferable source of neural activity with respect to PE for BMI applications. Regarding the algorithms here tested, SVM has proved to be the best algorithm in terms of pure performance, but an HMM-based classifier HMM has interesting strengths such as the robustness to neuronal loss and its high level of model explainability. The performance of LSTM, while still remarkable, did not clearly outperform the other methods as expected, probably suffering from an architecture not fully optimized and a limited amount of training data.

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Poster

389. Neurophysiology: Decoding and Neural Processing I

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Topic: E.05. Brain-Machine Interface

Support: post-doctoral fellowship for basic scientists (PF-PDF-1898)
Medical Research Foundation of Oregon New Investigator Grant

Title: Gaussian Modelled Beta Power in the Cortex Characterises Ageing, but not Parkinson's Disease

Authors: *A. KAREKAL[1], S. STUART[2], M. MANCINI[3], N. C. SWANN[1];
[1]Univ. of Oregon, Eugene, OR; [2]Northumbria Univ., Northumbria University, United Kingdom; [3]Oregon Hlth. and Sci. Univ., Portland, OR

Abstract: Ageing is a key risk factor for the progression of Parkinson's Disease (PD). PD is characterised by excessive synchrony of beta oscillations (13-30 Hz) in the basal ganglia thalamo-cortical network (Little & Brown, 2014). In humans, this manifests as elevated beta power in basal ganglia. However, cortical beta power is not reliably elevated in individuals with PD.We sought to disentangle how resting cortical beta power compares in populations of 21 older controls,15 younger controls, and in 41 individuals with PD (On levodopa) using a 32-channels scalp electroencephalogram (EEG) and a novel method of calculating beta power. Specifically, beta power is usually determined using conventional methods such as Fourier transforms that do not separate the periodic from aperiodic components. These components likely reflect different physiological phenomena and are indistinguishable in conventional analyses. To address these limitations, we used a Gaussian modelled beta power (Donoghue et al., 2020) to determine if sensorimotor beta power distinguishes healthy young from old subjects and from subjects with PD. Additionally, we looked at the distribution of beta power across the entire cortex. Our findings showed that Gaussian modelled beta power does not differentiate individuals with PD from the healthy young or old controls in the sensorimotor cortex.In addition, beta power was higher in healthy older versus younger controls. This effect was most pronounced in regions near the sensorimotor cortex including frontal and parietal areas where Gaussian beta power was significantly higher in healthy old controls as compared to the young controls ($p<0.05$, FDR corrected). In the rest of the cortex, beta power in the older controls was numerically higher, but did not surpass FDR corrected significance. Additionally, the aperiodic component, exponent was higher in PD than in younger controls in the right distal parietal region ($p<0.05$, FDR corrected).Our findings suggest that cortical Gaussian modelled beta power is modulated by age. These results should be further explored in longitudinal studies to determine if further increase in the periodic and aperiodic beta component could lead to pre-Parkinsonian state or other motor disabilities.

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Poster

389. Neurophysiology: Decoding and Neural Processing I

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Topic: E.05. Brain-Machine Interface

Support: EU GE-2-2-023A (REXO)
EU IT-2-2-023 (VAFES)

Title: Evaluation of occurrence to detection ratio of ErrPs for a closed-loop adaptation of BMIs utilizing reinforcement learning

Authors: *A. XAVIER FIDÊNCIO[1,2,3], C. KLAES[2], I. IOSSIFIDIS[3];
[1]Ruhr-University Bochum, Bochum, Germany; [2]Neurosurg., Knappschaftskrankenhaus Bochum, Bochum, Germany; [3]Inst. of Computer Sci., Ruhr West Univ. of Applied Sci., Mülheim an der Ruhr, Germany

Abstract: Neurorehabilitation devices can be used to help patients restore the lost mobility of upper-body limbs caused, e.g., by a spinal cord injury or a stroke. The rehabilitation sessions can require calibration of the device and the presence of a professional therapist to handle the system, which has motivated the search for systems that, by overcoming these limitations, could adapt to the individual patient's intentions and needs. Research on brain-machine interfaces (BMIs) has shown increased interest in integrating the so-called error-related potentials (ErrPs) to improve system performance. ErrPs reflect the event-related potentials associated with error processing and performance monitoring in the human brain and are known to be elicited as a response to self-made and also to external errors committed, for example, by the BMI. As they are implicitly generated and do not impose any extra workload on the subject, they provide an intrinsic and natural source of feedback for the development of adaptive BMIs. While error signals are an important source of additional and relevant information for BMIs, their effective use depends on their accurate detection, which is a limitation in the existing ErrP-based BMIs. We have designed a study to evaluate the occurrence of interaction ErrPs, expected during human-machine interactions when the BMI deliberately misinterprets the user's intention and executes the wrong command. Using a cursor control task in the form of a simple keyboard-controlled game, we have recorded data from 6 healthy subjects using a dry EEG system with 32 electrodes. Given the difficulties in obtaining a clear ErrP signal for all subjects in previous experiments, we included a recording with a gel-based EEG to evaluate whether the low signal-to-noise ratio of the dry system was an issue. The comparison shows that measured ErrPs displayed a similar waveshape in terms of observed peaks as expected, but differences in latencies and amplitude are visible. The single-subject analysis shows that while undergoing the same experimental task, a prominent ErrP is not clearly detectable for all subjects. This observation suggests that it is not entirely clear what the ratio between the occurrence of a prominent ErrP and its detection is.

From the learning perspective, we evaluate the boundary conditions for effective ErrP detection to establish the minimum threshold for the error classification to drive learning and adaptation in BMI systems.

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Poster

389. Neurophysiology: Decoding and Neural Processing I

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Topic: E.05. Brain-Machine Interface

Support: NIH Grant 5R01NS112535-03

Title: Aligning Neural Activity Recorded from Rats during Locomotion Across Time And Subjects

Authors: ***D. BASRAI**[1], G. ENGBERSON[1], D. SONG[1], L. E. MILLER[2], M. C. TRESCH[3]; [2]Physiol., [3]Biomed. Eng, Physical Med. and Rehab, Physiol., [1]Northwestern Univ., Chicago, IL

Abstract: Neural decoders can be trained to predict behavior based on the firing rates of recorded neurons. Trained decoders can be used to drive a brain-computer interface (BCI) to restore voluntary movements after spinal cord injury. However, because these decoders learn the co-variation patterns of the specific neurons the decoder was trained on, they are not robust to changes in the neurons being recorded. Their performance worsens as neurons turn over in time due to movement in implanted probes, and completely fails when applied to a different animal with a different set of recorded neurons. Retraining a decoder from scratch requires a significant amount of new training data, which can be difficult to generate post spinal cord injury. Work in non-human primates has suggested that the latent dynamics underlying neural population activity are preserved across time. (Gallego et. al 2020) These latent dynamics can be 'aligned' from a new dataset back to the original dataset the decoder was trained on, allowing the decoder to remain predictive of behavior, even if the new dataset is recorded from a different set of neurons. Here, we evaluate whether this process of neural alignment can allow a single decoder to be predictive of behavior across weeks in the same animal and when applied to a different animal. To do so, we implant 32-channel intracortical electrodes in the hindlimb representation of sensorimotor cortex of rats. We simultaneously record multi-unit neural activity and kinematics during treadmill locomotion over several weeks. We then apply canonical-correlation analysis (CCA) to align neural recordings across different times and subjects, and evaluate whether the decoder remains predictive of joint angles. Preliminary results show that the decoder remains predictive when applied to aligned neural data from the same animal recorded weeks later, as well as when applied to aligned neural recordings from a different animal. We are now examining the robustness of these results across data sets, including applying this approach to cortical data from spinal cord injured rats. If successful, this work could eliminate the need to

retrain decoders for BCIs that use cortical activity to drive stimulation of paralyzed muscles, enabling restoration of voluntary movement after spinal cord injury.

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Poster

389. Neurophysiology: Decoding and Neural Processing I

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Program #/Poster #: 389.12

Topic: E.05. Brain-Machine Interface

Support: William K. Bowes, Jr. Foundation

Title: A speech neuroprosthesis for generalizable spelling in a person with severe paralysis and anarthria

Authors: *S. L. METZGER[1], J. R. LIU[1], D. A. MOSES[2], M. DOUGHERTY[2], M. P. SEATON[2], K. T. LITTLEJOHN[3], J. CHARTIER[2], G. K. ANUMANCHIPALLI[3], A. TU-CHAN[2], K. L. GANGULY[2], E. F. CHANG[2];
[1]Grad. Program in Bioengineering, Univ. of California, Berkeley - Univ. of California, San Francisco, Berkeley, CA; [2]Univ. of California, San Francisco, San Francisco, CA; [3]Univ. of California, Berkeley, Berkeley, CA

Abstract: Many individuals who suffer from severe paralysis are unable to speak or type, hindering their ability to interact with their surroundings and communicate with others and thereby reducing quality of life. Brain-computer interfaces (BCIs) offer a promising pathway to restoration of communication. Researchers have shown that BCIs can decode visually evoked responses and attempted hand and arm movements into letters, allowing a paralyzed user to spell out intended messages without making overt movements, and allowing for flexible communication using large vocabularies. However, it is unclear if attempts to speak can be used to control a spelling BCI, which could afford benefits over other control modalities due to the relative naturalness and efficiency of speech.
Here, we developed a speech-based spelling BCI with a clinical-trial participant that has severe paralysis and anarthria (loss of the ability to articulate speech). As the participant attempted to silently say code words representing the 26 English letters (“alpha” for “a”, “bravo” for “b”, and so forth), we directly recorded neural signals from a 128-channel, high-density electrocorticography (ECoG) array implanted over the surface of his speech-motor cortex. Using deep artificial neural networks, we classified the attempted letter from the associated neural activity with a median single-trial classification accuracy of 54.2%. Using natural-language models and a 1,152-word vocabulary, we decoded prompted and freeform sentences that the participant attempted to silently spell in real time, with a median character error rate (CER) of 6.13% and speed of 29.4 characters per minute. By leveraging the broad spatial coverage

afforded by our custom ECoG array, which covers a portion of the hand knob area, the participant was able to use an attempted hand movement to volitionally disengage the spelling procedure. In offline analyses, we characterized the decoding contributions from low- and high-frequency signal components, compared the neural representations observed during silent and overt speech attempts, assessed the impact that using code words (instead of letters) as speech targets had on decoding performance, and validated simulated spelling performance with larger vocabulary sizes (achieving a median CER of 8.25% with over 9,000 words).
These results demonstrate the clinical viability of a spelling neuroprosthesis controlled by silent attempts to speak for real-time sentence generation, complementing our previous full-word decoding approaches and further highlighting the long-term efficacy of ECoG-based neural interfaces.

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Poster

389. Neurophysiology: Decoding and Neural Processing I

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Program #/Poster #: 389.13

Topic: E.05. Brain-Machine Interface

Support: NIH Grant UH3NS107714

Title: Restoring control of grasp force with a bidirectional intracortical brain-computer interface

Authors: *N. W. BRANTLY[1,2,7], B. DEKLEVA[3,2], E. V. OKOROKOVA[8], N. SHELCHKOVA[8], A. R. SOBINOV[9], A. MONSCHEUER[2,12], V. KARAPETYAN[4,2], P. WARNKE[10], J. A. GONZÁLEZ-MARTÍNEZ[5], L. E. MILLER[13,16,14,15,17], R. A. GAUNT[3,2,4,7], N. G. HATSOPOULOS[9,8,11], M. BONINGER[2,4,6], J. E. DOWNEY[9], S. J. BENSMAIA[9,8,11], J. L. COLLINGER[6,2,4,7];
[1]Bioengineering, [2]Rehab Neural Engin. Labs, [3]Physical Med. and Rehabil., [4]Dept. of Bioengineering, [5]Dept. of Neurosurg., [6]Dept. of Physical Med. and Rehabil., Univ. of Pittsburgh, Pittsburgh, PA; [7]Ctr. for the Neural Basis of Cognition, Pittsburgh, PA; [8]Committee on Computat. Neurosci., [9]Dept. of Organismal Biol. and Anat., [10]Dept. of Neurolog. Surgery,

[11]Neurosci. Inst., Univ. of Chicago, Chicago, IL; [12]Fac. of Psychology and Neurosci., Maastricht Univ., Maastricht, Netherlands; [13]Physiol., Northwestern Univ., Chicago, IL; [14]Dept. of Biomed. Engin., Northwestern Univ., Evanston, IL; [15]Dept. of Physical Med. and Rehabil., Northwestern Univ., Chicago, IL; [16]Dept. of Neurosci., Feinberg Sch. of Med., Chicago, IL; [17]Shirley Ryan AbilityLab, Chicago, IL

Abstract: Intracortical brain-computer interfaces (iBCIs) are being developed to restore arm and hand function for people with paralysis. While kinematic control of the hand has been demonstrated, the ability to modulate grasp force has been limited. To address this challenge, our group is conducting a multisite study with three human participants with tetraplegia, who each have two microelectrode arrays implanted in the arm/hand area of motor cortex and two arrays in the hand area of somatosensory cortex. We asked them to attempt to grasp a cylinder in a virtual reality (VR) environment using either a gentle or firm force to identify the neural population correlates of grasp force. We have consistently identified three prominent neural responses: an onset transient, a sustained response during hold, and an offset (i.e., release) transient component. This result inspired the development of a grasp state classifier to close the hand with a specified force when there is a high probability of grasp. We assessed decoder performance on a two-force grasp force adjustment task in a VR environment with and without visual feedback of force. The task includes four trial types: adjust up, adjust down, maintain low, and maintain high. All three participants used the decoder with continuous visual feedback of force (P2 - 77% successful, P3 - 98%, C1 - 93%). Two participants completed the task without force feedback, while the third was unable to do so, likely because the arrays are more in the arm area of motor cortex, and because signal quality has declined over the 7 years since implantation. Next, we attempted online grasp force decoding while providing tactile feedback using amplitude-modulated intracortical microstimulation (ICMS) of a single somatosensory electrode and discovered that task performance deteriorated for the two participants in which it was tested. This was unexpected since we previously demonstrated in one participant a substantial increase in reach and grasp task performance when ICMS feedback was provided with a kinematically-controlled iBCI. This reduction in performance was likely due to stimulation-induced changes in motor cortical activity, as many channels were inhibited or had a reduced difference in firing rate between low and high force conditions. Our team is currently conducting a systematic investigation of how ICMS in somatosensory cortex influences motor cortical activity in various behavioral contexts so that we may optimally design a neural decoder.

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Poster

389. Neurophysiology: Decoding and Neural Processing I

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Topic: E.05. Brain-Machine Interface

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NIH R01 HD071686
NSF CAREER award IOS1553252

Title: Sensory constraints on volitional modulation of motor cortex

Authors: *C. FERNANDEZ FISAC, S. M. CHASE;
Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Voluntary movement is driven by the primary motor cortex (M1), and individuals can learn to modulate even single neurons at will. Indeed, the fact that neural activity in M1 can be volitionally controlled makes it a powerful target for brain-computer interface (BCI) devices and their clinical applications. Yet M1 also encodes non-volitional information: it receives pronounced sensory inputs and contributes to sensory-driven motor responses. What does this duality in M1 imply for volitional control? To what extent do non-volitional signals restrict voluntary modulation of M1?

To answer this, we trained three macaque monkeys in a BCI paradigm that decoupled volitional modulation from specific aspects of sensory feedback. In the task, the firing rate of a single neuron—termed the *command neuron*—directly determined the position of a computer cursor along a one-dimensional axis. Altering the orientation and location of this movement axis created distinct sensory contexts for the subject without changing the neural requirements of the task. We leveraged this paradigm to assess monkeys’ ability to modulate individual neurons in M1 under different sensory contexts.

We found that sensory context persistently affected volitional control of single neurons in M1. For all three subjects, the ability to perform the task significantly changed based on movement orientation: rotating the feedback axis could render the same neural task effortless or problematic. Axis location within the workspace also affected the ability to modulate individual neurons, albeit to a lesser extent than orientation. Notably, the disparity in single-neuron control across sensory contexts was not resolved even after extended training in the task. We found that additional practice within a session or across multiple days was not sufficient to erase the interaction between movement orientation and the ability to voluntarily modulate individual neurons.

Our findings suggest that sensory context can limit the degree to which M1 activity is under volitional control. This interplay between sensory and volitional signals, which manifested at the level of individual neurons within M1, may constitute an additional constraint on motor learning. The notion that sensory inputs may impose bounds on the voluntary modulation of M1 could also have direct implications for the development of clinical neuroprosthetic devices.

Disclosures: C. Fernandez Fisac: None. **S.M. Chase:** None.

Poster

389. Neurophysiology: Decoding and Neural Processing I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 389.15

Topic: E.05. Brain-Machine Interface

Support: NSF award #1910526

Title: A comparison between different emotion elicitation stimuli in human emotion recognition using EEG signals

Authors: *N. KHAN, T. BRANDT, D. E. THOMPSON;
Electrical and Computer Engin., Kansas State Univ., Manhattan, KS

Abstract: Emotion recognition using electroencephalogram (EEG) is trending in neuroscience. EEG-based emotion estimation typically includes eliciting emotions through various stimuli. Here, we compare the effectiveness of different types of stimuli in creating a detectable affective state. We used 3 types of stimuli (pictures, facial images, and music) from IAPS, POFA, and DEAM [1-3]. Eight people experienced 240 stimuli and rated each stimulus 1-5 on 3 emotional axes (valence, arousal, and dominance). We performed binary classification (threshold at 3) in each emotional axis. We used magnitude squared coherence estimation (MSCE) as our feature, t-tests for feature reduction, Synthetic Minority Oversampling Technique (SMOTE) to reduce class imbalance, and a 3-layer neural network for classification. The SMOTE rate was 1, effectively doubling the minority class. Performance was evaluated using 5-fold cross-validation. For final comparisons, we used balanced accuracy (ACC_b) and its 95% credible intervals ($\alpha=0.05$) [4]. The results are in the figure. The highest mean ACC_b was achieved with music stimuli for the valence axis, with p-value 0.0078 (<0.05) between IAPS and music stimuli (Wilcoxon rank-sum). POFA and IAPS were statistically similar, although the POFA had severe class imbalance issues on the dominance axis. Finally, performance on all three axes is significantly greater than chance for all 3 types of stimuli.
References:1. Lang, P.J., Bradley, M.M., & Cuthbert, B.N. (2008). International affective picture system (IAPS): Affective ratings of pictures and instruction manual. Technical Report A-8.2. Ekman, P. and Friesen, W.V. (1976) Pictures of Facial Affect. Consulting Psychologists Press, Palo Alto, CA.3. A. Aljanaki, Y.-H. Yang, and M. Soleymani, “Developing a benchmark for emotional analysis of music,” *PLoS One*, vol. 12, no. 3, p. e0173392, 2017.4. K. H. Brodersen, C. S. Ong, K. E. Stephan, and J. M. Buhmann, “The balanced accuracy and its posterior distribution,” in *2010 20th International Conference on Pattern Recognition*, 2010, pp. 3121–3124.

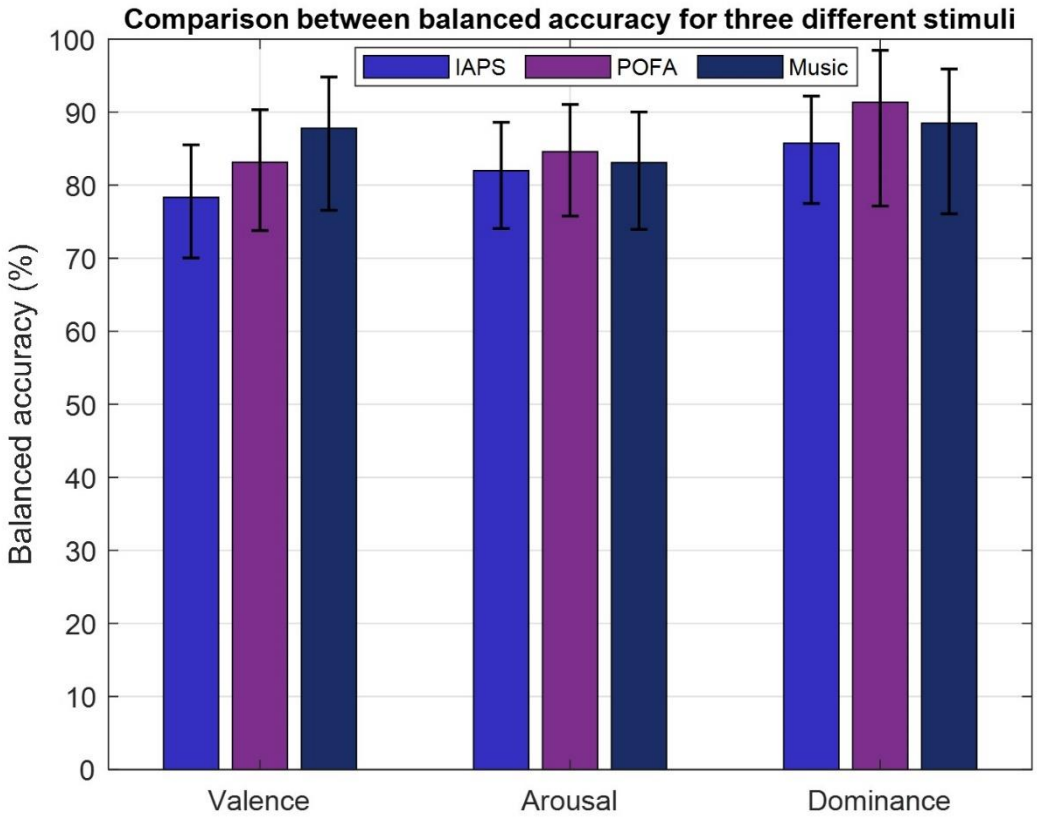

Figure 1. Mean balanced accuracy and credible intervals for three stimulus types.

Disclosures: **N. Khan:** None. **T. Brandt:** None. **D.E. Thompson:** None.

Poster

389. Neurophysiology: Decoding and Neural Processing I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 389.16

Topic: E.05. Brain-Machine Interface

Support: GE-2-2-023A (REXO)
IT-2-2-023 (VAFES)

Title: Contranet: a single end-to-end hybrid network for eeg-based and emg-based human machine interfaces

Authors: *O. ALI[1], M. SAIF-UR-REHMAN[2], T. GLASMACHERS[3], I. IOSSIFIDIS[2], C. KLAES[1];
[1]Dept. of Neurosurg., Knappschaftskrankenhaus Bochum, Bochum, Germany; [2]Dept. of Computer Sci., Ruhr-West Univ. of Applied Sci., Mülheim an der Ruhr, Germany; [3]Inst. für Neuroinformatik, Ruhr Univ. Bochum, Bochum, Germany

Abstract: Objective: Electroencephalography (EEG) and electromyography (EMG) are two non-invasive bio-signals, which are widely used in human machine interface (HMI) technologies (EEG-HMI and EMG-HMI paradigm) for the rehabilitation of physically disabled people. Successful decoding of EEG and EMG signals into respective control command is a pivotal step in the rehabilitation process. Recently, several Convolutional neural networks (CNNs) based architectures are proposed that directly map the raw time-series (EEG and EMG signal) into decision space (intended action of the user). Since CNNs are end-to-end learning algorithms, the process of meaningful features extraction and classification are performed simultaneously. However, these networks are tailored to the learn the expected characteristics of the given bio-signal. Henceforth, the implication of these algorithms is usually limited to single HMI paradigm. In this work, we addressed the question that can we build a single architecture which is capable of learning distinct features from different HMI paradigms and still successfully classify them. **Approach:** In this work, we introduce a single hybrid model called ConTraNet, which is based on CNN and Transformer architectures that is equally useful for EEG-HMI and EMG-HMI paradigms. ConTraNet uses CNN block to introduce inductive bias in the model and learn local dependencies, whereas the Transformer block uses the self-attention mechanism to learn the long-range or global dependencies in the signal, which are crucial for the classification of EEG and EMG signals. **Main results:** We evaluated and compared the ConTraNet with state-of-the-art methods on three publicly available datasets (BCI Competition IV dataset 2b, Physionet MI-EEG dataset, Mendeley sEMG dataset) which belong to EEG-HMI and EMG-HMI paradigms. ConTraNet outperformed its counterparts in all the different category tasks (2-class, 3-class, 4-class, and 10-class decoding tasks). **Significance:** Most HMI studies introduce the algorithms that are tailored to the characteristics of its expected bio-signal and validate their results on the dataset/s, which belong to only single paradigm. Contrarily, we introduced ConTraNet and validated the results on two different HMI paradigms, which contain the data of 2, 3, 4 and 10-classes. Furthermore, the generalization quality of ConTraNet remains equally good for both paradigms, which suggest that ConTraNet is robust to learn distinct features from different HMI paradigms and generalizes well as compared to the current state of the art algorithms.

Disclosures: O. Ali: None. **M. Saif-ur-Rehman:** None. **T. Glasmachers:** None. **I. Iossifidis:** None. **C. Klaes:** None.

Poster

389. Neurophysiology: Decoding and Neural Processing I

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Program #/Poster #: 389.17

Topic: E.05. Brain-Machine Interface

Support: H2020-EIC-FETPROACT-2019-951910-MAIA

Title: Computational Primitives in the Temporal Code of Parietal Circuits

Authors: L. PARRILLA[1], M. FILIPPINI[1,2], D. ZENDRIKOV[3], G. INDIVERI[3], *P. FATTORI[1,2];
[1]Biomed. & Neuromotor Sci., [2]Alma Mater Res. Inst. for Human-Centered Artificial Intelligence, Univ. of Bologna, Bologna, Italy; [3]Inst. of Neuroinformatics, Zurich, Swaziland

Abstract: Arm-reaching movements are orchestrated by complex activation dynamics in distributed networks of neurons which, by emitting spikes, multiplex information streams communicating through mean firing rates (rate coding) and precise spike-timing (temporal coding). Here we investigate the role of spatiotemporal dynamics of spike patterns in motor preparation by training a recurrent spiking neural network with in vivo parietal data. We demonstrate with modeling and simulation results how computational primitives as Winner Take All (WTA) networks can explain the transition from motor planning to motor actuation through increase in firing rate within γ-wave activity band.The model is trained in a staged supervised way, on a single cell recording dataset for a delayed fix to reach task performed by two Macaca Fascicularis. To demonstrate the role of precise spike timing in the temporal code of the parietal cortex, we use triplet Spike Timing Dependent Plastcity (tSTDP): a higher order Hebbian learning rule. To reduce the effect of inhomogeneity between neurons and their response to the different classes of inputs presented, we implement a synaptic scaling homeostatic mechanism. We show how this process is essential to create efficient inner representations from incomplete/unbalanced training data. We rely on biological features as homeostasis and recurrency with lateral competition to maintain robust computation within a noisy and inhomogeneous system as the modeled cortical network. We configure the model parameters such that changes in the input spike pattern distribution can produce synchronized increase in firing rates of neuronal pools tuned to a particular location in space, while maintaining sparse activity. This way, we show several parallels between our implementation and the parietal cortex such as how clustered representations in the synaptic weights can emerge from coincidence detection driven by Hebbian learning. We note how the output frequency of a single neural recurrent cluster, increases from 10-20Hz to more than 30Hz for their preferred location in space, and how this can be related to γ-wave activity due to the transition from motor planning to execution. This is the first attempt to model the temporal code of a parietal area (V6a) through recurrent spiking neural networks and canonical neural architectures such as WTA networks. These findings provide a biologically compatible computational framework to understand the transition from motor planning to motor actuation and can be directly implemented using spike-based electronic circuits in neuromorphic processors, for example to build efficient and adaptive neuroprosthetic systems.

Disclosures: L. Parrilla: None. **M. Filippini:** None. **D. Zendrikov:** None. **G. Indiveri:** None. **P. Fattori:** None.

Poster

389. Neurophysiology: Decoding and Neural Processing I

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Program #/Poster #: 389.18

Topic: E.05. Brain-Machine Interface

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NSF NCS BCS 1734916/1954107
NIH R01 MH118929
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Simons Foundation
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Title: Coordinated variability in prefrontal cortex reflects global and local neuronal processing

Authors: A. UMAKANTHA[1,2], R. C. WILLIAMSON[1,5,2], ***M. E. MCDONNELL**[1], B. M. YU[1,3,4], M. A. SMITH[1,4];
[1]Neurosci. Inst., [2]Dept. of Machine Learning, [3]Dept. of Electrical and Computer Engin., [4]Dept. of Biomed. Engin., Carnegie Mellon Univ., Pittsburgh, PA; [5]Sch. of Med., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: The activity of single neurons is variable, even across trials where sensory experiences are kept constant. Many processes can impact a neuron's activity, some of which reflect local processing (e.g., the activity of nearby neurons driven by similar inputs), others which reflect more global processing (e.g., the internal state of the brain), and finally others indicating noisy processes individual to each neuron. While all of these effects are important for facilitating behavioral outputs and achieving the correct result in a task, it is a major challenge for our field to tease apart the origins of the various influences on neuronal activity. To address this challenge, we investigated prefrontal cortex (PFC) activity in both hemispheres during a working memory task.
Specifically, we asked the following questions: 1) what aspect of neuronal variability is shared globally (across hemispheres of PFC) versus locally (within a hemisphere of PFC)? And 2) to what extent is global and local shared variability related to behavior and task encoding? To answer these questions we trained 3 male rhesus macaques to perform a memory-guided saccade task while recording from both hemispheres of PFC. We first found weak mean pairwise correlation between neurons across hemispheres. To understand the relationship of population-wide variability across hemispheres, we developed a novel dimensionality reduction technique, called pCCA-FA (probabilistic canonical correlation analysis - factor analysis). This allowed us to find dimensions of shared variance across hemispheres (global), similar to pCCA, as well as dimensions of shared variance within a hemisphere (local), similar to FA. Thus, we were able to parse neuronal variability into three different components: global, local, and independent (across neurons). Using our dual-hemisphere recordings, we found that the global shared variability of neurons was actually greater than local shared variability in both the amount as well as the dimensionality. This indicates that a surprising amount of the variability present in local groups of neurons originates from interactions with other brain regions. Finally, to see how global and

local shared variability are related to behavior and task encoding, we related each set of dimensions to the animals' pupil diameter during the task, as a proxy for arousal. We found that only the global, but not the local, shared variability strongly predicted the pupil diameter. Taken together, our results suggest that the shared variability of neurons in PFC is linked to both local computations confined to one hemisphere and, to a greater extent, global modulatory processes like arousal.

Disclosures: A. Umakantha: None. **R.C. Williamson:** None. **M.E. McDonnell:** None. **B.M. Yu:** None. **M.A. Smith:** None.

Poster

389. Neurophysiology: Decoding and Neural Processing I

Location: SDCC Halls B-H

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Program #/Poster #: 389.19

Topic: E.05. Brain-Machine Interface

Support: NSF 1533589
NEI R01EY015545
T&C Chen Brain Machine Interface Center
Caltech Division of Biology and Biological Engineering
The James G. Boswell Foundation

Title: Neural subspaces of imagined movements remain stable over several years in humans

Authors: *L. BASHFORD[1], I. ROSENTHAL[1], D. A. BJANES[1], S. KELLIS[1], K. PEJSA[1], B. LEE[2], C. LIU[2], B. W. BRUNTON[3], R. A. ANDERSEN[1];
[1]Caltech, Pasadena, CA; [2]USC, Los Angeles, CA; [3]Univ. of Washington, Seattle, WA

Abstract: Brain-Machine Interfaces (BMIs) decode neural activity and reproduce the user's intention to assist individuals with physical and neurological disabilities. However, recorded neural signals suffer from non-stationarity when recorded over days to years. This results in the degradation of BMI performance over time as the neural features originally trained change. The effects of nonstationarities in the neural signal can be mitigated. A promising solution currently being investigated is to use latent signals. Latent signals are typically derived from low-dimensional subspaces of the original high-dimensional single or multi-unit BMI recordings and have been shown to preserve information content while minimizing non-stationarity. The longitudinal stability of intracortically recorded neural activity is a challenge to the utility of decoders. The discovery of stable signals will maintain the performance of BMI devices without requiring users to frequently re-train decoders. This is especially important for individuals with degenerative diseases (e.g. amyotropic lateral sclerosis) where the loss of function over time may eventually prevent retraining.
Here we demonstrate the neural subspaces of imagined reaches in two human participants with intracortical BMIs remain stable over several years. The center-out task they performed

remained unchanged over that time and multi-unit neural activity was collected from the same arrays. To process the neural data, we first find a latent signal for each day on which the experiment occurred by performing Principal Component Analysis (PCA). We then align the latent signal for all pairs of days using Canonical Correlation Analysis (CCA). A Linear Discriminant Analysis (LDA) was then used to classify the target locations. Using the latent aligned signal, an LDA model trained on data from day 1 and tested on the data of any other day performs comparably to an LDA model that is trained and tested on data from the same day. If the raw multi-unit neural activity is used to train on day 1 and test on any other day the performance quickly falls to chance level. This result demonstrates the robust performance of latent aligned data in human neural decoding and demonstrates features of the neural signal that are stable over many years.

Disclosures: **L. Bashford:** None. **I. Rosenthal:** None. **D.A. Bjanes:** None. **S. Kellis:** None. **K. Pejsa:** None. **B. Lee:** None. **C. Liu:** None. **B.W. Brunton:** None. **R.A. Andersen:** None.

Poster

389. Neurophysiology: Decoding and Neural Processing I

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Program #/Poster #: 389.20

Topic: E.05. Brain-Machine Interface

Support: National Science Foundation under Grant #2127309 to the Computing Research Association for the CIFellows Project.

Title: Manifold Oblique Random Forests For Decoding EEG Signals Without Feature Engineering

Authors: *A. LI[1], R. PERRY[2], C. HUYNH[2], J. SHIN[2], S. S. KIM[4], J. A. GONZÁLEZ-MARTÍNEZ[5], S. SARMA[2], J. VOGELSTEIN[3];
[1]Columbia Univ., New York City, NY; [3]Johns Hopkins Univ., [2]Johns Hopkins Univ., Baltimore, MD; [4]Stonybrook Univ., Port Jefferson, NY; [5]Univ. of Pittsburg Med. Ctr., Pittsburg, PA

Abstract: Introduction: Decision forests, including random forests (RFs) and gradient boosting trees, have solidified themselves in the past couple decades as a powerful ensemble learning method in supervised settings, including both classification and regression. Artificial neural networks, such as convolutional neural networks (CNNs) on the other hand have produced astounding results when it comes to time-series and images. However, CNNs will generally require a tremendous amount of data. Manifold oblique random forests (or MORF), which are random forests imbued with the capability of learning manifolds more efficiently, analogous to CNNS. In this work, we highlight its capabilities in classification scenarios where there are low sample sizes and high dimensional structured data such as EEG time-series.
Methods: Using MORF, we analyze two intracranial EEG (iEEG) datasets: subcortical and cortical brain recordings from epilepsy 10 subjects undergoing iEEG monitoring for clinical

purposes (i) at rest and (ii) while performing a motor control task. Each dataset has a different classification task of: i) predicting up, down, left, right movements of the manipulandum, and ii) predicting surgical outcome of an epilepsy surgery (success or failure). The motor control task comprises of approximately hundreds of samples, while the epilepsy dataset comprises of ninety-one samples.
Results:MORF demonstrates superior area-under-the-curve (AUC) and Cohen's kappa performance over 5-fold cross-validation when compared to other classification models, including CNNs (pvalues < 0.05). On the motor control task, MORF was significantly (AUC=0.74 +/- 0.05) better than the CNN (AUC=0.51 +/- 0.04) and RFs (AUC=0.72 +/- 0.04) using a Wilcoxon paired sign test. When applied to predicting surgical outcomes using iEEG derived feature heatmaps, MORF outperforms CNNs and RFs in terms of Cohen's effect size (AUC=0.71 +/- 0.20), whereas CNNs perform at approximately chance level in terms of AUC (AUC=0.53 +/- 0.08). This is most likely due to overfitting due to high-dimensionality and low sample sizes.We have introduced highly optimized software for building oblique trees in scikit-learn as a Pull Request: https://github.com/scikit-learn/scikit-learn/issues/20819.
Conclusions: MORF is a new decision-tree based statistical learning model that can learn useful features derived from EEG data without significant feature engineering. With the inclusion of oblique-like trees into scikit-learn, we also plan on developing decision trees that operate over graphs, allowing us to integrate graphical methods from Bayesian statistics and causal inference.

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Poster

389. Neurophysiology: Decoding and Neural Processing I

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Program #/Poster #: 389.21

Topic: E.05. Brain-Machine Interface

Support: R01NS111982

Title: Movement fragments associated with discrete motor cortical population states are decoded with unique mappings

Authors: *R. BHATT[1], C. SPONHEIM[2,1], D. SHEETS[1], Z. GHULAM-JELANI[1], N. HATSOPOULOS[2,1];
[1]Committee on Computat. Neurosci., [2]Organismal Biol. and Anat., Univ. of Chicago, Chicago, IL

Abstract: Although the primary motor cortex (M1) is known to control movement, how exactly it produces complex movements is poorly understood. One possibility is that M1 creates complex movements using a set of elementary building blocks associated with simpler movements. Using a Hidden Markov Model, we previously showed that M1 population

dynamics transition between global states that segment complex reaching movements into accelerative and decelerative fragments of movement in particular directions (Kadmon Harpaz et al., 2018). Given these findings, we sought to determine whether the mapping between M1 activity and arm kinematics changed qualitatively between these global states. We recorded from M1 using chronically implanted, multi-electrode arrays in rhesus macaques trained to reach for a sequence of randomly positioned targets. We segmented the continuous movement at speed extrema for different directions resulting in accelerative and decelerative (i.e., accelerative polarities) fragments of different directions resulting in a total of 16 categories of fragments (i.e., 8 directions x 2 polarities). We then trained a Kalman Filter to decode hand velocity based on the neural activity (leading kinematics by 100 ms) for each category of fragments separately and tested how well each filter could predict hand velocity within and across fragment categories using cross-validation. We compared decoder performance using accelerative and decelerative fragments with two different controls where fragments were segmented based on accelerative extrema (i.e., velocity hills and valleys) and random segmentation. Using the fraction of variance accounted for (R^2) to assess decoding performance, we found that R^2 values were consistently higher within fragment categories for accelerative and decelerative fragments as compared to the two controls. Moreover, we found that while decoding of accelerative and decelerative fragments performed reasonably well within categories with positive R^2 values, decoding failed completely to generalize across categories resulting in negative R^2 values. These results suggest the presence of unique mappings between population activity in M1 and movement for clusters of movement segments associated with global population states within M1. This provides further support for the presence of discrete latent states within M1 that serve as elementary building blocks for creating complex movements.

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Poster

389. Neurophysiology: Decoding and Neural Processing I

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Program #/Poster #: 389.22

Topic: E.05. Brain-Machine Interface

Support: NINDS U01NS098969
NINDS UH3NS114439
NIDCD U01DC016686

Title: Towards Online DNN-based Classification of Syllable-level Speech from Sensorimotor Electrocorticography

Authors: ***S. LUO**[1], Q. RABBANI[3], A. BUSH[4], V. PETERSON[4], C. COOGAN[2], M. ANGRICK[2], M. RICHARDSON[4], H. HERMANSKY[3], N. E. CRONE[2];
[1]Dept. of Biomed. Engin., [2]Dept. of Neurol., The Johns Hopkins Univ. Sch. of Med., Baltimore,

MD; [3]Dept. of Electrical & Computer Engin., The Johns Hopkins Univ., Baltimore, MD; [4]Massachusetts Gen. Hosp., Massachusetts Gen. Hosp., Boston, MA

Abstract: One important objective of implantable brain computer interface (BCI) is to give subjects the freedom to control a computer system that assists them in their daily needs. A classification model based on speech commands will provide a fast and natural way to control such a system. Here, we investigate the feasibility of online classification of syllable-level neural signals using electrocorticographic data collected during an overt speech production task. We designed a real-time-ready decoding architecture that first maps neural signals into Perceptual Linear Prediction (PLP) spectrum, an intermediate acoustic representation, and then classifies the vowel and consonant components of the syllables separately from this acoustic space. Using a hybrid Time Delay Neural Network (TDNN) and Densely Connected Convolutional Network (DenseNet) model, we classified 12 syllable class plus rest using only electrodes over sensorimotor cortex. Our results indicate that jointly optimizing the loss for acoustic representation reconstruction and syllable classification produced better decoding accuracy than an identical architecture optimized for classification alone. These findings suggest that abstract PLP estimation of the spectral envelope of acoustic signals was a better intermediate representation for syllable classification than more precise acoustic representations. Based on these results, we believe PLP spectrum makes for a promising target for acoustic representation in developing more robust speech BCI systems. We hope this hybrid TDNN-DenseNet speech decoding model will provide the groundwork for an online BCI system driven by the classification of naturally produced syllabic commands.

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Poster

389. Neurophysiology: Decoding and Neural Processing I

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Program #/Poster #: 389.23

Topic: E.05. Brain-Machine Interface

Support: Dutch Science Foundation SGW-406-18-GO-086

Title: Considerations for implanting Speech BCI based on 7T fMRI

Authors: *F. D. GUERREIRO FERNANDES, Z. V. FREUDENBURG, N. F. RAMSEY, M. A. H. RAEMAEKERS;
Neurol. and Neurosurg., UMC Utrecht, Utrecht, Netherlands

Abstract: Background
Brain-Computer Interfaces (BCI's) have the potential to reinstate lost communication faculties. Results from speech decoding studies indicate that a usable speech BCI based on activity in the

sensorimotor cortex (SMC) requires subdurally implanted electrodes. However, the characteristics for a successful speech implant have yet to be investigated. We address this topic in a high-field fMRI study by assessing the decodability of spoken words based on BOLD responses in the SMC (correlating well with electrophysiology [1]), focusing on 3 characteristics: extent of coverage, implant location, depth/surface recording.
Methods
12 subjects conducted a 7T fMRI experiment in which they pronounced 6 different words over 6 runs. fMRI scans (TR = 1.4s, 1.6mm resolution) were processed using SPM12 and custom scripts. For all classification procedures, the volumetric pre-processed fMRI data of each run was mapped to the pial cortical surface. We divided the SMC into areas with equal number of vertices based on 3 metrics; sulcal depth, position along the dorsal/ventral axis, exposed/non-exposed portions of the cortex. Machine learning classification was performed on unique vertices in these SMC divisions using multiclass SVM.
Results & Discussion
(1)The results showed no preference for either sulcal or gyral portions of the SMC for word classification. Even when including sulcal information to the gyral BOLD activity, classification was not substantially facilitated. This suggests there is no essential contribution of neuronal sources deep in the sulcus, which might be difficult to measure in detail by surface electrodes due to distance. Surface electrodes may thus suffice.
(2)No hemispheric preference for classification was found, nor was bilateral decoding substantially better than unilateral decoding. These results predict no substantial differences in performance between unilateral (left vs right SMC) speech decoding, nor a substantially improvement of a bilateral decoding.
(3)Decoding was highest for the ventral 25% of SMC, presenting itself as the predicted optimal location for the implant.
(4)The SVM-searchlight analysis showed a similar pattern of classification throughout the ventral portion of the SMC, without differences between locations linked to different articulators. As no link was observed between classification performance and the topographic representations of individual articulators, the activity differentiating the speech activity of different words is assumed to be relatively widely distributed, which might require enough electrodes to cover the lower SMC.
[1] Siero et al (2014), 10.1016/j.neuroimage.2014.07.002

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Poster

389. Neurophysiology: Decoding and Neural Processing I

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Program #/Poster #: 389.24

Topic: E.05. Brain-Machine Interface

Title: Analysis of different motor attempts from intracortical microelectrode arrays in a completely locked-in state patient

Authors: *K. LEE[1], J. RAMOS DA CRUZ[1], D. IBANEZ SORIA[1], A. TONIN[1], A. ESPINOSA[1], N. F. RAMSEY[2], J. B. ZIMMERMANN[1];
[1]Wyss Ctr. for Bio and Neuroengineering, Geneva, Switzerland; [2]Brain Ctr. Rudolf Magnus, Utrecht, Netherlands

Abstract: Development of brain-machine interfaces (BMIs) for patients who cannot move any body part, i.e., in completely locked-in state (CLIS), is very challenging since no ground truth for attempted behavior or reliable communication channel is available. In this single-case study, a CLIS patient diagnosed with amyotrophic lateral sclerosis (ALS) was implanted with two 64-channel microelectrode arrays in the dominant left primary motor cortex and adjacent superior frontal gyrus. We explored different attempted movements to assess the reliability and strength of neural responses to be used for communication BCI.
The patient was instructed to try moving different body parts even though he was completely paralysed. The attempted movements included horizontal and vertical eye movements, hands, tongue, feet, and a movement chosen by the patient that he prefers for indicating "yes". We computed the power of different frequency bands of the 128 channels as features: spike band (300-7500 Hz), high gamma (120-300 Hz), low gamma (50-120 Hz), broad gamma (50-300 Hz), beta (10-30 Hz), low frequency (0.5-5 Hz). Power values were standardized and the median of the response period of each trial was computed per channel and per frequency band as a feature, which was reduced to 50 dimensions using PCA and then visualized using t-distributed stochastic neighbor embedding (t-SNE), a non-linear dimensionality reduction method.
Data showed a separation of clusters between eyes and other parts. The feet cluster was also distinct from other clusters but had a higher variability compared to other movement types. For a movement type associated with "yes", we speculated that the patient was performing eye movements since it was the strategy that he employed before transitioning into CLIS, and he confirmed during the BCI communication. Our results provided evidence that the "yes" movement was indeed overlapping with the cluster that contains horizontal eye movement attempts but not vertical eye movement attempts. Compared to similar experiments performed for example with participants in the BrainGate trials, we found attempted movement-related activity to be less stereotypical. This may be affected by the patient's unknown cognitive state when the experiments were performed due to advanced progress in paralysis.

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Poster

389. Neurophysiology: Decoding and Neural Processing I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 389.25

Topic: E.05. Brain-Machine Interface

Title: Multiscale neural reassociation in BMI learning

Authors: *Y. ZHAO[1], H. STEALEY[2], E. CONTRERAS-HERNANDEZ, Sr.[4], H.-Y. LU[3], M. BAKER[5], Y.-J. CHANG[6], S. R. SANTACRUZ[7];
[1]Biomed. engineering, [3]Biomed. Engin., [2]Univ. of Texas at Austin, Austin, TX; [4]Biomed. Enginering, Univ. of Texas at Austin, Dept. of Biomed. Engin., Austin, TX; [5]Biomed. Engin., Univ. of Texas, Austin, Austin, TX; [6]Dept. of Biomed. Engin., Univ. of Texas At Austin, Austin, TX; [7]Biomed. Engin., The Univ. of Texas at Austin, Austin, TX

Abstract: Title: Multiscale neural reassociation in BMI learningYi Zhao, Hannah Stealey, Enrique Contreras-Hernandez, Hung-Yun Lu, Megan Baker, Yin-Jui Chang, Samantha R. Santacruz Department of biomedical Engineering, University of Texas at Austin, Austin, TxAbstract: Behavior is driven by cooperation of a population of neurons. In a Brain-machine interface (BMI) reaching task, the relationship between neural activity and output behavior is easy to obtain. However, how the mechanism of neural pattern changes when task is changing is not clear. In this study, we explore the reassociation between neurons in a subpopulation in the same brain area as well as the coordinated activity changes between different brain areas, such as: Caudate Nucleus (Cd), dossal lateral prefrontal cortex (dlPFC). Two rhesus macaques are trained with BMI center-out task. Implanted chronic arrays gather the neural activities of both spiking data and local field potential (LFP) from the motor cortex and premotor cortex. And the spiking signals from chronic arrays are the input of the BMI. Neural recordings from Cd and dlPFC are obtained by the invasive V-probes. At beginning of the task, we use Kalman Filter to build the mapping between neural activities from M1 and premotor cortex and behavior. In the perturbation block, we shuffled specific percentage of neurons in the population with their Kalman gain from the mapping. After comparing the neural activities from normal center-out task to perturbation center-out task, we can know the changes in neural activities from same brain regions and across the brain regions. For the recording data, we will compare the intrinsic differences between neurons and the network changes between neurons and brain regions. This work will have important implications in understanding how the neurons reassociate in multiscale level (same brain region and across regions) help learning in different tasks.

Disclosures: Y. Zhao: None. **H. Stealey:** None. **E. Contreras-Hernandez:** None. **H. Lu:** None. **M. Baker:** None. **Y. Chang:** None. **S.R. Santacruz:** None.

Poster

389. Neurophysiology: Decoding and Neural Processing I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 389.26

Topic: E.05. Brain-Machine Interface

Support: NIH R01-NS109257

Title: A human-operated real-time intracortical brain-computer interface simulation platform

Authors: T.-H. LIN[1], P. AWASTHI[2], L. E. MILLER[3], ***Z. C. DANZIGER**[1];
[1]Florida Intl. Univ., [2]Florida Intl. Univ., Miami, FL; [3]Northwestern University, Feinberg Sch. of Med., Northwestern Univ., Chicago, IL

Abstract: Prototyping and rigorously evaluating invasive intracortical brain-computer interface (iBCI) decoders in closed-loop with human users is an essential step for their clinical translation; offline tests cannot evaluate a user's ability to correct errors and adapt to the decoder. However, the severely limited participant pool for iBCIs makes this impossible to achieve. To address this challenge, we developed a hybrid human participant / machine learning iBCI model that lets us test decoders in controlled experiments across statistically rigorous numbers of naive users. The iBCI model combines an artificial neural network (trained on non-human-primate cortical firing rates) with human participants able to generate emulated M1 firing rates through finger movements captured non-invasively using a data glove. The emulated activity can be input to any iBCI decoder and used to operate an external device. A human participant controlling a cursor with a high-dimensional (19 DoF), non-intuitive input-space of finger joint angles is conceptually similar to that of a human controlling a cursor with a high-dimensional, non-intuitive input-space of motor cortex firing rates. The great advantage of our approach is that readily-recruited, able-bodied participants can use the decoders for closed-loop control, preserving the relevant learning dynamics between humans and decoders. We validated the proposed iBCI model using both offline measures of the emulated neurons and performance measures of subjects during closed-loop cursor control.
In offline results, we found the neural emulator generated firing patterns that matched training and validation datasets (22 recording sessions from 2 monkeys) using multiple measures (e.g., similar low dimensional projections of population activity and peri-event histograms). In online experiments, participants used cursor velocity, decoded from the emulated firing rates using a steady state Kalman filter, to perform a center-out target acquisition task, facilitating direct comparisons to prior human iBCI studies. We ran 25 healthy participants, each on four separate days. Their performance was consistent with human iBCI users with brain implants in studies by Kim et al., 2008, Kim et al., 2011, and Simeral et al., 2011 across seven different behavioral measures. These encouraging results suggest that this real-time firing rate emulation process can provide statistically robust sample sizes for rapid prototyping and optimization of decoding algorithms while keeping human users in the loop, ultimately improving iBCI control.

Disclosures: T. Lin: None. **P. Awasthi:** None. **L.E. Miller:** None. **Z.C. Danziger:** None.

Poster

389. Neurophysiology: Decoding and Neural Processing I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 389.27

Topic: E.05. Brain-Machine Interface

Support: HHMI funding

Title: Low-latency extracellular spike assignment for high-density electrodes at single-neuron resolution

Authors: *C. LAI[1], D. KIM[1], B. LUSTIG[1], S. TANAKA[1], L. NARAYAN[1], B. BARBARITS[1], O. PAULSEN[2], A. K. LEE[1], T. D. HARRIS[1];
[1]HHMI/Janelia, Ashburn, VA; [2]Dept. of Physiology, Develop. and Neurosci., Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Real-time neural signal processing is essential for closed-loop neural perturbation and Brain-Machine Interface (BMI) applications. Most of these applications are spike rate-based, in which the cell type and precise temporal information of individual neuron spiking are ignored. We report a novel hardware-software hybrid system that assigns single-neuron identities to individual spikes of a large number of neurons recorded by tetrodes or silicon probes. Our platform uses a single Field Programmable Gate Array (FPGA) chip for data acquisition and processing, which enables 1 ms spike assignment latency. Our online spike assignment method uses multichannel spike waveforms to produce results as accurate as offline spike sorting. We validate the high accuracy using two in vivo data sets that contain ground-truth intracellularly or juxtacellularly recorded spikes. This platform allows us to rapidly inactivate a downstream region based on single-neuron spikes from an upstream region in vivo, potentially before these spikes reach the downstream region. We also show that this platform enables us to perform population decoding at single-neuron, single-spike resolution with low latency. This new system is suitable for a broad spectrum of research and clinical applications.

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Poster

389. Neurophysiology: Decoding and Neural Processing I

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Program #/Poster #: 389.28

Topic: E.05. Brain-Machine Interface

Support: NIMH Grant R00 MH117264
NIH Grant R01 NS053603
NSF Grant IIS-1835345

Title: Self-supervised segmentation of EMG dynamics uncovers the structure of unconstrained behavior

Authors: *R. YANG[1], A. ULMER[1], X. MA[1], L. MILLER[1,2,3,4], A. KENNEDY[1];
[1]Dept. of Neurosci., Northwestern Univ., Chicago, IL; [2]Dept. of Biomed. Engin., Northwestern Univ., Evanston, IL; [3]Dept. of Physical Med. and Rehabil., Northwestern Univ., Chicago, IL; [4]Shirley Ryan AbilityLab, Chicago, IL

Abstract: Traditionally, sensorimotor studies using monkeys were mostly performed in highly constrained conditions such that only one limb or even a single joint could move. Such settings allow the precise relationship between neural activity and individual motor acts to be characterized, but due to practical constraints, only a small number of movement parameters can be investigated, and any structure in neural activity unrelated to the target action is removed, minimized or averaged out of the data. More critically, if there are nonlinearities or context dependencies in the motor system, models that capture the relationship between neural activity and isolated motor acts will not generalize to more complex and naturalistic settings. With the advent of high channel-count wireless neural recording, we can collect a remarkably large amount of data from many simultaneously recorded neurons as monkeys perform a variety of unconstrained behaviors. Such settings are critical if we are to begin to explore the motor control system in naturalistic settings. However, such massive continuous recordings pose analytical challenges, as they lack well-defined events with which to delineate behavior start and end times, and trials for the unstructured behaviors performed by the monkey are hard to define. One solution to this dilemma is to identify the animal's actions via manual labeling, however, this process is time-consuming and often yields inconsistent results when performed by different labelers. In this work, we overcome the need for human-defined annotations of animals' actions by collecting EMG signals from a group of muscles in the monkey's arm and hand during unconstrained behavior in a large plastic telemetry cage where the animal may freely interact with multiple behavioral devices, while simultaneously performing wireless recording of M1 neural activity. We find that EMG signals are consistent in repeated episodes of specific experimenter-defined behaviors, but may change dramatically across behaviors. To further characterize the richness of animal behavior in this experimental setting, we developed a self-supervised algorithm to learn a low-dimensional representation of recorded EMG signals. We then automatically segmented this representation into discrete movement categories, thereby increasing the resolution and granularity with which actions could be identified. We used this rich description of animals' actions to characterize the relationship between motor activity and M1 neural population dynamics in the unconstrained setting.

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Poster

390. Neurophysiology: Decoding and Neural Processing II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 390.01

Topic: E.05. Brain-Machine Interface

Support: NIH NINDS R01 NS123663
NIH NEI F30 EY032799
NIGMS T32 GM008042
Tianqiao and Chrissy Chen Institute

Title: A Functional Ultrasound Brain-Machine Interface: Online, Closed-Loop Decoding of Eight Movement Directions

Authors: *W. S. GRIGGS[1], S. NORMAN[1], C. RABUT[2], T. DEFFIEUX[4], M. TANTER[5], V. N. CHRISTOPOULOS[6,3], C. LIU[7], M. G. SHAPIRO[2], R. A. ANDERSEN[1,3];
[1]BBE, [2]CCE, [3]T&C Chen Brain-Machine Interface Ctr., Caltech, Pasadena, CA; [4]Inserm DR6, Physmed Inserm U1273, Physmed Inserm U1273, Paris, France; [5]Inst. Langevin Ondes Et Images, Inst. Langevin Ondes Et Images, Paris, France; [6]Univ. of California, Riverside, Univ. of California, Riverside, Riverside, CA; [7]USC, Los Angeles, CA

Abstract: Large numbers of people live with chronic paralysis. Brain-machine interfaces (BMIs) can be transformative for these people. By translating complex brain signals into computer commands, BMIs can bypass neurological impairments, enabling users to move robotic limbs and control computers. State-of-the-art BMIs have already made this future a reality in university-based clinical trials. However, these BMIs typically require highly invasive electrodes inserted into the brain. Device degradation limits the BMI's longevity to 3-5 years, the implants require invasive surgeries in which electrodes are implanted into the brain, and the field of view of brain activity is small. These factors limit their widespread use. Advances for the next generation of BMIs include being longer lasting, less invasive, and scalable. Functional ultrasound (fUSI) is a recently developed neuroimaging technique that meets these criteria. fUSI uses ultrafast pulse-echo imaging to sense changes in Cerebral Blood Volume (CBV). It has excellent spatiotemporal resolution (<100 μm; 100 ms) and high sensitivity to slow blood flow (~1 mm/s velocity) across a large and deep field of view (several centimeters). In this present study, we demonstrate the first online, closed-loop functional ultrasound brain-machine interface (fUSI-BMI). We used the Power Doppler time series (updating at 2 Hz) generated by fUSI to measure real-time changes in CBV in the non-human primate (NHP) posterior parietal cortex, an important area for the transformation of visuospatial information into motor planning and execution. We then fed these data into linear classifiers based on principal component and linear discriminant analyses to predict planned movement timing and direction. These predictions were used to control a behavioral task in real-time while the NHP did not produce overt movement. To create the training set for our linear classifier, the monkeys performed memory-guided eye or hand movements to eight targets at the start of each recording session. After 100 successful trials, we trained the linear classifiers and switched to BMI control mode where the monkey planned making movements but did not execute those movements. Using this online, closed-loop fUSI-BMI, we found that we could decode movement initiation and eight directions of movement significantly above chance level (Monkey P - 42 ± 4%, Monkey L - 21 ± 3%, binomial test, $p<0.001$) in real-time. This work establishes, for the first time, the feasibility of an ultrasound-based BMI and prepares for future work on a next generation of minimally-invasive BMIs that can restore function to patients with neurological impairments.

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Poster

390. Neurophysiology: Decoding and Neural Processing II

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Topic: E.05. Brain-Machine Interface

Support: NIH/NRSA Grant NS105595
Boswell Foundation
T&C Chen Brain-machine Interface Center
NIH/NINDS Grant U01NS098975
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Title: S1 represents multisensory contexts and somatotopic locations within and outside the bounds of the cortical homunculus

Authors: I. A. ROSENTHAL[1], L. BASHFORD[1], S. KELLIS[1], K. PEJSA[1], B. LEE[2], C. LIU[2], R. A. ANDERSEN[1];
[1]Caltech, Pasadena, CA; [2]USC, Los Angeles, CA

Abstract: Tactile sensations are important for dexterous, adaptable actions, as well as creating a sense of embodiment. The responsiveness of primary somatosensory cortex (S1) to physical tactile stimuli is well documented but the extent to which it is modulated by vision has been a long-standing question. Additionally, recent literature has suggested that tactile events are represented in S1 in a more complex, generalized manner than its long-established topographic organization (Muret et al., 2022). Here, we use human electrophysiology to characterize S1's responses to touches in two locations in the presence or absence of vision. A C5-C6 tetraplegic patient was implanted with two Utah microelectrode arrays in the arm region of S1. Neural activity was recorded during 1s stroking touches to the forearm (evoking numb sensation) or the thumb (naturalistic sensation). Touch conditions included visually observed physical touches, physical touches without vision, and visual touches without a physical element (virtual reality was used to display touches being performed without any contact to the patient's real body). Two major findings emerged from this dataset. The first finding was that vision strongly modulates S1 activity, but only if there is also a physical element to the touch. Visually observed physical touch trials were decodable from all other conditions (>84% accuracy, chance = 50%), including physical touch trials without vision (84-92%). Of the 96 channels measured, 38% were tuned to visually observed physical touches, and 16% were tuned to physical touches without vision. Observed touches with no physical element could not be used to decode touch locations, and <2% of channels were tuned to these trials. These findings suggest that S1 is modulated by visuotactile integration but that passive observation of non-physical touches may not be sufficient to recruit S1 neurons. The second finding was that despite the location of the recording arrays being in a putative arm area of S1, neural activity encoded both arm and finger touches in physical touch conditions. 36% of channels were tuned to arm conditions; 22% of channels were tuned to finger conditions. Tuned channels were tuned either solely to the arm (17%) or the arm and finger (20%), but rarely solely to the finger (2%), and the tuned response to arm conditions began before the tuned response to finger conditions. The unequal representation of arm and finger touches supports the idea that S1 encodes tactile events primarily through its well-

documented topographic organization, as well as in a more general manner encompassing larger areas of the body.

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Poster

390. Neurophysiology: Decoding and Neural Processing II

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Program #/Poster #: 390.03

Topic: E.05. Brain-Machine Interface

Support: The T & C Chen Brain-machine Interface Center at Caltech
The Boswell Foundation
The USC Neurorestoration Center
Longeviti LLC

Title: Ultrasonic Brain-Machine Interfaces are Enabled by Sonolucent "Windows" to Human Brain Function

Authors: *S. L. NORMAN[1], C. RABUT[1], W. S. GRIGGS[2], M. TANTER[3], C. LIU[4], M. G. SHAPIRO[1], R. A. ANDERSEN[2];
[2]BBE, [1]Caltech, Pasadena, CA; [3]Equipe Physique des Ondes pour la Médecine INSERM U979, CNRS UMR7587, Inst. Langevin Ondes Et Images, Paris, France; [4]USC, Los Angeles, CA

Abstract: Brain-machine interfaces (BMIs) interpret neurophysiological signals from the brain, enabling users to communicate and control devices without physical movement. In previous work, we used functional ultrasound imaging (fUSI) of the brain to decode motor planning activity in non-human primates with single-trial resolution and in real time. Less invasive, high resolution and scalable BMIs for human use is an important direction for this work. However, ultrasound imaging through adult human skull bone cannot yield the signal quality required for BMIs. Here, we present a novel approach to performing fUSI imaging through a sonolucent skull reconstruction material based on polymethyl methacrylate. First, we characterized signal degradation by imaging *in vitro* B-mode and Doppler phantoms and *in vivo* craniotomized rats. We found that signal quality worsened in proportion to cranioplasty thickness. Implant thickness had a minimal effect on both contrast (~2.5 dB/mm) and resolution (no detectable change). We then collected *in vivo* clinical data from a patient who had part of their skull removed as part of a decompressive craniectomy procedure following traumatic brain injury. In the ensuing months, we imaged the brain through the scalp, i.e. without the skull. We then performed the cranioplasty and placed the sonolucent portion of the cranioplasty over a brain region previously identified to be associated with motor activity (fMRI finger-tapping task). Finally, we collected the first fUSI images in a skull-reconstructed awake and behaving human. Together, these results represent the first steps toward chronic "transcranioplastic" imaging and fUSI-BMI in humans.

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Poster

390. Neurophysiology: Decoding and Neural Processing II

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Topic: E.05. Brain-Machine Interface

Support: NIH/NEI R01EY015545
NIH/NEI UG1EY032039
Tianqiao and Chrissy Chen Brain-machine Interface Center at Caltech
Boswell Foundation
Swartz Foundation

Title: Shared allocentric and egocentric neural representations in posterior parietal cortex

Authors: *J. GAMEZ[1], T. AFLALO[1,2], K. KADLEC[1], C. GUAN[1], X. ZOU[1], K. W. PEJSA[1], E. R. ROSARIO[3], A. A. BARI[4], N. POURATIAN[4], R. A. ANDERSEN[1,2];
[1]Div. of Biol. and Biol. Engin., [2]T&C Chen Brain-machine Interface Ctr., Caltech, Pasadena, CA; [3]Res. Inst., Casa Colina Hosp. and Centers For Healthcare, Pomona, CA; [4]UCLA, Los Angeles, CA

Abstract: Visually guided target-oriented motor actions begins with the encoding of a visual image in the retina and ends with the control of an effector's muscles. In this process, the role of the posterior parietal cortex (PPC) in body-centered egocentric sensorimotor transformations has been studied extensively. However, the position of an object can also be represented in a landmark-centered allocentric reference frame. We previously found that body-centered and landmark-centered representations are encoded in the activity of the neural population in PPC. Here we studied if these representations share the same neural code and if the representations are specific to the body part that performs the motor action. As part of a brain-machine interface clinical trial, we recorded from the PPC and the motor cortex (MC) of one human tetraplegic participant, implanted with one 96-channel Blackrock NeuroPort array in each area. The participant performed a task where he had to decide if an object was located to the left or the right of his gaze (egocentric condition) or a landmark (allocentric condition), providing his answer using saccades or attempted thumb movements. This task had perceptual, memory, motor planning, and motor execution phases. The task phase progression was designed to dissociate the spatial decision from the motor response, requiring a cognitive process to transform visual information into an abstract spatial representation. Using a population level across-condition analysis on saccade trials, we found that in PPC the cross-decoding strength of cognitive spatial information between allocentric and egocentric conditions increases monotonically across the trial, peaking during the motor execution phase. This suggests an increasing shared neural representation of this abstract spatial information between both conditions. In contrast with

saccade responses, attempted thumb movement trials showed a decrease in the coding strength of cognitive spatial information during motor planning and execution for both allocentric and egocentric conditions. Interestingly, MC showed no cognitive spatial coding for saccades, but it did show weak coding for this information during attempted thumb movement trials for both conditions. Our results support the idea that PPC contains an effector-dependent neural population representation of allocentric and egocentric information that becomes independent of the original perceptual reference frame across perceptual-cognitive-motor transformations.

Disclosures: **J. Gamez:** None. **T. Aflalo:** None. **K. Kadlec:** None. **C. Guan:** None. **X. Zou:** None. **K.W. Pejsa:** None. **E.R. Rosario:** None. **A.A. Bari:** None. **N. Pouratian:** None. **R.A. Andersen:** None.

Poster

390. Neurophysiology: Decoding and Neural Processing II

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Topic: E.05. Brain-Machine Interface

Support: NIH R01EY015545
NIH UG1EY032039 (NIE)
NIH T32NS105595 (NRSA)
Tianqiao and Chrissy Chen Brain-machine Interface Center at Caltech
The Boswell Foundation

Title: Coding for movements across the body in human Motor Cortex and Posterior Parietal Cortex

Authors: *K. KADLEC[1], T. AFLALO[1,2], J. GAMEZ[1], C. GUAN[1], E. ROSARIO[3], N. POURATIAN[4], R. ANDERSEN[1,2];
[1]BBE, Caltech, Pasadena, CA; [2]T & C Chen Brain Machine Interface Ctr., Pasadena CA, CA; [3]Casa Colina Hopsital, Pomona, CA; [4]UT Southwestern, Dallas, TX

Abstract: Even simple movements rely on the recruitment of multiple brain areas. Understanding these regions' contributions is a classic problem in motor control. One fundamental question that has been historically investigated is how brain regions relate to the control of different parts of the body. Surprisingly, studies recording from single neurons within the same motor regions have yielded highly variable results. For example in motor cortex (MC), early work shows a close link between cortical location and the effector controlled; in other words an effector-specific organization. Recent work, however, shows evidence for a more complex motor representation such as behavioral maps, indicative of a functional organization in MC. In posterior parietal cortex (PPC), much like MC, nonhuman primate work points toward effector specific, anatomically segregated patches of cortex. In contrast, work in humans reveals a more overlapping representation of effectors, which supports a functional rather than effector

based representation. How do we consolidate results highlighting separation of effector representations in cortical motor areas with recent findings of effector overlap? We have a chance to bring some clarity to this question as part of an ongoing brain-machine interface study, where we recorded from single neurons in human PPC and MC (hand knob) as the subject attempted movements from head to foot (left and right side). MC codes for effectors across the body, but with a strong preference for the right hand and wrist (2x encoding strength). The population structure for these movements has a biomechanical relationship between effectors (e.g. right and left thumb). Further, during simultaneous movements, the hand and wrist suppress other effector representations. PPC codes for the whole body as well, however, unlike MC, there is similar tuning strength across effectors. Single neurons code for movements of random combinations of effectors, but code the direction of movement the same across those effectors. In PPC, simultaneous movements are represented, but the combined movements are not linearly predictable from the individual effector components. The results of emphasized hand and wrist tuning in MC support literature highlighting effector preferences, but do not dismiss findings of whole body representation. The exact role of this coding in control is unclear, because during combined movements, we see minimal representation for the non-preferred effector. In PPC, results support a more functional than effector based organization with many effectors localized to the same region of cortex.

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Poster

390. Neurophysiology: Decoding and Neural Processing II

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Topic: E.05. Brain-Machine Interface

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NIH/NEI Grant R01EY015545
T&C Chen Brain-Machine Interface Center
Amazon AI4Science Fellowship
Boswell Foundation

Title: Compositional decoding of individual finger movements from human posterior parietal cortex

Authors: *C. GUAN[1], T. AFLALO[1], K. KADLEC[1], J. GÁMEZ[1], E. R. ROSARIO[2], A. BARI[3], N. POURATIAN[4], R. A. ANDERSEN[1];
[1]Caltech, Caltech, Pasadena, CA; [2]Casa Colina Hosp. and Centers for Healthcare, Pomona, CA; [3]UCLA, Los Angeles, CA; [4]UT Southwestern Med. Ctr., Dallas, TX

Abstract: Restoring dexterous hand control is a top priority for individuals with tetraplegia. Brain-machine interfaces (BMIs) enable paralyzed individuals to control assistive devices, but most prior hand BMIs enable only a limited number of fixed grasps. Here, we used intracortical recordings from the left posterior parietal cortex (PPC) to enable two participants to control the individual fingers of a BMI hand. One of the participants was also implanted with an electrode array near the hand area of the left motor cortex, allowing us to compare between simultaneously recorded brain regions. Both PPC and MC single neurons modulated selectively for individual finger movements of the right (contralateral) hand, suggesting that dexterous hand control may recruit cortical areas beyond the motor cortex. Online control accuracy (participant 1: 86%; participant 2: 92%; chance = 17%) exceeded previous state-of-the-art finger BMIs. Finally, we recorded neural activity during finger movements of all ten fingers. A compositional neural code linked corresponding finger movements of the left and right hands while enabling classification of all ten fingers (offline accuracy, participant 1: 69%; participant 2: 64%; chance=10%). Future neural decoders can exploit this representational geometry to generalize decoding across hands.
Additional keywords: digits, paralysis, brain-computer interface (BCI), compositionality, partially mixed selectivity, neuroprosthetics

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Poster

390. Neurophysiology: Decoding and Neural Processing II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 390.07

Topic: E.05. Brain-Machine Interface

Support: T&C Chen Brain-machine Interface Center
NIH/NINDS Grant U01NS098975
NIH/NINDS Grant U01NS123127

Title: Decoding speech and internal speech from populations of single units from the supramarginal gyrus in a tetraplegic human

Authors: *S. K. WANDELT[1], D. A. BJANES[2], L. BASHFORD[3], I. ROSENTHAL[4], K. PEJSA[2], B. LEE[5], C. LIU[5], R. A. ANDERSEN[6];
[1]Caltech, [3]Biol. and Biol. Engin., [4]Computation and Neural Systems, [2]Caltech, Pasadena, CA; [5]USC, Los Angeles, CA; [6]BBE, Calif Inst. of Technol., Pasadena, CA

Abstract: Speech is a natural and intuitive way for humans to express their thoughts and desires. Neurological diseases like amyotrophic lateral sclerosis (ALS) and cerebral brain lesions can lead to the loss of this ability, leaving patients without any means of communication. Brain-Machine Interfaces (BMIs) are devices that offer a promising technological path to restoring communication by recording neural activity related to speech from the cortex. Intracortical

speech decoding has predominantly been attempted with electrocorticography (ECoG) (Rabbani, Milsap and Crone, 2019), stereo-electroencephalographic (sEEG) depth arrays (Herff, Krusienski and Kubben, 2020), and from small-scale Utah arrays located in the motor cortex (Stavisky et al., 2019; Wilson et al., 2020) and the posterior parietal cortex (Wandelt et al., 2022). While important advances in overt speech, attempted speech (Moses et al., 2021) and mimed speech (Anumanchipalli, Chartier and Chang, 2019) decoding have been made, results for internal speech decoding are sparse and have yet to achieve high functionality (Proix et al., 2022). We hypothesized internal speech would modulate single unit activity in the supramarginal gyrus (SMG), due to its involvement in vocalized speech and other language processes.
In this work, a C5-C6 tetraplegic patient implanted with Utah arrays in the SMG performed an internal and vocalized speech task, following Martin et al., 2016. Trials were composed of six phases, beginning with a brief inter-trial interval, followed by an auditory or written cue to one of eight words (6 words, 2 pseudowords). Then, after a delay period, the subject was instructed to internally say the word, and after a second delay, to vocalize the word.
We found single units tuned to words during cue, imagined and vocalized speech phases. Internal speech decoding, while slightly less accurate than speech, reached up to 72% classification accuracy for eight words (chance = 12.5%) and over 90% for four words (chance = 25%). Shared representations between imagined and vocalized speech were demonstrated through overlapping tuning curves and through cross-classification analysis. These findings suggest robust SMG modulation during internal speech, indicating that internal speech BMIs can be built using signals from single brain areas, and adding evidence that a multipurpose BMI could be viable from a single implant in the PPC.

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Poster

390. Neurophysiology: Decoding and Neural Processing II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 390.08

Topic: E.05. Brain-Machine Interface

Support: ARO W911NF-16-1-0368

Title: Learning and inference for switching dynamical systems with multiscale neural observations

Authors: *C. Y. SONG[1], H.-L. HSIEH[2], B. PESARAN[3], M. M. SHANECHI[1];
[1]USC, [2]Electrical and Computer Engin., USC, Los Angeles, CA; [3]Ctr. for Neural Sci., NYU, New York City, NY

Abstract: Neural population dynamics during naturalistic behavioral setups and over longer time periods can exhibit regime changes, which correspond to changes in task stage or internal states.

Further, these dynamics involve multiple spatial and temporal scales that can be measured with different neural modalities such as small-scale spiking activity and large-scale local field potentials (LFP). Thus, studying population dynamics and enhancing brain-machine interfaces in these naturalistic setups can benefit from novel methods that simultaneously tackle two challenges. First, these methods should describe the different spatial and temporal properties of multiple neural activity scales and fuse information across these scales - from the fast binary nature of spiking activity to the slower continuous-valued field potentials. Second, these methods should also model and detect abrupt switches in such multiscale neural dynamics. Prior methods address either multiscale observations or regime-switching but not both together. Here, we address both challenges simultaneously. We do so by developing an unsupervised learning algorithm and inference methods for a Switching Multiscale Dynamical System (SMDS) model. We show the success of SMDS in both simulations and in monkey spike-LFP datasets during a saccadic eye movement task with various stages from fixation to execution. Using extensive numerical simulations, we show that, compared to known ground truths, our learning algorithm identifies accurate model parameters using only multiscale neural observations. We then show in both numerical simulations and in the neural dataset that, once model parameters are learned, our inference algorithms can accurately and robustly estimate both the saccade direction and the task-relevant regimes purely from multiscale neural data. Finally, we show that SMDS successfully fuses information across spiking and LFP activity scales to better detect regimes and decode behavior compared to single-scale switching methods.

Disclosures: **C.Y. Song:** None. **H. Hsieh:** None. **B. Pesaran:** None. **M.M. Shanechi:** None.

Poster

390. Neurophysiology: Decoding and Neural Processing II

Location: SDCC Halls B-H

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Program #/Poster #: 390.09

Topic: E.05. Brain-Machine Interface

Support: DoD MURI – Grant number: W911NF-16-1-0368

Title: Dynamic modeling and quantification of shared interactions across brain regions

Authors: *T. JANI[1], B. PESARAN[2], M. M. SHANECHI[1];
[1]Electrical and Computer Engin., USC, Los Angeles, CA; [2]Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Most tasks carried out by the brain rely on interactions among several regions. Prior work studying these interactions has often utilized static modeling methods that do not consider the dynamic nature of the neural data. Further, when modeling dynamics in interactions, a main remaining challenge is that shared dynamics between brain regions may be mistaken for or masked by dynamics within regions. We address this challenge by developing a model that prioritizes the learning of shared neural dynamics over other dynamics, which current dynamic

methods of multiregional interactions do not achieve. We use our method to study shared dynamics between multiregional motor cortical activity from two monkeys performing naturalistic 3D reach, grasp, and return movements. To prioritize shared dynamics, we formulate the problem within the framework of preferential subspace identification (PSID), a recent dynamic dimensionality reduction method. While PSID was originally developed to model behaviorally relevant neural dynamics, we show that its general framework can be adapted to the novel application of modeling shared dynamics between populations of neurons in different brain regions. Doing so, we can explicitly prioritize the learning of a latent state relevant to the shared dynamics between regions over learning those relevant to within-region dynamics - that is, we can preferentially model shared dynamics. We show that this preferential dynamic modeling better describes the shared dynamics compared to both static and non-preferential dynamic methods. Further, the model more accurately finds the dimensionality of shared dynamics between regions. Finally, the method can quantify the strength of interactions within and between neural populations. Doing so, the method identifies the dominant interaction pathways between bilateral premotor and primary motor cortical areas, which we find are consistent with prior evidence about their functional roles. Our results show that preferential dynamical modeling can solve the challenge of learning shared dynamics between brain regions with priority such that they are not masked by, confounded by, or mistaken for within-region dynamics.

Disclosures: **T. Jani:** None. **B. Pesaran:** None. **M.M. Shanechi:** None.

Poster

390. Neurophysiology: Decoding and Neural Processing II

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 390.10

Topic: E.05. Brain-Machine Interface

Support: NIH Director's New Innovator Award (DP2), Contract DP2MH126378

Title: Describing neural population dynamics on an intrinsic geometric multidimensional manifold

Authors: ***H.-L. HSIEH**[1], H. ABBASPOURAZAD[1], B. PESARAN[2], M. M. SHANECHI[1]; [1]Electrical and Computer Engin., USC, Los Angeles, CA; [2]Ctr. for Neural Sci., NYU, New York City, NY

Abstract: Neural population activity exhibits low-dimensional dynamics that may be described in terms of a low-dimensional latent manifold. A prominent feature that has been observed in low-dimensional projections of neural population activity across diverse datasets is rotations over time. We hypothesize that these rotations suggest that neural population activity evolves over nonlinear manifolds that contain a hole, yet can be multi-dimensional. Testing this hypothesis requires developing new methods that identify whether such intrinsic geometry exists

in neural data, and if so, can explicitly incorporate the geometry in a dynamical model to better predict neural dynamics. We thus developed a data-driven method to learn this multi-dimensional nonlinear manifold with holes, designed a manifold-specific PCA-like algorithm to sort the contribution of different manifold dimensions, and a machine learning algorithm to fit the intrinsic dynamical model on top of this manifold. We applied this geometric framework to motor cortical population activity in non-human primates performing motor tasks. We found that the manifold-based intrinsic coordinate predicts the neural population activity better than the linear Euclidean embedding coordinate; this suggests that the intrinsic manifold coordinate is a more natural space in which to describe neural dynamics. Interestingly, the manifold-based intrinsic coordinate was better than the Euclidean coordinate not only in predicting the neural activity (the signal), but also in describing the neural deviation around this prediction, i.e., the neural noise. These results suggest that the nonlinear manifold-based intrinsic coordinate provides a more natural space to describe the dynamics of neural population activity. This geometric dynamical modeling framework provides a novel tool to study how the intrinsic geometry of neural population activity and how it gives rise to behavior.

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Poster

390. Neurophysiology: Decoding and Neural Processing II

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Program #/Poster #: 390.11

Topic: E.05. Brain-Machine Interface

Support: NIH Director's New Innovator Award DP2-DP2MH126378

Title: Multimodal neural network models of neural population activity with flexible inference

Authors: *E. ERTURK, H. ABBASPOURAZAD, M. SHANECHI;
Electrical and Computer Engin., USC, Los Angeles, CA

Abstract: Recent work in neuroscience has shown the power of neural networks in capturing nonlinear latent structure in neural population activity. Despite much progress, prior neural network models have characterized only a single modality of neural activity, mostly spiking activity. However, behavior is encoded across multiple spatial and temporal scales of brain activity that are measured with different modalities including discrete spiking activity and continuous local field potentials (LFP). As such, leveraging multiple neural modalities can not only extract more accurate latent factors for neural population activity, but also allow for studying nonlinear neural dynamics across multiple scales. Here, we develop a novel neural network model that can extract the underlying latent factors/dynamics from multiple neural modalities recorded simultaneously. In addition to supporting multiple neural modalities and capturing nonlinearities, our model also allows for flexible inference - that is, both causal and

non-causal inference even in the presence of missing neural observations. We first validate our method on nonlinear dynamical simulations by showing that it infers the true dynamical latent manifold from noisy observations. We then apply our method to a non-human primate motor dataset, and show that it improves behavior decoding compared to neural network models that take single modalities as observations. This novel multimodal neural network model can capture nonlinearity, support multimodal observations and enable flexible inference. Doing so, the model can be used to extract more accurate latent factors by fusing multiple modalities, to study the similarity and dissimilarity of the latent dynamics across modalities, and to investigate the link from these modalities to behavior.

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Poster

390. Neurophysiology: Decoding and Neural Processing II

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Program #/Poster #: 390.12

Topic: E.05. Brain-Machine Interface

Support: DoD Bilateral Academic Research Initiative (DoD BARI), grant number W911NF1810434

Title: Multimodal subspace identification for learning of latent dynamics in neural population activity

Authors: *P. AHMADIPOUR, O. G. SANI, Y. YANG, M. M. SHANECHI;
Electrical and Computer Engin., USC, Los Angeles, CA

Abstract: Behavior is encoded across multiple spatiotemporal scales of neural activity measured with various neural activity modalities, such as small-scale neuronal spiking activity and large-scale field potential activity. Developing methods that can identify a low-dimensional subspace in which multimodal neural dynamics can be modeled is thus important across diverse neuroscience and neurotechnology applications. However, prior subspace identification (SID) methods are designed for a single signal modality rather than for multimodal signals. Subspace identification for multimodal neural signals is challenging because spike counts are discrete-valued while field potentials are continuous-valued, thus exhibiting different statistical distributions. Here we address this challenge and develop a computationally efficient multimodal SID method. We then validate multimodal SID using both comprehensive numerical simulations and a non-human primate spike-LFP dataset during a random target reaching task (Zenodo, 2020, doi: 10.5281/zenodo.583331). We also compare with common expectation maximization (EM) methods, including a recently developed multimodal EM (TNSRE, 2019, doi: 10.1109/tnsre.2019.2913218), which work by iteratively optimizing the likelihood function. We show that multimodal SID accurately learns the dynamical model parameters from multimodal observations. Further, multimodal SID aggregates information across modalities and improves

performance compared to using a single modality. Finally, multimodal SID is much faster compared with prior EM methods, while also being more accurate. Taken together, multimodal SID provides a new tool for investigating how behavior is encoded across spatiotemporal scales of neural activity and for improving brain machine interfaces by aggregating information.

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Poster

390. Neurophysiology: Decoding and Neural Processing II

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Program #/Poster #: 390.13

Topic: E.05. Brain-Machine Interface

Support: DoD Bilateral Academic Research Initiative (DoD BARI), grant number W911NF1810434

Title: Developing a confidence-based brain-computer interface for enhanced decision accuracy

Authors: *N. SADRAS, O. G. SANI, P. AHMADIPOUR, M. M. SHANECHI;
Electrical and Computer Engin., USC, Los Angeles, CA

Abstract: When making decisions, humans can evaluate how likely they are to be correct via their confidence. Indeed, it has been shown that decision confidence is predictive of decision accuracy. In light of this, we envisioned a confidence-based brain-computer interface (BCI) that can decode a user's confidence level from electroencephalogram (EEG) signals prior to a decision and can subsequently provide feedback to improve their performance. To realize such a BCI, we first investigated the neural correlates of confidence in a carefully designed stimulus discrimination task. We compared data from two versions of the task, one with a post-stimulus gap and one without, to determine whether the neural representation of confidence is stimulus locked (pre-decision) or response locked (post-decision). This distinction is important since a BCI can only intervene and improve a user's performance if confidence can be decoded pre-decision. Our analysis showed that in the presence of a post-stimulus gap, the neural encoding of confidence is indeed stimulus locked. We then assessed the ability of several decoding algorithms to predict confidence from single trial pre-decision EEG activity and found that a nonlinear support vector machine (SVM) classifier performs best. Lastly, we developed a simulation to show that SVM-decoded confidence can be used by a BCI to improve a user's performance. This improvement was highest when the decision task was difficult and had a high cost of error. Our results demonstrate the viability of a confidence-based EEG BCI for enhanced decision accuracy.

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Poster

390. Neurophysiology: Decoding and Neural Processing II

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Topic: E.05. Brain-Machine Interface

Support: DoD MURI W911NF-16-1-0368
NIH R01 R01MH123770

Title: Modeling the shared subspace between Poisson neural population activity and continuous behavior signals

Authors: *L. L. OGANESIAN, O. G. SANI, M. M. SHANECHI;
Electrical Engin., USC, Los Angeles, CA

Abstract: Modeling the shared dynamics between Poisson neural observations and continuous behavior signals can help reveal how computations through neural population dynamics give rise to a specific behavior. Prior methods have extracted this shared subspace for continuous Gaussian neural observations, such as field potentials or binned spike counts (Nat Neurosci, 2021, doi:10.1038.s41593-020-00733-0; bioRxiv 2021.09.03.458628). Spiking activity, however, can be better represented as a collection of binary time-series changing at a millisecond scale, whereas the corresponding behavior of interest can be changing smoothly and continuously over slower timescales. Extracting the shared subspace between Poisson and continuous observations is challenging and not addressed by prior methods in system identification theory. Here we address this challenge by developing Poisson preferential subspace identification (Poisson-PSID). Poisson-PSID models both continuous behavioral and discrete population spiking activity in terms of low-dimensional latent states, thereby dissociating and prioritizing their shared subspace. We demonstrate the method both in simulations and in neural population activity recorded from non-human primates during a movement reaching task. First, in extensive numerical simulations we validate the new algorithm and identify the regimes of population spiking activity that are best modeled using Poisson vs. Gaussian observations. Second, in real neural data we show that Poisson-PSID decodes motor behavior more accurately and with fewer latent dimensions than Poisson learning algorithms that simply model neural activity alone. Our results suggest that modeling the shared subspace between Poisson neural observations and continuous behavior signals can help study the neural basis of specific behaviors or cognitive processes of interest, and enhance brain-machine interfaces.

Disclosures: L.L. Oganesian: None. **O.G. Sani:** None. **M.M. Shanechi:** None.

Poster

390. Neurophysiology: Decoding and Neural Processing II

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Program #/Poster #: 390.15

Topic: E.05. Brain-Machine Interface

Support: NIH Director's New Innovator Award (DP2), Contract DP2MH126378

Title: Dynamical flexible inference of nonlinear embeddings of neural population activity

Authors: *H. ABBASPOURAZAD[1], E. ERTURK[2], B. PESARAN[3], M. M. SHANECHI[2]; [2]Electrical and Computer Engin., [1]USC, Los Angeles, CA; [3]Ctr. for Neural Sci., NYU, New York City, NY

Abstract: Neural population activity displays complex dynamical patterns that underlie our behaviors. These dynamical patterns can be modeled in terms of low-dimensional latent factors that evolve in time and explain behavior and neural activity. Much progress has been made by developing nonlinear neural network models of population activity that extract its latent factors. However, an unresolved challenge is to develop neural network models that not only allow for nonlinear latent factor modeling, but also enable flexible inference of these latent factors. To enable flexible inference, the model should be capable of both causal/real-time and non-causal inference simultaneously, and allow for inference in the presence of missing neural measurements. If realized, flexible inference of latent factors allows them to be seamlessly and accurately extracted, whether in real-time in neurotechnology applications or in causal perturbation experiments in neuroscience, whether non-causally to improve accuracy in basic science studies, or whether with partially missing neural measurements. Here, we develop a neural network model that addresses this challenge by enabling both flexible inference and accurate nonlinear description for neural population activity. We validate our method in nonlinear simulations and compare it to benchmark methods on neural data recorded across different brain regions during diverse behavioral tasks. We show that our method enables flexible inference capabilities in nonlinear modeling. Moreover, and despite enabling this new capability, the method also performs more accurately than linear and nonlinear benchmark methods in terms of neural prediction, behavior prediction, and extracting the latent manifold structure. By enabling the new capability of flexible inference in nonlinear neural network modeling and by enhancing neural description, this method can be used for diverse neurotechnology and neuroscience applications.

Disclosures: H. Abbaspourazad: None. **E. Erturk:** None. **B. Pesaran:** None. **M.M. Shanechi:** None.

Poster

390. Neurophysiology: Decoding and Neural Processing II

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Program #/Poster #: 390.16

Topic: E.05. Brain-Machine Interface

Support: NIH R01: R01MH123770

Title: Disentangling the effect of input from intrinsic neural dynamics in preferential dynamical modeling of neural-behavioral data

Authors: *P. VAHIDI, O. G. SANI, M. M. SHANECHI;
Electrical and Computer Engin., USC, Los Angeles, CA

Abstract: Studying neural population dynamics helps understand the computations that give rise to behavior. Much progress has been made in modeling neural population dynamics in terms of low-dimensional latent states. Beyond these efforts that non-preferentially model all dominant dynamics in neural data, recent work has shown the importance of preferentially modeling those neural dynamics that are behaviorally relevant, e.g. by developing a method termed PSID. However, a standing challenge is to incorporate the effect of measured inputs in preferential models. Doing so is important because it allows the model to further dissociate those neural dynamics that are intrinsic to a given brain region from those that are due to dynamic inputs to that region -- such as sensory inputs or recorded neural activity from upstream regions. Here, we develop a novel analytical projection-based method, termed input-PSID or IPSID, that can incorporate the effect of measured inputs in preferential dynamical modeling. By doing so, IPSID can dissociate intrinsic behaviorally relevant neural dynamics from other intrinsic neural dynamics as well as from input dynamics. We also extend IPSID for scenarios where some downstream regions of the input that affect behavior are not reflected in the neural recordings. We first simulate a brain that performs different behavioral tasks but has fixed intrinsic dynamics. We show that IPSID consistently finds the same intrinsic behaviorally relevant dynamics regardless of which task is used to collect the training data. We then apply IPSID to motor cortical population activity from two publicly-available datasets in which monkeys make reaching movements following task instruction sensory inputs. We take these task instructions as inputs in IPSID. We find that IPSID identifies distinct intrinsic behaviorally relevant dynamics that are more predictive of behavioral and neural data compared to prior methods. Taken together, we provide a new tool to dissociate intrinsic behaviorally relevant neural dynamics from other intrinsic neural dynamics as well as from input dynamics.

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Poster

390. Neurophysiology: Decoding and Neural Processing II

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Topic: E.05. Brain-Machine Interface

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NSF NCS BCS 1533672
NIH T32 NS07391
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Title: Constraints on the temporal sequencing of neural population activity

Authors: A. D. DEGENHART[1], E. M. GRIGSBY[3], ***E. OBY**[4], A. MOTIWALA[2], N. T. MCCLAIN[4], P. MARINO[5], A. P. BATISTA[5], B. M. YU[1];
[2]Electrical and Computer Engin., [1]Carnegie Mellon Univ., Pittsburgh, PA; [3]Biomed. Engin., [5]Bioengineering, [4]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Recent insights about the brain computations involved in sensory, motor, and cognitive processes such as decision making, motor control, and olfaction, conceptualize time-evolving neural population activity as trajectories in a low-dimensional space. Network models have posited that such trajectories evolve according to flow fields that arise from the underlying neural connectivity. However, it is unclear whether experimentally observed neural trajectories are actually constrained by flow fields. A key prediction of a flow field framework is that for any given initial state of neural population activity, the activity evolves only along the direction prescribed by the flow fields. Here we used a brain-computer interface (BCI) to test whether neural trajectories in motor cortex are constrained to follow paths as if set by a flow field, or if instead neural trajectories can be flexibly generated.
We recorded spiking activity of a population of ~90 neural units from three Rhesus monkeys, each implanted with a Blackrock multielectrode array in the motor cortex. The monkeys were trained to control a computer cursor by modulating their neural activity using a BCI that mapped the neural population activity to cursor position. This position BCI mapping provides animals with direct visual feedback of a 2D projection of their neural trajectories. Using a BCI mapping that captured movement intention, animals performed a two-target task, moving a cursor between diametrically opposed pairs of targets. Cursor trajectories in this task were highly overlapping. However, in other dimensions, neural trajectories exhibited distinct paths for each target, suggestive of an underlying flow field. To test whether this temporal structure could be violated, we provided the animal with feedback of a projection of neural activity where the trajectory separation between the distinct paths was strongest. When the monkey controlled the BCI cursor in this view, the trajectory separation persisted, consistent with the hypothesis that the temporal structure cannot be readily altered, hence implying it is a manifestation of underlying neural circuitry. As a more stringent test of this idea we directly challenged the animals to reverse their natural sequence of neural activity. Monkeys showed only a modest ability to modify cursor trajectories when incentivized to do so and within single sessions never approached the time-reversed sequence of neural activity. This suggests that the sequences of neural population activity observed in the cerebral cortex cannot be flexibly generated due to constraints imposed by underlying neural circuitry.

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Poster

390. Neurophysiology: Decoding and Neural Processing II

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NIH R01 HD071686
NSF NCS BCS 1533672
NIH T32 NS07391
Simons Foundation 543065
Burroughs Wellcome Fund
DSF Charitable Foundation 132RA03

Title: Characterizing neural population dynamics in primary motor cortex in the absence of arm movement

Authors: *A. MOTIWALA[1], E. R. OBY[2], E. M. GRIGSBY[3], A. D. DEGENHART[5], N. T. MCCLAIN[2], A. P. BATISTA[4], B. M. YU[1];
[1]Carnegie Mellon Univ., Pittsburgh, PA; [3]Biomed. Engin., [4]Bioengineering, [2]Univ. of Pittsburgh, Pittsburgh, PA; [5]Carnegie Mellon Univ. Library, Pittsburgh, PA

Abstract: Activity in the motor cortex has been shown to have highly structured time evolution during arm movements. However, it is difficult to determine in the context of overt arm movements whether the structure observed in population activity reflects the mechanical constraints of the arm or constraints due to the network architecture. We recorded activity of ~90 simultaneously recorded neural units using a multielectrode array in the motor cortex. Using a brain computer interface (BCI), we show that population activity exhibits highly structured time evolution even in the absence of overt arm movements. We characterize the geometry of neural population activity to address three questions: 1. what aspects of neural activity can be readily modified by animals based on task demands, 2. why activity is correlated along some sets of dimensions, and 3. why some features of population trajectories are not easily altered, even when required for task success.
We show that the overall geometry of motor cortical activity can be decomposed into several distinct population activity patterns and can be well described in terms of a dynamical system whose parameters are shared across all BCI target conditions. The dynamical model allowed us to decompose the population activity on individual experimental trials into several distinct ‘input driven’ and ‘recurrent’ model components. When animals controlled a cursor using a BC mapping that was well aligned with ‘input’ dimensions of the model, we observed that they were able to change their neural activity to flexibly control the directions in which the cursor moved. We also found that target dependent ‘inputs’ of the dynamics model tend to be confined to a smaller number of dimensions than those spanned by the population trajectories. This suggests that recurrent dynamics are responsible for propagating inputs through the population. In this

case, we would expect activity along 'input' dimensions to be correlated with changes in activity along dimensions that are recruited through the 'recurrent dynamics'. Since we expect 'recurrent dynamics' to reflect underlying network connectivity, constraints due to 'recurrent dynamics' may be difficult to violate on a short time scale. When animals are given cursor control using a BC mapping that is aligned with 'recurrent components', they are able to change the shape of cursor trajectories only modestly. And these changes are yoked to changes in activity along 'input' dimensions. In sum, by characterizing the geometry of population activity and by describing it in terms of a dynamical system, we are able to gain a more mechanistic understanding of observed features of population activity.

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Poster

391. Posture and Gait: Aging, Injury, and Disease I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 391.01

Topic: E.06. Posture and Gait

Support: Sentara Foundation

Title: Older persons with Parkinson's disease exhibit altered trunk and head acceleration patterns during standing balance

Authors: *S. MORRISON[1], **N. REILLY**[2], J. MOXEY[1], D. M. RUSSELL[1], A. A. GRUNSFELD[3];
[1]Rehabil. Sci., Old Dominion Univ., Norfolk, VA; [2]Womack Army Med. Ctr., Fort Bragg, NC; [3]Neurol., Sentara Hosp., Charlottesville, VA

Abstract: To maintain optimal balance control during many everyday tasks, stabilization of the head is critical for ensuring optimal visual and vestibular function. However, the combination of aging and age-related neurological diseases such as Parkinson's disease (PD) can severely impact a person's balance ability, often contributing to their increased risk of falling. This study was designed to examine differences in falls risk, postural motion, and trunk and head acceleration between twenty-five older persons diagnosed with Parkinson's disease and twenty-five neurologically healthy, older adults during standing balance tasks. Each person's falls risk was measured using the Physiological Profile Assessment (PPA), a validated tool which includes tests of vision, sensation, reaction time, posture, and leg strength. Values from each test are combined to provide an overall risk score with higher scores denoting greater falls risk. Balance was assessed under the conditions where vision (i.e., eyes open or closed) and the standing support surface (i.e., a firm or pliable surface) were altered. During the balance tasks, overall motion of the center of pressure (COP) were collected using a force platform and assessed. Body accelerations were collected using triaxial accelerometers affixed to the head and lower trunk

segments. For the acceleration signals, differences in amplitude (i.e., RMS) and attenuation from the trunk to the head (in the medio-lateral (ML) and anterior-posterior (AP) directions) were assessed. As expected, the results revealed that the PD individuals exhibited increased falls risk compared to the healthy elderly. The PD group also demonstrated greater postural (COP) motion and increased acceleration for the trunk and head compared to the healthy older adults. Reduced attenuation of acceleration between the trunk and head was also seen for the PD persons. Overall, these findings suggest that during standing balance tasks, individuals with PD exhibited a reduced ability to accommodate upper body accelerations. These changes could be linked to a decline in head control, which could be an important contributing factor to the increased falls risk commonly found for individuals with PD.

Disclosures: S. Morrison: None. **N. Reilly:** None. **J. Moxey:** None. **D.M. Russell:** None. **A.A. Grunsfeld:** None.

Poster

391. Posture and Gait: Aging, Injury, and Disease I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 391.02

Topic: E.06. Posture and Gait

Support: NSF Grant 1815506

Title: Effects of Mock Head Mounted Display Weight on Spatiotemporal Gait Parameters in Younger and Older Adults

Authors: *A. S. PADILLA, M. TOEPFER, A. PEER, K. PONTO, K. A. PICKETT, A. H. MASON;
Univ. of Wisconsin, Madison, Univ. of Wisconsin, Madison, Madison, WI

Abstract: Previous studies have compared walking in virtual (VR) and natural environments, however, these studies have primarily focused on the distinct visual characteristics of the environments. It remains unclear whether previously reported changes in spatiotemporal gait parameters when walking in VR were influenced by the weight of the VR headset. In this study, we compared walking while wearing a mock head mounted display (HMD) to walking without the HMD. Our mock HMD was designed to match the mass and weight distribution of an HTC Vive Pro VR headset, but was see-through to allow for visual feedback of the natural environment. We were interested in quantifying changes in spatiotemporal gait parameters in these two conditions across younger and older adults. ***Method:*** Twenty younger adults (mean = 23.67 years) and 20 older adults (mean = 73.15 years) were asked to walk over a 20m GAITRite gait mat in two different Conditions:(i) without HMD for 10 trials (ii) with HMD for 10 trials. A 2x2 repeated measures ANOVA was used to examine the main effects and interactions of Age Group and Condition on cadence, step extremity ratio (SER), heel to heel base of support (BOS), percent time in double support (%DS) and normalized velocity. ***Results:*** There were no main

effects of Age Group on any of the spatiotemporal gait measures ($p>0.05$). In contrast, there were main effects of Condition on cadence ($F_{1, 38}=15.93$, $p<.001$), SER ($F_{1, 38}=67.68$, $p<0.001$), BOS ($F_{1, 38}=7.97$, $p=.008$), %DS ($F_{1, 38}=28.92$, $p=.001$) and normalized velocity ($F_{1, 38}=5.61$, $p=.023$). Specifically, cadence (112.153 ± 1.42) and step extremity ratio (.782 ± .011) were lower and %DS was greater (25.05 ± .456) for the HMD On condition than the HMD Off condition (cadence = 113.67 ± 1.4; step extremity ratio = .806 ± .011; %DS = 24.18 ± .471). While base of support was also greater for the HMD On condition (9.86 ± .36) than the HMD Off condition (9.56 ± 0.36), there was also a significant interaction between Group and Condition ($F_{1,39}=10.14$, $p=0.003$) for this measure. The interaction indicated that young adults used the same BOS of support for both the HMD Off and On conditions (9.9 ± 0.5 cm), whereas the older adults used a larger base of support for the HMD On condition (9.9 ± 0.5 cm) compared to the HMD Off condition (9.2 ± 0.5 cm). Finally, normalized velocity was less for the HMD On condition (1.503 ± .031) than the HMD Off condition (1.529 ± .031). ***Conclusion:*** The results of the current study suggest that the weight of the head mounted display may contribute to more conservative gait in virtual compared to natural environments. Our results also suggest that older adults may compensate for this additional destabilizing weight by increasing base of support.

Disclosures: **A.S. Padilla:** None. **M. Toepfer:** None. **A. Peer:** None. **K. Ponto:** None. **K.A. Pickett:** None. **A.H. Mason:** None.

Poster

391. Posture and Gait: Aging, Injury, and Disease I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 391.03

Topic: E.06. Posture and Gait

Support: NRF-2021R1A6A3A13039817
NRF-2021R1A2C3004572

Title: Adssl1, a muscle specific enzyme, suppresses skeletal muscle aging by inducing myogenic fusion

Authors: *S. WON, J. LEE;
Dept. of Hlth. Sci. and Technology, Samsung Advanced Inst. of Hlth. Sci. & Techn, Sungkyunkwan Univ., Seoul, Korea, Republic of

Abstract: Compared to young myoblasts, older myoblasts have a defective fusion with multinucleated myotubes. Failure of myogenic cell fusion prevents damaged muscle from being rescued, resulting in muscle mass loss and sarcopenia. Therefore, it is important to elucidate the mechanism that allows myoblasts to fuse in order to understand the pathology of aging-related muscle disorders. Studies to data suggest that up to 75% of gene expression is associated with aging. Studies using rat revealed that the expression of *Adenylosuccinate synthase like 1* (*Adssl1*) decreased with aging in skeletal muscle. On the other hand, it has been suggested that the

expression of *Adssl1* is increased when satellite cells are activated to replenish portion of damaged tissue as a result of muscle injury. In the present study, we investigated whether Adssl1 is required for myogenic fusion to elucidate the role of Adssl1 in muscle aging. Through a loss-of-function study of *Adssl1* using the mouse myoblast line C2C12, we confirmed that depletion of *Adssl1* downregulates myogenic markers, including myogenin and desmin, suggesting that Adssl1 is required for myoblast fusion. Next, we investigated the relationship between age-related gait defects and decreased expression of *Adssl1* using 3 month (n=4) and 22 month old (n=8) C57BL6/J mice. We performed the rota-rod test, which has been used to evaluate the walking ability of mice, to identify genetic factors that influence age-related muscle atrophy and gait. Most of the old mice showed gait disturbance, but 2 out of 8 did not show a significant decrease in their ability to walk compared to the young mice. Therefore, old mice were divided into two groups according to their walking ability. We then examined changes in gene expression in the gastrocnemius (GA) muscle, which acts as an accelerator that propels the body forward while walking. As expected, expression of the *Adssl1* gene was reduced in aged mice with walking disability. In addition, we found that the expression of *Adssl1* was not reduced in aged mice without severe gait difficulties. These results provide evidence for a correlation between *Adssl1* expression and muscle aging leading to impaired gait. Taken together, our findings suggest that elucidating the mechanism of myogenic fusion regulation by Adssl1 is important for understanding the mechanisms of muscle aging inhibition.

Disclosures: S. Won: None. **J. Lee:** None.

Poster

391. Posture and Gait: Aging, Injury, and Disease I

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Program #/Poster #: 391.04

Topic: E.06. Posture and Gait

Support: Noel Foundation Grant

Title: Patient centered neuro-rehabilitation post-COVID-19: Case Study Series

Authors: *S. TINSLEY, M. MORGAN;
Louisiana State Univ. Hlth. - Shreveport, Louisiana State Univ. Hlth. - Shreveport, Shreveport, LA

Abstract: Purpose/Hypothesis: In June of 2020, the LSUHS SAHP neurologic clinical faculty began treating patients with significant post-COVID-19 sequalae including neuropathies, debility/fatigue, pulmonary dysfunction, dizziness, pain, and upper motor neuron symptoms. Since this was very early in the COVID-19 pandemic, we developed a standard set of assessments across multiple domains of the International Classification of Functioning, Disability and Health (ICF) model to assess functional disabilities following the recommendations from the SARS pandemic of 2003. The purpose of these case studies is to

describe how patient-centered care, revolving around the patient's goals, values, and individual physical needs, resulted in improved outcomes in patients with very different residual impairments during post-COVID recovery. **Case Description:** A standard set of assessments across multiple domains including endurance, oxygen consumption, cognition, strength, fatigue, sleep and participation were established as part of the LSUHS Post-COVID Recovery Program. A series of patients are highlighted to demonstrate the varied sequalae that patients may exhibit post COVID infection and how a patient-centered approach can result in overall improvements at all levels of the ICF model even with such varied functional disabilities. Each patient had a different course of disease expression and medical intervention. All patients were referred to the LSUHS Faculty Practice Clinic for rehabilitative care to address their specific impairments after their initial infection resolved. Each therapeutic program was developed specifically to address their specific individual impairments and disabilities. Program effectiveness was evaluated by using Minimal Detectable Change (MDC) and/or Minimal Clinical Important Difference scores based on standardized norms. **Results:** All patients showed significant improvement in both subjective and objective assessments including fatigue, cognition, endurance, strength, functional mobility, sleep and participation. **Conclusions:** Using outcome measures based on findings during the SARS-2003 outbreak, these patients treated made significant progress in all functional outcome measures, with the most significant and meaningful change for each patient being improvement in the Patient Specific Functional Scale emphasizing the importance of patient centered care. **Clinical Relevance:** Results from these case studies displays that patient-centered care aimed at addressing specific impairments and disabilities can have positive outcomes after infection with COVID-19.

Disclosures: S. Tinsley: None. **M. Morgan:** None.

Poster

391. Posture and Gait: Aging, Injury, and Disease I

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Topic: E.06. Posture and Gait

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Jascha Foundation (No. 2021-0110)
"Søster og Verner Lipperts Fond"
"Parkinsonforeningen"
"Frimodt-Heineke Fonden"

Title: Motor assessment in translational porcine models by pressure-sensitive gait mat analysis

Authors: *J. B. STEINMÜLLER[1,2], K. H. BINDA[3,4], T. P. LILLETHORUP[3,4], B. SØGAARD[1], D. ORLOWSKI[1], A. LANDAU[3,4], C. R. BJARKAM[2], J. C. H. SØRENSEN[1], A. N. GLUD[1];
[1]CENSE, Dept. of Neurosurg. & Dept. of Clin. Med., Aarhus Univ. Hosp. & Aarhus Univ.,

Aarhus N, Denmark; [2]Dept. of Neurosurg. & Dept. of Clin. Med., Aalborg Univ. Hosp. & Aalborg Univ., Aalborg, Denmark; [3]Dept. of Nuclear Med. & PET-Center, Aarhus Univ. Hosp., Aarhus, Denmark; [4]Translational Neuropsychiatry Unit, Aarhus Univ., Aarhus, Denmark

Abstract: The increasing use of minipigs in neuroscience has resulted in numerous translational models of neurologic diseases, many of which implicate the motor system. Induced pathologies manifest as motor deterioration, which may be determined from neurological examination. As such assessments may be both investigator-dependent and bias-susceptible, quantitative methods constitute useful alternatives to detect motor deterioration and monitor progression, e.g., in chronic models of Parkinson's disease (PD). Accordingly, gait analysis has been used to evaluate motor function of minipigs, but often involves extensive camera-sensor-based research setups. To investigate a methodologically simpler alternative, we used a pressure-sensitive gait mat (GAIT4Dog® / GAITFour®, CIR Systems Inc., NJ, US) to characterize normal quadruped gait parameters of 7 healthy, female minipigs (age 7-10 months, weight 19.2-26.5 kg). Then, we induced a unilateral lesion using stereotaxic microinjections of 6-hydroxydopamine in the right medial forebrain bundle resulting in a hemi-parkinsonian phenotype (n=5). We compared with saline sham lesions (n=2) as controls and repeated the gait analysis to uncover pathological gait dynamics. We determined symmetric gait characteristics of step length, stride length, stance time, and stance % across the four extremities, but found a frontally placed center of gravity seen by a total pressure index skewed towards the frontal limbs. We found significant variation of gait parameters across healthy animals. Post-lesion gait dynamics were characterized by a significantly reduced mean velocity from 102.4 cm/sec (*SD* = 8.53) at baseline to 90.42 cm/sec (*SD* = 11.82) (*P* = 0.0275). Also, in the ipsilateral right limbs we found a significantly increased median stance time from 0.295 sec [IQR = 0.258-0.343] at baseline to 0.32 sec [IQR = 0.298-0.418] (*P* = 0.006) in the PD animals, but not in the controls. The step length was ipsilaterally affected in the PD animals, where the mean step length decreased from 26.21 cm (*SD* = 2.20) at baseline to 25.53 cm (*SD* = 2.34) (*P* = 0.008). These findings are consistent with the clinical bradykinesia that can be seen in PD animals. While pathological gait dynamics in some animals were not clinically evident, we managed to detect even subtle motor deterioration in the gait analysis. Even so, we emphasize the importance of using the same investigators to obtain the sufficient scientific rigor and validity of gait data. In conclusion, our findings suggest that pressure-sensitive gait mat analysis is a sensitive, useful, and reliable tool to monitor motor deterioration in translational porcine models of neurological disorders.

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Poster

391. Posture and Gait: Aging, Injury, and Disease I

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Program #/Poster #: 391.06

Topic: E.06. Posture and Gait

Support: NIH/NIGMS U54GM104942
NIH Award Number 1 R03 HD099426-01A1

Title: Spatiotemporal Modification and Asymmetric Loading of Lower Limbs Causes Sensorimotor Adaptation in Humans

Authors: *E. HERRICK[1], S. YAKOVENKO[1,2,3,4];
[1]Chem. and Biomed. Engin., [2]Human Performance, [3]Neurosci., West Virginia Univ., Morgantown, WV; [4]Rockefeller Neurosci. Inst., Morgantown, WV

Abstract: Chronic limb asymmetry during locomotion can lead to musculoskeletal trauma and can be prevented by targeted training. Adaptation to walking on a split-belt treadmill with a large limb speed difference is an example of rehabilitation that targets limb asymmetries caused by a preference to an uninjured side. Yet, the strategy of using a limb speed difference is behaviorally related to turning and not to limb preference. Here, we studied imposed kinematic and kinetic constraints of stepping using a passive orthosis and feedback about limb loading to modify limb dynamics and to induce and manipulate stepping asymmetry. We hypothesized that both these constraints would cause persistent locomotor asymmetry by affecting limb preference. Also, we hypothesized that applying the constraint to the opposite limb after the period of asymmetric walking would increase the rate of symmetrical gait recovery. Uninjured healthy adults (N = 23) walked at 1.0 m/s on a treadmill instrumented with force plates in three conditions: unconstrained, constrained, and washout unconstrained. We used an asymmetric index based on double stance times to quantify the subject's gait asymmetry in each condition. Our results showed that a purely spatiotemporal gait asymmetry imposed by the kinematic constraint did not result in persistent adaptation ($p = 0.29$). However, when combined with an asymmetric loading between the limbs, the condition does induce a persistent adaptation ($p = 0.03$). This suggests that uninjured locomotor systems can cope with purely kinematic asymmetries without the use of persistent adaptations, and that loading is a key variable for evoking adaptations that require prolonged de-adaptation. Moreover, the reversal of asymmetry in subjects with induced asymmetric adaptation resulted in faster than control recovery ($p = 0.02$). This suggests that the principles of constraint-induced movement therapy for upper limbs post-stroke can also be applied in rehabilitation for lower-limbs.

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Poster

391. Posture and Gait: Aging, Injury, and Disease I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 391.07

Topic: E.06. Posture and Gait

Title: Vestibular processing for balance control during walking in Parkinson's disease

Authors: *M. ARCODIA[1], A. SANSARE[2], J. J. JEKA[1], H. REIMANN[1];
[1]Dept. of Kinesiology and Applied Physiol., [2]Physical Therapy, Univ. of Delaware, Newark, DE

Abstract: Vestibular processing for balance control during walking in Parkinson's disease**M. ARCODIA**[1], A. SANSARE[2], J. J. JEKA[1], H. REIMANN[1]; [1]Dept. of Kinesiology and Applied Physiol., [2]Physical Therapy, Univ. of Delaware, Newark, DE
Motivation: Parkinson's disease (PD) is a neurodegenerative disorder that results in neural processing deficits of the sensory system. Impaired processing of the sensory system can lead to loss of balance and increased fall risk. The goal of this study is to understand how individuals with PD use vestibular information for balance control during walking. **Research Question:** How does PD affect the processing of vestibular information for balance control during walking?**Methods/Approach:** Nine healthy older adults and eleven individuals with PD walked on an instrumented treadmill for 10 two-minute trials. During the trials, subjects received intermittent stimulation to their vestibular system via a small electrical current to electrodes placed on the subject's mastoid processes. This causes a perceived fall to the side depending on direction of the electrical current. We quantified the overall center of mass (CoM) response to the vestibular stimulation by taking the integral of the lateral CoM trajectory response following stimulation. We also analyzed the magnitude and timing of the peak CoM change. **Results:** Following vestibular stimulation, both groups shifted their CoM in the opposite direction of the perceived fall. The were no statistically significant differences of overall CoM displacement and peak CoM value between groups. However, the time to reach the peak CoM trajectory for the PD group was greater than the healthy older adult group. **Conclusion/Impact:** The time delay in response to a vestibular perturbation is consistent with other findings that individuals with PD have difficulty producing force in a timely manner, i.e. while they are capable of generating the appropriate motor action, they do so slower than neurotypical controls. Individuals with PD had similar overall CoM displacement and peak CoM value which suggests vestibular processing is largely intact in PD. Since motor responses following a vestibular perturbation is similar between PD and older adults, we must study how other sensory processing systems, such as vision, effect balance control during walking in PD.

Disclosures: M. Arcodia: None. **A. Sansare:** None. **J.J. Jeka:** None. **H. Reimann:** None.

Poster

391. Posture and Gait: Aging, Injury, and Disease I

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Topic: E.06. Posture and Gait

Support: NINDS K12 NS080223
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Michael J Fox Foundation

Title: Cortical and Pallidal Neural Changes During Turns in People with Parkinson's Disease

Authors: *J. BATH, K. H. LOUIE, J. BALAKID, H. FEKRI AZGOMI, D. WANG;
UCSF, San Francisco, CA

Abstract: Patients with Parkinson's Disease (PD) often face gait and balance impairments, such as difficulties with turning, which can lead to increased falls and reduced quality of life. To develop effective therapies, we first need to understand the **neural circuitry involved in turn regulation, and how it becomes altered in PD.** We performed multisite neural recordings from two PD patients (one male and one female) implanted with investigational bidirectional Summit RC+S neural interfaces. These were attached to subdural electrodes over the cortex (premotor and motor areas) and deep brain stimulation (DBS) leads in the pallidum. Participants performed L-directed 180-degree turns in between bouts of normal overground walking while cortical and subcortical local field potentials (LFPs) were recorded, while the patient was off dopamine medication. Corresponding biomechanical data was aligned to neural data with turn intervals and inter-turn steps marked for each turn using the angular velocities of the pelvis and shanks. LFP spectral power was analyzed around inter-turn heel-strike and toe-off events for three frequency bands (alpha, beta, theta) at all cortical and pallidal areas. Intrasubject comparisons were performed using repeated measures one-way ANOVA testing of the subject's turns at each neural location and for the three frequency bands examined. We found that off-medication, L-directed 180-degree turns were associated with LFP power changes in both subjects among alpha (8-12 Hz), beta (13-30 Hz), and theta (4-8 Hz) frequency bands at the pallidal and cortical locations, differentiating between heel-strike and toe-off inter-turn gait events. Specifically, the globus pallidus internus (GPi) showed greater spectral power in the alpha band during toe-off compared to heel-strike inter-turn gait events. Conversely, greater spectral power was seen during heel-strike compared to toe-off in the beta band at the GPi and the alpha band at the premotor cortex. Statistical analysis showed that the differences in power between toe-off and heel-strike did not reach significance but trended towards it, with next steps to process additional patient turns for increasing statistical power.These exploratory results show that there is dynamic, inter-turn neural modulation occurring at pallidal and cortical areas across various frequencies coinciding with heel-strike and toe-off during turns, potentially mediating differences in turn performance among people with PD. This information could be used in the future as controls for adaptive neuromodulatory interventions to improve patient turn performance and safety and facilitate return to physical independence.

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Poster

391. Posture and Gait: Aging, Injury, and Disease I

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Program #/Poster #: 391.09

Topic: E.06. Posture and Gait

Support: NIH/NINDS K12 NS080223
Burroughs Wellcome Fund Career Award for Medical Scientists
Michael J Fox Foundation

Title: Pallidal and Cortical Local Field Potentials Show Gait Related Changes in Parkinson's Disease Patients During Free Walking

Authors: *K. H. LOUIE[1], J. BALAKID[1], J. BATH[1], H. FEKRI AZGOMI[1], J. CHOI[2], P. STARR[1], D. WANG[1];
[1]Neurolog. Surgery, Univ. of California - San Francisco, San Francisco, CA; [2]Applied Physiol. & Kinesiology, Univ. of Florida, Gainesville, FL

Abstract: The supraspinal network activities involved during walking is poorly understood in humans. Currently, there are a limited number of studies on gait-related changes in basal ganglia and cortical local field potentials (LFP), especially within the pallidum. In this study we recorded sensorimotor cortical and pallidal LFPs from patients with Parkinson's disease (PD) during natural, overground walking using the Medtronic RC+S bidirectional neurostimulator. Six PD individuals consented to participate in this study. Four individuals underwent bilateral implantation of deep brain stimulation leads targeting the globus pallidus interna (GPi), and subdural cortical paddles overlying the primary motor (M1) and premotor (PM) cortices. Two individuals underwent unilateral implantation in the left GPi, with subdural cortical paddles overlaying the M1 and primary sensory (S1) cortices. Participants walked overground at a self-selected speed for 200 steps. Subcortical and cortical LFPs were simultaneous recorded at 500 Hz. Additionally, gait kinematics were measured using external sensors (Delsys and Xsens). LFP time frequency and subcortical-cortical coherence analysis were performed and synchronized to each participants gait cycle. We found cyclical, gait-cycle changes in LFP power and subcortical-cortical coherence in the theta/alpha (4-12 Hz), beta (13-30 Hz), and low gamma (30-50 Hz) band frequencies. Increases in theta/alpha power was mainly associated with subcortical areas, while beta and low gamma increases were mainly associated with M1. Power increases in the subcortical area occur during weight acceptance of the contralateral leg, whereas M1 power increases occur prior to heel strike of the contralateral leg. Increases in coherence were seen predominately in the alpha band during contralateral leg weight acceptance. These results show that there are changes in motor cortical and GPi low frequency LFP power and coherence related to the gait cycle in PD patients. These dynamic changes may be used as control signals for future adaptive DBS applications targeted towards gait improvement.

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Poster

391. Posture and Gait: Aging, Injury, and Disease I

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Program #/Poster #: 391.10

Topic: E.06. Posture and Gait

Support: NIH Grant 1R01AG073157-01

Title: Assessing head stabilization during the instrumented Timed Up and Go test: comparison to level walking

Authors: *C. AGATHOS, A. VELISAR, N. SHANIDZE;
The Smith-Kettlewell Eye Res. Inst., San Francisco, CA

Abstract: The Timed Up and Go (TUG) is a common clinical functional balancetest, involving standing, walking and turning . In research, it is often used to complement findings on sensorimotor changes, notably in the context of vestibular dysfunction (e.g., Nishi et al., 2017). Thanks to wearable technology, the instrumented TUG is increasingly used to obtain objective postural and gait measures, sensitive to mobility changes, e.g., for identifying fall risk in older adults (Weiss et al., 20010). Head stabilization is important for providing a stable reference for the visual and vestibular systems and to maintain stable gaze (Assaiante & Amblard, 1995). To maintain stability, the trunk and lower limbs attenuate accelerations otherwise experienced by the head (Ratcliffe & Holt, 1997). Stabilizing the head in space is primarily driven by vestibular signals (Bronstein, 1988) and those with vestibular dysfunction tend to adopt more rigid strategies, blocking the head on the trunk (Pozzo et al., 1991). With aging, this rigid stabilization is also evident during turns (Forsell et al., 2017) and among fall-prone older adults (Wright et al., 2012). Examining head stabilization during TUG may thus provide a dual benefit: as a measure of functional balance and to reveal adaptive changes in vestibular integration. It is unknown, however, whether body coordination during TUG is representative of daily life. We thus sought to determine whether head stabilization as measured during TUG is comparable to natural behavior, focusing on the walking phase of TUG and comparing head and trunk movement to normal walking. Seven participants (age range: 30-60) performed the TUG and walked on a predefined trajectory at their habitual pace. Wireless inertial measurement units were attached to their head, chest and right ankle. Sensors were synchronized via a low energy electromagnetic pulse. Ankle data served for event detection. We examined acceleration in the horizontal plane for the head and chest. Rotation angles in pitch and roll in world coordinates were extracted for both segments from gyroscope data, calculated with respect to a reference posture. Anchoring indices were calculated based on these rotations to indicate the stabilization strategy used: head articulated in space or rigid on the trunk (Amblard et al. 1997). Data were compared using paired (non)parametric tests. While we did find a significant difference in chest acceleration in the horizontal plane, there was no difference in the head stabilization strategies used by our participants between the two tasks. Only one participant showed a rigid stabilization strategy in both tasks in our sample

Disclosures: C. Agathos: None. **A. Velisar:** None. **N. Shanidze:** None.

Poster

391. Posture and Gait: Aging, Injury, and Disease I

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Program #/Poster #: 391.11

Topic: E.06. Posture and Gait

Support: Blue Cross Blue Shield of Michigan Foundation

Title: Impact of uilateral brachial plexus injury on dynamic balance control in children

Authors: E. A. BIN MULAYH[1], R. N. LOGUE COOK[2], L. J. S. YANG[3], *S. BROWN[1];
[1]Univ. of Michigan Sch. of Kinesiology, Ann Arbor, MI; [2]Univ. of Michigan, Ann Arbor, MI;
[3]Neurosurg., Michigan Med., Ann Arbor, MI

Abstract: Introduction: Unilateral birth-related damage to the brachial plexus - a complex network of nerves innervating the shoulder and upper limb - is associated with muscle weakness and altered somatosensation that often persists despite conservative or neurosurgical treatment. To what extent such asymmetric arm deficits impact postural control in this population is not well understood. **Methods:** Twelve participants with neonatal brachial plexus palsy (NBPP) who were conservatively treated (mean age: 11.6 ± 3.2y) and 10 who underwent surgical reconstruction of the brachial plexus (mean age: 10 ± 1.1y) stood on a force plate and traced a maze using their outstretched arm. The maze display was mounted at chest height and the task was performed with the unaffected and the affected arm. Dependent measures were center of pressure (COP) displacement and smoothness. Sixteen age-matched controls (mean age: 11.5 ± 2.4y) were included for comparison purposes. Assessments were followed by one week of wrist accelerometry to capture self-initiated arm movement. Relative movement of the two arms was expressed as symmetry ratios. **Results:** When performing the task with their affected arm, total COP displacement was greater in the surgical NBPP group compared to the nonsurgical and control groups ($p<0.001$). COP displacement in the surgical group was 69% greater ($p<0.001$) and more irregular ($p<0.05$) when the task was performed by the affected compared to the unaffected hand. Lastly, COP sway area was predictive of accelerometry-based movement magnitude but only in the surgical NBPP group ($p<0.05$). **Conclusions:** These findings demonstrate that long term impairment due to NBPP is associated with greater postural sway during the performance of goal-directed arm movements, particularly in cases where nerve reconstruction as occurred. In such cases, impaired postural control may contribute to a reduction in arm use in real world settings and underscores the importance of whole body assessment when considering rehabilitation strategies.

Disclosures: E.A. bin Mulayh: None. **R.N. Logue Cook:** None. **L.J.S. Yang:** None. **S. Brown:** None.

Poster

391. Posture and Gait: Aging, Injury, and Disease I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 391.12

Topic: E.06. Posture and Gait

Support: NIH Grant TL1TR002375
Virginia Horne-Henry Fund
Grand Challenges Seed Fund, School of Education, University of Wisconsin-Madison

Title: Parameterization of frequency-dependent lower-limb coordination during human standing after stroke

Authors: J. BARTLOFF[1], K. NICHOLS[2], W. OCHS[3], J. FOX[1], *K. GRUBEN[4];
[1]Univ. of Wisconsin, Madison, WI; [2]Univ. of Wisconsin Madison, Madison, WI; [3]Biomed. Engin., Northwestern Univ., Chicago, IL; [4]Univ. Wisconsin, Madison, WI

Abstract: Postural instability disproportionately afflicts humans with history of stroke, incurring substantial social, economic, and medical costs. A key impediment to fall prevention is insufficient understanding of the coordination mechanisms that enable humans to remain upright while standing. Traditional measures based on the center of pressure (CP) of the ground on foot force (F) are inadequate to explain angular momentum control, and the imprecision in joint torque and muscle activity estimations precludes capturing the subtle adjustments that maintain posture. Our approach analyzes F to summarize the control of sagittal-plane translational and rotational body motion. We quantify the relationship between the CP and the ratio of horizontal F to vertical F. Within narrow frequency bands (0.5 Hz width from 0.25 to 6.25 Hz), that relationship is nearly linear, indicating that the F vectors pass near a fixed point in space. The height of that intersection point (IP) varies with frequency, being located above the center of mass (CM) for <~2Hz and below the CM at higher frequencies. In studies of 10 young humans (<30 years) without history of stroke and 9 older (>30 years) humans with stroke history, we show that a three-parameter exponential relationship can sufficiently parameterize (shape, IP height offset, and frequency offset) the frequency dependence of the behavior. The paretic limb differed from the non-paretic limb in only the height parameter ($p=0.0036$), with the non-paretic limb being higher than the paretic limb. The paretic limb did not differ from the young control participants in any of the parameters. This observation supports the hypothesis that stroke causes a shift in relative muscle activation across the hip, knee, and ankle that produces F with an abnormal orientation which can lead to various compensatory behaviors that characterize post-stroke behavior. However, the coordination coupling with frequency is not disrupted by stroke. These characterizations may be a useful measure of deficit and suggest an explicit target for rehabilitation that is currently not addressed.

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Poster

391. Posture and Gait: Aging, Injury, and Disease I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 391.13

Topic: E.06. Posture and Gait

Support: Newcastle Biomedical Research Centre
Newcastle University

Title: Greater complexity of leg muscle activity during walking in females and people with Parkinson's disease: an insight into different motor control strategies?

Authors: A. AMRAPALA[1], J. EELTINK[2], L. ALCOCK[3], C. WATSON[4], ***A. PANTALL**[4]; [1]Fac. of Med., Chulalongkorn Univ., Bangkok, Thailand; [2]Leiden Univ. of Applied Sci., Leiden, Netherlands; [3]Clin. Ageing Res. Unit, [4]Newcastle Univ., Newcastle upon Tyne, United Kingdom

Abstract: Gait disturbance, a common feature in Parkinson's disease (PD), is associated with increased risk of falls, immobility, and decreased quality of life. Gait electromyography (EMG) may yield information regarding underlying motor neural networks. Nonlinear variability metrics, which are measures of signal changes at different timepoints, are related to underlying control systems. Complexity is a measure of nonlinear variability. The aim of this study is to assess how complexity of gait EMG is affected by 1) ageing and Parkinson's disease, 2) biological sex, and 3) muscle. The clinical relevance of the study is to understand motor control of gait in PD. EMG complexity may also be an important PD biomarker and indicator of disease progression as well as response to interventions. 40 participants were involved; 19 people with PD, 9 healthy older adults (OA)>60 yrs and 12 healthy young adults (YA) 20-40 yrs. Participants walked overground for 5 minutes. EMG signals from the leg muscles lateral gastrocnemius (LG), medial gastrocnemius (MG), soleus (SO) and tibialis anterior (TA) were recorded. Gait events were detected with a motion capture system. Nonlinear variability was calculated using multiscale entropy course-grained to timescales 1 to 40 with optimised parameters m = 2 and r = 0.15. The complexity index (CI) was then determined. A linear mixed effects model assessed the effect of group (PD, OA, YA), sex (male, female), muscle (LG, MG, SO, TA) and interaction effects. Significance was set at $p<0.05$. People with PD had greater complexity compared to OA and YA (PD - 41.78, OA - 33.32, YA - 31.89), females had higher complexity then males (F - 39.97, M -31.35), and the CI of the 2 joint muscle LG was significantly higher than its 1 joint synergist SO (LG - 37.82, SO - 31.90). There was a significant interaction effect for Group*Sex with males displaying a difference in CI between PD (39.71) and OA (24.60), whereas females displayed no significant difference between PD (43.85) and OA (42.04). The interaction effect Group*Sex*Muscle was significant. In males, LG, MG, and TA complexity was greater in PD than in OA. Greater complexity of EMG in people with PD suggests altered motor control. The difference in CI between males and females indicates different neuropathological processes. This may explain different symptoms presenting in females with PD including greater dyskinesia. The complexity of muscles differs which may relate to their role (one or two joint) and muscle fibre phenotype (slow type I, fast type II). In summary, EMG complexity may reveal information about neural mechanisms and the different effect that PD has on males compared to females as well as muscle function.

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Poster

391. Posture and Gait: Aging, Injury, and Disease I

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 391.14

Topic: E.06. Posture and Gait

Support: 1R21NR017484-01A1

Title: Stepahead: wearable real-time audio feedback system for gait and balance rehabilitation in parkinson's disease

Authors: *N. MUTHUKRISHNAN[1], D. D. PATEL[2], H. A. SHILL[3], J. J. ABBAS[4], N. KRISHNAMURTHI[5];
[1]Ctr. for Adaptive Neural Systems, [2]Arizona State Univ., Tempe, AZ; [3]Muhammad Ali Parkinson Center, Barrow Neurolog. Institute, St. Joseph's Hosp. and Med. Ctr., Phoenix, AZ; [4]Inst. for Integrative and Innovative Res., Univ. of Arkansas, Fayetteville, AR; [5]Ann & Robert H. Lurie Children's Hosp. of Chicago, Chicago, IL

Abstract: Real-time feedback (RTF) of gait and balance measures has been investigated as a technique to influence walking patterns and postural stoop in Parkinson's disease (PD). We have previously investigated the effects of visual feedback of step length and back angle during treadmill walking and observed that people with PD could follow real-time feedback and utilize it to modulate their gait and stooped posture favorably in a manner that transferred, at least acutely, to overground walking. That study, and most others with RTF in the literature, utilized a laboratory-based optical motion capture system and a feedback monitor. Recent advances in wearable sensors can be leveraged to develop wearable real-time feedback (WRTF) system that can evaluate movements and provide feedback during daily activities to improve walking and posture in PD. The work presented here addresses the challenges of obtaining accurate gait and posture measurements in real-time from wearable sensors and investigates the effects of real-time auditory feedback of step length and back angle on gait and posture in people with PD. We have developed a smartphone-based application to detect gait events and calculate measures such as step length and trunk uprightness in real-time using data streaming from wearable sensors (inertial measurement units) that were placed on the feet and T7-T8 vertebrae region of the back. Gait and posture measures were calculated from 60-meter walking trials from people with and without PD. Preliminary results demonstrate reliable gait event detection and subsequent calculation of step length, step time, and back angle. In an ongoing study, we are testing the validity of the measures from the wearable system against laboratory-based gold-standard measurements as well as evaluating the immediate effects of auditory feedback provided by the WRTF system based on the computed measures in improving step length and reducing postural stoop during overground walking by people with PD.

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Poster

391. Posture and Gait: Aging, Injury, and Disease I

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 391.15

Topic: E.06. Posture and Gait

Support: SimGait Sinergia project funded by the Swiss National Science Foundation, grant agreement No. 177179

Title: Studying the effect of neural spasticity and contracture in the hamstring muscles on cerebral palsy crouch gaits using neuromuscular simulations

Authors: *A. DI RUSSO[1], S. ARMAND[2], A. IJSPEERT[1];
[1]Biorobotics Lab., EPFL - École polytechnique fédérale de Lausanne, Lausanne, Switzerland;
[2]Kinesiology Lab., Univ. Hosp. of Geneva, Geneva, Switzerland

Abstract: Cerebral palsy (CP) patients present various gait impairments due to neurological and biomechanical pathologies. This condition leads to the problematic investigation of the effect of each impairment on the patient's gait deviation. Neuromuscular simulations are a powerful tool to perform what-if scenarios and isolate the effect of neuromechanical properties. However, current neural controllers rely on a state machine that activates specific sensory feedback mechanisms at specific times of the gait cycle. These controllers are not designed to study neuropathological conditions like spasticity, and additional components should be included, considering facilitatory and inhibitory mechanisms. This study focuses on replicating the spasticity and contracture of the hamstrings muscle typical of CP patients walking in crouch gait. We use a novel neuromuscular controller implemented in the simulation framework SCONE, accounting for sensory inhibitory and excitatory mechanisms given by Ia, IIa, Ib sensory fibers, Renshaw cells, and feed-forward patterns generated by central pattern generators (CPGs). A previously developed musculoskeletal model for sagittal plane simulations of human locomotion is used to replicate gait behaviors. A CMA-ES algorithm optimizes the neural parameters to minimize metabolic effort and promote gait stability. Spasticity is modeled by augmenting the inputs from Ia sensory neurons and by deactivating reciprocal inhibition between the hamstring muscle and its antagonists. On the other hand, biomechanical contractures are modeled by modifying the optimal fiber length and passive stiffness parameters of the Hill-type model. First preliminary results show that the only spastic condition maintained knee flexion between 20 and 50 degrees through the whole gait cycle and ankle dorsiflexion between 0 and 17 degrees. Adding hamstrings contracture by increasing passive stiffness by 20% leads to a slightly increased high knee flexion and a reduced maximum ankle plantarflexion. More significant knee flexion is achieved by decreasing the optimal fiber length by 30%. These results suggest that spastic condition alone is sufficient to generate key characteristics of crouch gait. The addition of contracture is essential to simulate the muscle biomechanics measured in clinical studies. The investigation of isolated effects of neurological and biomechanical impairments using neuromuscular simulations provided information that could help clinical decision-making by evaluating the causes of pathological conditions and assisting in choosing neurological or orthopedic surgeries.

Disclosures: **A. Di Russo:** None. **S. Armand:** None. **A. Ijspeert:** None.

Poster

391. Posture and Gait: Aging, Injury, and Disease I

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Program #/Poster #: 391.16

Topic: E.06. Posture and Gait

Support: NIH T32-NS082128-06
NIH R21NS119849

Title: Deep brain stimulation reduces obstacle clearance variability in Essential tremor patients

Authors: *Y. CHOI[1], B. YACOUBI KEYHANI[1], S. DELMAS[1], J. KIM[1], J. HUBBARD[1], M. S. OKUN[2], E. A. CHRISTOU[1,2];
[1]Applied Physiol. and Kinesiology, Univ. of Florida, Gainesville, FL; [2]Departments of Neurol. and Neurosurg., Norman Fixel Inst. for Neurolog. Dis., Gainesville, FL

Abstract: The cardinal symptom of Essential tremor (ET) is bilateral upper limb tremor. However, more than 40% of ET patients also exhibit gait and balance disturbances. Obstacle clearance is a challenging daily activity, and, in healthy older adults, variable foot clearance increases their fall risk. To our knowledge, no study has examined the ability of ET patients to clear an obstacle. Here, we examined the ability of ET patients undergoing thalamic deep brain stimulation (DBS) treatment to clear an obstacle while walking. We hypothesized that ET patients will exhibit a more variable foot clearance with DBS OFF than healthy adults (HA) because of the presence of tremor and that reduction of tremor with DBS ON will improve their ability to clear an obstacle. Thus, the purpose of this study was to compare HA and ET with DBS OFF and determine the effectiveness of DBS in improving obstacle clearance. Thirteen ET patients (63.5±6.8 yrs; F=5) who underwent DBS surgery in the ventral intermediate (VIM) nucleus of the thalamus and 5 HA (30.4±15.0 yrs; F=4) performed overground walking for 10 m while clearing the obstacle. A foamed block (height 6.35 x length 13 x width 10 cm) was located at 5 m of the walkway. We quantified the following: 1) the distance of the leading and trailing foot from the obstacle using video analysis software (Kinovea Inc); 2) distance variability from the obstacle across the three trials; 3) tremor during the obstacle clearance as the sum of power from 4-8 Hz of the filtered acceleration signal for each wearable sensor (lumbar, sternum, left ankle, right ankle, left wrist, right wrist) (APDM Inc.) using a wavelet analysis. ET patients exhibited more variable foot clearance (greater distance variability) than HA (p=0.01). Based on the distance variability, we separated the ET patients into a high variability group (HVG; 29.4±7.6%, N=6) and low variability group (LVG; 12.6±3.5%, N=7). The HVG comprised of individuals who exhibited distance variability greater than HA (10.7±5.2%), whereas the LVG comprised of individuals who exhibited distance variability within the HA range. For the HVG, the distance variability of the leading foot significantly reduced with DBS ON (p=0.04), whereas for the LVG distance variability did not significantly change with DBS ON (p=0.2). The DBS-

induced reduction of distance variability for the HVG associated with upper limb tremor suppression ($R^2 = 0.75$, $p<0.01$). Our findings provide novel evidence that a significant portion of ET exhibit greater variability in obstacle clearance than HA and that neurostimulation appears to be an effective treatment to reduce this exacerbated variability by suppressing wrist tremor.

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Poster

391. Posture and Gait: Aging, Injury, and Disease I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 391.17

Topic: E.06. Posture and Gait

Support: PRSS Grant of the CRIUGM

Title: Concentrating to avoid falling: evidence of an interaction between proprioception and the attentional demand for dynamic postural control in sedentary seniors

Authors: *M. J. VERMETTE[1,4], F. PRINCE[2], L. BHERER[3,4], J. Y. MESSIER[1,4];
[1]Sch. of Kinesiology and Sci. of Physical Activity, [2]Dept. of Surgery, Univ. de Montréal, Montréal, QC, Canada; [3]Dept. of Psychology, Univ. de Montréal, Montreal, QC, Canada; [4]Ctr. Recherche Inst. Geriatrie, Montréal, QC, Canada

Abstract: Falls and postural instabilities among seniors have been attributed to both a decline in proprioceptive function as well an inability to efficiently allocate attentional resources to balance during multi-task conditions. This study aims to explore the interaction between proprioception and the attentional demand for dynamic postural control in sedentary seniors. Older (n=21) and younger (n=17) sedentary adults performed a postural stability limit task in five experimental conditions that varied the availability of vision and the presence of a secondary attentional task: (a) attentional task, (b) postural task with eyes open, (c) postural task with eyes closed (d) postural task with eyes open and secondary attentional task and (e) postural task with eyes closed and secondary attentional task. Ground reaction force data was collected at 200 Hz using an AMTI force platform and center of pressure (COP) was analyzed. The functional limits of stability were quantified as the maximum COP excursion during voluntary leaning in the anteroposterior and mediolateral axes. Our results revealed significantly smaller limits of stability in seniors compared to young adults in both the anteroposterior and mediolateral axes across all sensory-attentional conditions ($p< 0.05$). However, in conditions where vision was removed, both groups similarly decreased their mean stability limits. Interestingly, the performance scores of seniors were significantly lower than young adults in the secondary attentional task, particularly while concurrently performing the postural task in the eyes closed condition ($p< 0.05$). Accordingly, seniors showed greater dual-task costs (DTC) of the attentional task than young adults in all conditions ($p< 0.05$). Hence, this between group

difference was significantly larger in the no vision condition along the mediolateral axis. Our results suggest that seniors have a reduced ability to concurrently cope with high proprioceptive and attentional demands for dynamic postural control due to limited neuronal resources. Understanding the interaction between proprioception and attention in dynamic postural control is crucial to designing future interventions aimed at combating fall-risk in seniors. Our ongoing study is currently assessing the impact of a 12-week specialized proprioceptive training program on the proprioceptive sensitivity and the postural control of seniors.

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Poster

391. Posture and Gait: Aging, Injury, and Disease I

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Program #/Poster #: 391.18

Topic: E.06. Posture and Gait

Support: NIH NINDS R01NS096083

Title: Energetic costs of walking, but not arm reaching, are elevated in persons with mild multiple sclerosis

Authors: *R. J. COURTER[1], R. M. ENOKA[1], A. A. AHMED[2];
[1]Integrative Physiol., Univ. of Colorado Boulder, Boulder, CO; [2]Mechanical Engin., Univ. of Colorado, Boulder, CO

Abstract: Why does multiple sclerosis (MS) - an inflammatory disease of the central nervous system (CNS) - involve significant movement slowing in walking, arm reaching, and saccades? Similar to its heterogenous symptoms, there are many potential causes of movement slowness in MS that appear to depend on the location of demyelination in the CNS. If we approach this question from a neuroeconomics perspective - where choice of movement speed is a consideration of both the effort required and the reward gained - then slower speeds could be due to higher effort costs or lower reward valuation. A dissection of the influence of MS on effort costs and reward valuation may illuminate those factors that drive movement slowness. Here, we focused on effort costs and hypothesized that the costs of walking would be higher in MS, largely due to reductions in physiological fitness, whereas the costs of seated reaching would be no different due to its low cardiorespiratory demand.
Participants with MS (pwMS) (n = 13; 46 +/- 8yrs) and age- and sex-matched controls (HCs) (n = 11; 47 +/- 12yrs) were strictly pre-screened and first performed a battery of questionnaires and physical assessments. On two separate days, metabolic rates were measured via indirect calorimetry while participants walked on a treadmill at five speeds (0.6 up to 1.60 m/s) and while performing out-and-back reaching movements with the dominant arm at five speeds (approx. 0.1 m/s up to 0.8 m/s) for at least five minutes each.
Neither age, height, nor weight differed between pwMS and HCs. PwMS self-rated low levels of

mobility impairment using walking-related questionnaires. Nonetheless, time to walk 25ft as quickly as possible was slower for pwMS ($p = 0.0022$).
Net normalized power (W/kg) increased with walking speed ($p < 2e\text{-}16$). A significant main effect suggested that pwMS used 20.54% (95% CI: [3.33, 40.60]) more metabolic power for walking at a given speed than HCs ($p = 0.0185$). A group-by-speed interaction was trending significant ($p = 0.0709$) and suggested that differences in net normalized power between pwMS and HCs began to converge at faster walking speeds.
Gross power (W) increased with reaching speed ($p < 2e\text{-}16$). Interestingly, there was no main effect of group, suggesting that pwMS and HCs had similar costs of reaching at a given speed ($p > 0.05$), unlike when walking.
Individuals with MS who have high mobility and low disability may require more energy expenditure to walk, but not for seated reaching movements. Our findings suggest that movement slowness in MS may not be caused by higher effort costs alone and implicates changes in reward valuation as a contributor to slowness.

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Poster

391. Posture and Gait: Aging, Injury, and Disease I

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Program #/Poster #: 391.19

Topic: E.06. Posture and Gait

Support: NINDS (R01NS105502)
GMCMD-T32 (5T32NS041234-20)

Title: Motor phenotype of a mouse model for a human kainate receptor genetic disorder

Authors: *B. WEBB[1], S. PANDIYAN[2], G. T. SWANSON[3];
[1]Northwestern Univ., Evanston, IL; [3]Pharmacol., [2]Northwestern Univ., Chicago, IL

Abstract: Deleterious mutations in ionotropic glutamate receptor genes are causative for numerous non-syndromic neurodevelopmental disorders (NDDs). Individuals with NDDs caused by a point mutation in the glutamate receptor ionotropic, kainate subunit 2 (*GRIK2* c.1969 G>A, GluK2 p.A657T) exhibit imbalance and uncoordinated/ataxic gait. Kainate receptors are expressed throughout the mammalian CNS and play distinctive roles in mediating cellular excitability, synaptic transmission, and synaptic development. We are working to identify the mechanistic basis for this impaired motor control using a mouse model – GluK2(A657T) – with an analogous G>A mutation in *Grik2*. We first tested the hypothesis that GluK2(A657T) animals display motor deficits analogous to those observed in the human disorder.
We probed 4-week-old GluK2(A657T) heterozygous mutant mice (n=11) and their wild type (WT) littermates (n=15) of both sexes for ataxia and motor deficits using the Guyenet ataxia phenotype scoring system, which incorporates tests of balance along a ledge, aberrant posture

referred to as kyphosis, and hindlimb clasping tests. During the ledge test, animals are placed on the ledge of a cage and scored based on their ability to walk without paw slips or dismount smoothly. For the kyphosis test, animals were scored on the presence and severity of spine curvature during locomotion. The hindlimb clasping test involved scoring animals based on the presence and severity of hindlimb clasping behavior. All tests were performed by two separate, blinded experimenters on a scale of 1-4 of increasing severity. Each animals' scores on these three tests were averaged to determine their composite ataxia score. The averaged score on the three tests was higher in the GluK2(A657T) compared to WT littermates (Glu2(A657T): 1.7 ± 0.5; WT: 0.4 ± 0.4, unpaired two-tailed t-test, $p<0.0001$). In 10-minute-long open field tests of movement and gait, GluK2(A657T) mice traveled a lower distance (849 ± 314 cm) compared to WT (1122 ± 293 cm) ($p= 0.0264$, unpaired two-tailed t-test), and this deficit was more pronounced during the first minute in the arena. There was no difference between genotypes in the percent time spent in the middle of the field. We plan to supplement these data with more rigorous characterization of gait and traversal differences field using a machine learning tracking analysis.
Our results confirm that heterozygous GluK2(A657T) animals display motor deficits and ataxia and therefore are suitable models for this component of the analogous human genetic disorder. We anticipate that these experiments will yield insight into the role of kainate receptors in genetic developmental disorders.

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Poster

391. Posture and Gait: Aging, Injury, and Disease I

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Topic: E.06. Posture and Gait

Support: NIH R01 AA028879
P60 AA006420
T32AA007456.

Title: Pparδ Role in motor responses and gene expression in mice striatum

Authors: *L. B. BERTOTTO[1], K. LIN[1], O. ALCORAN[1,2], E. P. ZORRILLA[1];
[1]Dept. of Mol. Med., The Scripps Res. Inst., La Jolla, CA; [2]Div. of Human Biol., UCSD, San Diego, CA

Abstract: Medium spiny neurons (MSNs) comprise around 95% of striatal neurons and are known to be involved in motor and motivated behavior regulation. Indirect pathway MSNs (iMSNs) are putatively related to promote apetitive ("Go") behavior, as opposed to direct pathway MSNs (dMSNs) that are thought to promote avoidance ("No Go") behavior. Peroxisome proliferator-activated receptor delta (PPARδ) is a bioactive lipid-sensing

transcription factor abundantly expressed in energy demanding peripheral tissues that feedback lipid signaling to the dentral nervous systrem and is the isotype responsible for most of the brain's PPAR activation and expression. Furthermore, PPARδ is highly expressed in MSNs and its role within the striatum is yet unknown. We hypothesized that mice presenting a double PPARδ knockout (KO) in neurons expressing the dMSN marker dopamine receptor 1 (Drd1) or expressing the iMSN marker adenosine A2A receptor (Adora2a) will have impaired locomotor behavior and altered gene expression in the ventromedial (NAc) and dorsolateral (DLS) striatum. To test our hypothesis, we investigated weight and motor responses (hind limb clasping, ledge test, gait and kyphosis) in double floxed PPARδ (n = 8/sex), Adora2a-cre (n = 8/sex), Drd1-cre (n = 5-8/sex), double PPARδ cKO x Adora2a-cre (n = 5-8/sex) and double PPARδ cKO x Drd1-cre (n = 7-8/sex) female and male C57BL/6 mice. In the same animals, we investigated expression of pathway markers and immediate-early gene of interest (*Drd1*, *Drd2*, *Adora2a*, *Penk*, *Pdyn*, *Tac1*, *Egr1*) in the NAc and DLS. Results show that mice performance in the hindlimb clasping test is significantly affected by genotype and sex. After pairwise comparison, Adora2a-cre mice showed a significantly better response relative to all other genotypes but double PPARδ cKO x Adora2a-cre mice, highlighting the importance of cre mice as a control in behavioral research. Collectively, our study sheds light on the neurobiological role of PPARδ in direct and indirect pathway MSNs of the NAc and DLS.

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Poster

391. Posture and Gait: Aging, Injury, and Disease I

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Topic: E.06. Posture and Gait

Support: NIH Grant R03 HD104217-01
Magistro Family Foundation Research Grant from the Foundation for Physical Therapy Research

Title: Comparing biofeedback paradigms to reduce kinematic impairment post-stroke: Preliminary data

Authors: *S. A. KETTLETY, M. L. KELLY, M. BONILLA YANEZ, J. M. FINLEY, K. A. LEECH;
Div. of Biokinesiology and Physical Therapy, USC, Los Angeles, CA

Abstract: Kinematic impairments are common post-stroke and are associated with increased metabolic cost and fall risk. One approach to address kinematic impairments post-stroke is biofeedback-based gait training, which has improved single gait kinematic impairments. However, kinematic impairment post-stroke spans multiple joints, indicating the need to understand overall gait kinematics. The effect of previously studied biofeedback paradigms on

overall gait kinematics is unclear. This work aims to determine the visual biofeedback paradigm that provides the largest reduction in interlimb asymmetry (an overall gait asymmetry metric) in people post-stroke. Due to previous work demonstrating a relationship between propulsion and interlimb asymmetry, we hypothesized that propulsion biofeedback would provide the greatest reduction in interlimb asymmetry. Twelve participants post-stroke completed three days of biofeedback training using step length, paretic propulsion, and interlimb asymmetry visual biofeedback. Each day, a single biofeedback paradigm was used, and the order was randomized. We collected kinematic and force data while participants walked on a treadmill for a two-minute baseline trial and four, five-minute biofeedback trials. To determine whether participants successfully changed the targeted gait variable, we compared the magnitude of the biofeedback variable across all trials to baseline values. We fit a robust linear mixed-effects model on each biofeedback paradigm with a fixed effect for trial and a random intercept. To evaluate how changes in the targeted gait variable influenced interlimb asymmetry, we fit robust linear mixed-effects models with a fixed effect for the change in magnitude of the targeted gait variable, a random slope, and a random intercept. For the propulsion analyses, we excluded participants with a baseline propulsion asymmetry < 0.04 ($n = 3$). We excluded participants with a baseline step length asymmetry < 0.04 from the step length analyses ($n = 3$). Across all trials, participants used step length biofeedback to reduce step length asymmetry and propulsion biofeedback to increase paretic propulsion (both $p < 0.001$). Participants did not use interlimb asymmetry biofeedback to reduce interlimb asymmetry compared to baseline ($p > 0.05$). We found that a decrease in step length asymmetry was associated with a reduction in interlimb asymmetry ($\beta = 1.2$, $p < 0.0001$). We found no relationship between change in normalized propulsion and change in interlimb asymmetry ($p = 0.06$). This suggests that step length biofeedback may be the most effective for reducing overall gait asymmetry, as measured by interlimb asymmetry.

Disclosures: S.A. Kettlety: None. **M.L. Kelly:** None. **M. Bonilla Yanez:** None. **J.M. Finley:** None. **K.A. Leech:** None.

Poster

391. Posture and Gait: Aging, Injury, and Disease I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 391.22

Topic: E.06. Posture and Gait

Title: Effects of Multi-Obstacle Contexts on Obstacle Negotiation Strategies in Healthy Older Adults under Dual-Task Conditions

Authors: *J.-E. YUN;
Korea Univ., Korea Univ., Seoul, Korea, Republic of

Abstract: Background: Performance of obstacle crossing is an attentionally demanding task due to the need for motor planning and gait regulation, particularly among older adults. Despite extensive studies on age-associated changes in obstacle negotiation strategies, relatively little is

known about adaptive mechanisms in the elderly regarding multiple obstacle crossings with different execution demands.
Research question: For better understanding of avoidance strategies employed by the elderly, the current study investigated adaptive mechanisms related to planning and implementation of more complex multi-obstacle contexts. Do older adults use a more conservative strategy such as prolonged step duration or elevated foot height when crossing obstacles with increased task demands of obstacle negotiation?
Methods: Eleven healthy older and 11 young adults participated in the experiment. We examined how the presence and physical property of the second obstacle influenced the planning and adjustments for obstacle avoidance performance. Spatiotemporal characteristics of the stepping movement were analyzed using a 3D motion capture system. Results: Older adults showed a longer stance time before crossing the first obstacle than young adults when the task complexity increased. These stepping characteristics were more evident in the dual-task condition. However, their foot clearance and crossing speed were not influenced by the level of task complexity.
Significance: These findings suggest that healthy elderly participants may have difficulty in developing the motor plan rather than implementing the stepping strategies under more complex obstacle constraints. A general cognitive decline with advancing age or adaptation of compensatory adjustment to enhance postural stability may underlie such altered obstacle negotiation behaviors in older adults.

Disclosures: J. Yun: None.

Poster

391. Posture and Gait: Aging, Injury, and Disease I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 391.23

Topic: E.06. Posture and Gait

Support: NIH Grant R01AG072756

Title: Individual differences in prefrontal cortical inputs to motor cortical outputs during tandem stance are associated with balance ability

Authors: *C. F. MASON[1], A. J. LOPEZ[1], T. J. GLOVER[1,2], L. H. TING[1,2], M. R. BORICH[1,2], T. M. KESAR[1];
[1]Dept. of Rehabil. Med., Emory Univ., Atlanta, GA; [2]The Wallace H. Coulter Dept. of Biomed. Engin., Georgia Inst. of Technol., Atlanta, GA

Abstract: The objective of this project is to characterize cognitive-motor circuit interactions underlying standing balance control. Cognitive involvement in balance increases with age to compensate for aging-related impairments in motor circuits underlying balance function. Neural correlates of balance ability are poorly understood but important to improving cognitive-motor interactions that underlie fall risk. We used non-invasive neurostimulation to characterize

cognitive-motor circuit interactions from a primary cognitive area, the dorsolateral prefrontal cortex (DLPFC), to the primary motor cortex (M1) during functionally relevant standing balance tasks in young adults (YA). Data were collected in 10 YA (18-35 years) during standing balance tasks with lower (quiet standing or QS) and higher (tandem stance or TS) levels of difficulty to characterize how M1 excitability of the soleus muscle is modulated by DLPFC activation during balance challenge. Individual balance ability was assessed using the narrowing beam walking test. We used dual-site transcranial magnetic stimulation (TMS) to assess the causal and directional relationship, or effective connectivity from DLPFC to M1 (DLPFC-M1). To perform dual-site TMS, the DLPFC was targeted with a TMS conditioning pulse 10ms prior (or 0ms in control condition) to a secondary TMS test pulse to M1. The TMS pulse to M1 elicits a motor evoked potential (MEP), a measure of motor excitability. DLPFC-M1 is assessed by comparing conditioned MEP amplitudes (TMS-DLPFC + TMS-M1) to unconditioned MEPs (TMS-M1) by calculating: 100*(conditioned MEP/unconditioned MEP). Due to the DLPFC's known inhibitory effects on M1 in upper limb muscles, we hypothesized that DLPFC conditioning would decrease M1 excitability and that DLPFC-M1 would be stronger in individuals with lower balance ability. Interim analyses show that DLPFC conditioning decreased MEP amplitudes compared to unconditioned MEPs in each balance condition. DLPFC-M1 did not differ between QS vs TS conditions. Greater DLPFC-M1 was correlated with lower balance ability during TS but not during QS. These results suggest that DLPFC-M1 is higher in YA with low balance ability during difficult standing balance tasks. Measuring DLPFC-M1 during functionally relevant conditions provides a unique task-specific probe into the modulatory role of the DLPFC in standing balance control. Our long-term goals are to develop neurophysiological biomarkers to predict falls risk, and precision (p)rehabilitation strategies to reduce falls.

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Poster

391. Posture and Gait: Aging, Injury, and Disease I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 391.24

Topic: E.06. Posture and Gait

Support: W81XWH-17-1-0424

Title: Effects of exercise intolerance on prefrontal cortex activity after mild traumatic brain injury

Authors: P. ANTONELLIS, W. LIU, K. CAMPBELL, J. WILHELM, N. PETTIGREW, M. MANCINI, *L. KING;
Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Every year 2.8 million people in the United States are diagnosed with a mild traumatic brain injury (mTBI). After sustaining an mTBI there are alterations in brain function, including dysfunction of the autonomic nervous system, that may contribute to exercise intolerance. The prefrontal cortex (PFC) has been found to be an integral part of the central autonomic network. Thus, changes in oxygenated hemoglobin (HbO_2) in the PFC, suggest activation changes of the PFC, may be a marker of autonomic dysfunction and directly linked to exercise intolerance. While incremental exercise in healthy people is accompanied by moderate-to large HbO_2 increases in the PFC, the neurophysiological processes mediating exercise intolerance after mTBI remain poorly understood. Our aim was to examine the differences in PFC activity during exercise in people with and without exercise intolerance after mTBI. Ten people with subacute mTBI were included in this study (mean ± SD; age 34.7 ± 10.9 yrs, 6 F, BMI 24.2 ± 4.3, 53.1 ± 18.9 days post mTBI). Exercise intolerance was assessed using the Buffalo Concussion Treadmill Test (BCTT). Intolerance was defined as an increase of ≥ 3 symptoms during testing and PFC activity during exercise was simultaneously measured using a mobile, functional, near-infrared spectroscopy (fNIRS) system (Octamon, Artinis). Seven subjects (70%) were exercise intolerant and three (30%) were exercise tolerant. The exercise intolerant subjects started at higher levels of PFC activity (0.16 ± 0.35) compared with exercise tolerant subjects (-0.43 ± 0.83) during the initial 2 minutes of the BCTT (effect size = 1.15). Furthermore, the exercise intolerant subjects had a large decrease in PFC activity during the last minute of the BCTT compared to the initial 2-minute treadmill walk (Delta = -3; effect size: -0.85). The exercise tolerant subjects had a large increase in PFC activity during the last minute of the BCTT compared to the initial 2-minute treadmill walk (Delta = 1.1; effect size: 1.22). These results suggest that people with exercise intolerance after mTBI may have abnormalities in PFC activity during exercise and may be unable to increase their PFC activity further to get to their maximum exercise level. Although these findings indicate dysregulation of PFC activity in people with exercise intolerance after mTBI, further work is needed to determine the relationship between PFC activity and exercise intolerance after mTBI.

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Poster

391. Posture and Gait: Aging, Injury, and Disease I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 391.25

Topic: E.06. Posture and Gait

Support: NIA Grant AG006457
VA Merit Award RX001075

Title: Resting state functional connectivity of postural sway in older adults

Authors: *A. RAGOTHAMAN[1], O. MIRANDA-DOMINGUEZ[2], J. G. NUTT[1], G. R. HARKER[1], I. ARPAN[1], P. CARLSON-KUHTA[1], M. MANCINI[1], F. B. HORAK[1]; [1]Neurol., Oregon Hlth. and Sci. Univ., Portland, OR; [2]Pediatrics, Univ. of Minnesota, Minneapolis, MN

Abstract: Postural sway is a sensitive measure to quantify balance impairments in older adults (OA) and involves multisensory integration. Sway increases when standing on an unstable surface compared to stable surface and worsens when eyes are closed. Cortical control of postural sway is still unknown.

Our aim was to determine whether resting state functional connectivity can predict postural sway ratio (sway standing on foam/firm surface) in eyes closed and eyes open conditions.

The study consisted of 53 OA (age 68.15 $\pm$ 8.5). An inertial sensor worn at the lumbar level measured postural sway area while standing on foam and firm surface, with eyes open and closed. MRI data collected from each participant was denoised, processed and quality checked. Resting state-fMRI data was reported for 333 cortical and 19 subcortical regions of interest (ROIs) grouped into 12 cortical and 1 subcortical functional networks. Associations between functional connectivity (FC) and sway ratio were characterized using partial least square regression predictive modeling, with a training (n=38) and independent test (n=15) dataset. Models in the training sample were optimized by hold-3-out cross validation and 10,000 null-data simulations and performance of the best model was validated in the test dataset.

Postural sway was significantly higher on foam compared to firm surface in both the eyes open (p.adj = 1.2e-07) and eyes closed (p.adj = 4.5e-15) conditions and different across all conditions globally (p.adj < 2.2e-16). FC between Salience and Ventral Attention networks predicted sway ratio in eyes closed condition, while Default and Somatosensory networks predicted sway ratio in eyes open condition (Fig 1).

Our findings suggest that cortical networks are involved for postural control in OA. OA use more attention related networks when standing with eyes closed, possibly to compensate for the loss of visual input. While these results need to be validated in a larger sample, they suggest that different cortical networks may be associated with balancing on stable surface when vision is available versus not available.

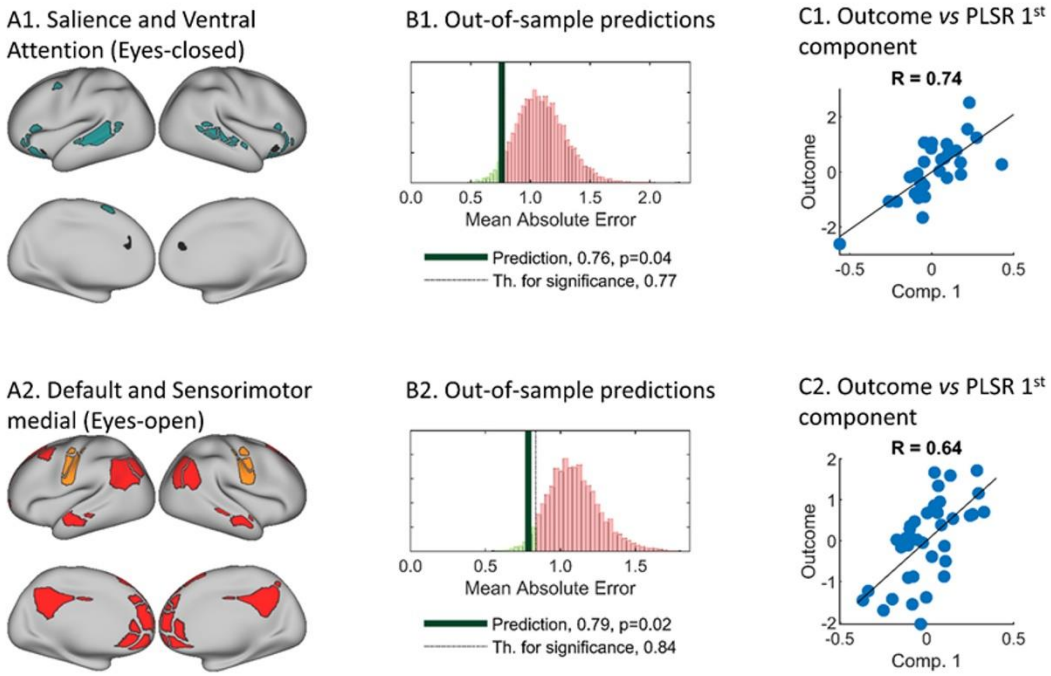

Figure 1 Functional systems significantly associated with sway scores. A. Location of each functional system pair in a very inflated projection of the cortex. B. Mean absolute error of predicted scores when models were trained with data from the training dataset and tested on the independent dataset. Distribution of mean absolute errors when models were trained using null-hypothesis data and used to determine significance is shown as background. C. Scatter plot showing the correlation between the first component of the Partial Least Square Regression (PLSR) models versus sway scores.

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Poster

392. Motor Unit Recordings, Kinematics, and EMG

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 392.01

Topic: E.09. Motor Neurons and Muscle

Support: Simons Foundation Grant 8310000132
NIH Grant 8310000097

Title: Motor Unit Coordination Across Multiple Skilled Behaviors In Mice

Authors: *M. WILLIAMS[1], K. A. THOMAS[1], B. CHUNG[1], M. ZIA[3], A. P. KEIM[4], E. AZIM[5], M. BAKIR[3], S. J. SOBER[2];
[2]Biol., [1]Emory Univ., Atlanta, GA; [3]Georgia Inst. of Technol., Atlanta, GA; [4]Univ. of California San Diego, San Diego, CA; [5]MNL-E, Salk Inst. for Biol. Studies, La Jolla, CA

Abstract: To interact with the outside world, the brain must flexibly control and coordinate muscle groups to create complex movements. However, we cannot fully understand how the nervous system achieves the goal of complex motor behavior without observing the precise spiking patterns at the output of the motor system, the motor unit. Previous studies of spiking patterns have enabled a richer understanding of motor control and learning; evidence also suggests that motor units are coordinated differently across tasks. We used custom electrode arrays that enable the simultaneous, chronic recording of multiple individual motor units to study this coordination across skilled behaviors. We examined motor unit coordination in the mouse forelimb during bimanual force control and locomotion under various conditions.
Many learned and innate behaviors require the precise coordination of muscle groups, but we do not understand how the central nervous system controls single motor units to achieve complex muscle pattern activations. We trained mice in a bimanual forceplate task wherein they must generate a precise combination of forelimb forces in order to receive a water reward. The mice were then implanted with a high-density electrode array in each triceps muscle. Simultaneous recordings of both output forelimb force and single motor unit spiking enable the study of muscle coordination both within and across limbs at the level of the single motor unit. The long term goal of this study is to determine how descending signals from the brain are translated by motor units into complex behaviors. The next step in this study is to use optogenetic techniques to probe the role of different cortical and subcortical structures during bimanual coordination.
Locomotion is a kinematically-rich behavior that requires continuous coordination across multiple muscles across the limbs. However, the coordination between individual motor units has yet to be characterized during locomotion. Throughout locomotion, mice flexibly adjust their behavior by modifying stride features, both temporally and spatially. Even at a set speed, limb kinematics demonstrate variability along these axes. To examine how motor unit coordination generates different locomotor strategies, we recorded single motor units from the mouse forelimb during free locomotion at different speeds. We analyzed differences in spike patterns across strides to determine how their variability contributes to locomotor kinematics. These results along with those for the force control task allow us to better understand how individual motor units contribute to the coordination of task-specific movements.

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Poster

392. Motor Unit Recordings, Kinematics, and EMG

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 392.02

Topic: E.09. Motor Neurons and Muscle

Support: Northwestern Medicine Department of Physical Therapy and Human Movement Sciences

Title: Quantitative analysis of human motor unit discharge properties across the female menstrual cycle

Authors: *S. T. JENZ[1], J. A. BEAUCHAMP[2], T. LULIC-KURYLLO[3], F. NEGRO[4], C. K. FRANZ[5], C. HECKMAN[1], G. E. PEARCEY[6];
[1]Dept. of Neurosci., [2]McCormick Sch. of Engin., Northwestern Univ., Chicago, IL; [3]Dept. of Clin. and Exptl. Sci., Univ. degli Studi di Brescia, Brescia, Italy; [4]Univ. degli Studi di Brescia, Dept. of Clin. and Exptl. Sci., Brescia, Italy; [5]Biologics, Shirley Ryan AbilityLab, Chicago, IL; [6]Sch. of Human Kinetics and Recreation, Mem. Univ., St. John's, NL, Canada

Abstract: Since biological studies have historically excluded females, there is a strong understanding of neural control of movement in males, but a poor understanding in females. There is great need to study female motor physiology, as many neurological diseases progress differently between the sexes. Studies that have included females have found motoneuronal discharge patterns and neuromuscular fatigue differ between the sexes but the mechanisms underlying these differences are not clear. Descending monoaminergic input from brainstem modifies motoneuronal discharge and is broadly understudied in the human motor system, yet interactions between sex hormones and monoamine signaling throughout the nervous system have been reported. The sex hormones estradiol and progesterone fluctuate across the menstrual cycle in females and these fluctuations are associated with aspects of motor control, including motoneuron discharge rates and fatigue. This suggests that fluctuating sex hormones may impact motoneuron function. To understand if sex hormonal and monoaminergic signaling interactions underlie sex differences in motoneuron discharge properties in humans, we examined motor unit (MU) discharge properties and hormone levels across the menstrual cycle (during menses, follicular phase, and luteal phase) in females, and at time-matched days for males. On each day, blood plasma levels of estradiol, progesterone, and testosterone were measured and high-density surface EMG with blind source separation was used to identify spike times of tibialis anterior (TA) MUs during isometric dorsiflexion contractions. Persistent inward currents (PICs; a proxy for the level of neuromodulatory drive) were estimated using the paired-MU analysis technique, which quantifies discharge rate hysteresis (ΔF) by obtaining the discharge rate of a lower-threshold MU (reporter unit) at the onset and offset of a higher-threshold MU (test unit). Preliminary data suggest that estimates of PICs are greater in females (N=10) than males (N=10) in the TA (female = 5.66 ± 0.223, male = 5.20 ± 0.222), however ongoing data is required to determine if these parameters are affected by sex hormones. Whether or not we identify sex and hormonal effects on MU discharge, findings from this work will not only further the scientific knowledge of how these sex hormones influence nervous system function but will highlight the critical importance for additional studies focusing on female participants out of need, necessity, and equity.

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Poster

392. Motor Unit Recordings, Kinematics, and EMG

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 392.03

Topic: E.09. Motor Neurons and Muscle

Support: NSERC Grant RGPIN-2020-06977
NSERC Grant RGPAS-2020-00038
CFI/BCKDF Grant 32260

Title: Motor unit discharge rates during a submaximal fatiguing contraction with a constant level of integrated electromyographic activity

Authors: J. R. MAGNUSON, B. H. DALTON, *C. J. MCNEIL;
The Univ. of British Columbia, Kelowna, BC, Canada

Abstract: A submaximal, sustained fatiguing contraction induces numerous changes within the neuromuscular system. Most notably, motor units (MUs) are recruited progressively to overcome contractile impairments and maintain the requisite torque of the task. As many evoked indices of neural excitability are influenced by the relative activation of the motoneuron pool, it can be difficult to isolate fatigue-related changes to excitability. For this reason, it is becoming increasingly common to investigate fatigue during a submaximal task with constant output from the motoneuron pool; i.e., matched electromyographic (EMG) activity. However, it is unknown how MU behavior is modulated as fatigue develops during a matched-EMG contraction. To address this knowledge gap, 10 participants (5 females; 30.8 ± 5.9 years old) performed a sustained 10-min isometric elbow flexion contraction at the level of integrated biceps brachii EMG recorded at 20% maximal voluntary contraction torque. Four fine-wire intramuscular recording electrodes were used to sample discrete MU action potential trains from the biceps brachii during the fatiguing contraction. A total of 69 MUs were recorded across the entirety of the first minute of the contraction, with a mean discharge rate of 15.2 ± 4.4Hz. During the last minute of the contraction, 76 MUs were recorded. These MUs had a mean discharge rate of 13.8 ± 4.5Hz, which was not different to the value during the first minute ($p > 0.05$). However, when considering only those MUs successfully tracked across the entire 10-min contraction (48 of the initial 69), there was a significant decrease in discharge rate from the first (16.4 ± 4.7Hz) to last (15.0 ± 4.9Hz) minute ($p < 0.01$). Based on these results, we can conclude that the nervous system not only relies on recruitment of new MUs to maintain motoneuron pool output (i.e., the EMG target), but also continually activates certain MUs throughout the entire contraction. For these latter MUs, the reduced discharge rate supports published work that contends the fatigue-related decrease of the cervicomedullary motor evoked potential reflects an impairment of intrinsic motoneuron excitability due to repetitive activation.

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Poster

392. Motor Unit Recordings, Kinematics, and EMG

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 392.04

Topic: E.09. Motor Neurons and Muscle

Support: GE-2-2-023A
IT-2-2-023

Title: Linking muscle activity and motion trajectory

Authors: *M. SCHMIDT[1], T. GLASMACHERS[2], I. IOSSIFIDIS[1];
[1]Inst. of Computer Sci., Ruhr West Univ. of Applied Sci., Muelheim an der Ruhr, Germany;
[2]Inst. for Neural Computation, Ruhr Univ. Bochum, Bochum, Germany

Abstract: Coordinated arm movements enable us to perform a variety of tasks that require both strength and a wide range of motion as well as precision. For this, the action must be well planned and timed. Therefore, information about the target and the current body state from the sensory systems is as important as the integration of previous experiences. These experiences might be represented as pre-learned inverse dynamics that generate associated muscle activity. We propose a generative model that predicts the upper limb muscle activity driven by various motion parameters. This model is motivated by the motion planning process in the central nervous system. The generative model is based on a recurrent neural network predicting muscle activity or complex upper limb motions. Our approach achieves remarkable agreement in predicting different subjects and abstracts well for new motions and muscle activities that have not been trained before. The high inter-subject variation of the recorded muscle activity is successfully handled using a transfer learning approach, resulting in a good fit for a new subject. To gain a deeper understanding of the link between motion trajectory and muscle activity, we reverse our problem and test the opposite prediction from muscle activity to motion parameters. The abstraction performs comparably well for new subjects and motions. The ability of this approach to predict muscle activity and motion trajectory efficiently has implications for the fundamental understanding of movement control and use for rehabilitation of neuromuscular diseases with myoelectric prostheses and functional electrical stimulation.

Disclosures: M. Schmidt: None. **T. Glasmachers:** None. **I. Iossifidis:** None.

Poster

392. Motor Unit Recordings, Kinematics, and EMG

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 392.05

Topic: E.09. Motor Neurons and Muscle

Support: Coulter Foundation

Title: Braided Multi-Electrode Intramuscular EMG probes for Single Motor Unit Recordings

Authors: *T. KIM[1], T. S. SMITH[1], A. BORISYUK[1], B. BINDER-MARKEY[2], S. F. GISZTER[1];
[1]Neurobio. & Anat., Drexel Univ. Col. of Med., Philadelphia, PA; [2]Physical Therapy & Rehabil., Drexel Univ. Col. of Nursing & Hlth. Professions, Philadelphia, PA

Abstract: The braided multi-electrode probe (BMEP) is an ultrafine microwire bundle in a tubular micro braid form, which was initially designed as a novel neural probe to chronically record neural signals for neural interfaces in brain, spinal cord, and peripheral nerves. The main advantages of BMEPs are that the BMEP provides not only high mechanical flexibility to follow tissue motions and deformation, but also 6 ~ 24 channels for recording/stimulation within a 200um or smaller diameter footprint. Long (~10cm) probes are possible, and, combined with flexibility and individual 9.6um microwires, BMEPs are easily applied to Electromyography (EMG) and especially single unit applications. The volume of muscle fibers is bigger than neural axons and the amplitude of EMG signals is thus relatively bigger than neural signals. We have explored several ways to use BMEPs in single unit and aggregate EMG applications. Laser ablation techniques allow us to expose a controlled impedance recording sites on the body of microwires, not only the tips of microwires, by ablating plastic insulation material with a precision laser, adjust the area size and shape of recording sites on braided microwires and systematically allocate the recording sites on various positions of microwires. Using laser ablation, we made tested recording sites of ~150um length on each of the 9.6um Nichrome braid wires, removing the 3um thickness of polyimide insulation, and successfully recorded good SNR single motor units with the BMEPs. The BMEPs designed for EMG are currently built in two different primary forms: braided microwires on a needle for acute recording and braided microwires designed like a suture thread for chronic recording in frogs and rats. We demonstrate features and relations between single motor units recorded from BMEPs and aggregated EMG signals recorded from classic stainless-steel wire electrodes. We discuss further new designs and applications of the BMEP EMG probes and coupling with neural recordings.

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Poster

392. Motor Unit Recordings, Kinematics, and EMG

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 392.06

Topic: E.09. Motor Neurons and Muscle

Support: Simons-Emory International Consortium on Motor Control; Simons Foundation
McKnight Foundation - Technological Innovations in Neuroscience
NIH R01-NS109237

Title: Next-generation electrode arrays for high-resolution muscle recording in rodents, songbirds, primates, and humans

Authors: *B. CHUNG[1], M. ZIA[3], K. A. THOMAS[1], J. A. MICHAELS[4], T. OYA[4], M. KASHEFI[4], M. WILLIAMS[2], K. NAGAPUDI[1], A. PRUSZYNSKI[5], M. BAKIR[3], S. J. SOBER[2]; [2]Biol., [1]Emory Univ., Atlanta, GA; [3]Georgia Inst. of Technol., Atlanta, GA; [5]Physiol. and Pharmacol., [4]Western Univ., London, ON, Canada

Abstract: Neural circuits produce an astonishing variety of motor behaviors by precisely controlling muscle activation. Understanding how a nervous system produces these behaviors requires high resolution recordings of populations of neurons in the brain as well as the motor system output - individual motor neurons that activate groups of muscle fibers, called "motor units," generating force and movement. Recent developments in neural recording technologies enable new insights for neural activity at high spatial and temporal resolution. Traditional methods for recording motor units are far less precise than those available for neural recordings and are typically limited in their ability to record during complex behavior.

We present a design and fabrication platform that enables us to quickly iterate multiple configurations for high-density multielectrode arrays. We obtain high-resolution electromyogram (EMG), including isolated spike trains of multiple individual motor units, across a variety of muscle groups and species, including: mouse forelimb, songbird expiratory, monkey arm, and human arm. Multielectrode arrays are fabricated on a flexible polymer with 32 contacts arranged in groups that are implanted together or separately. Micron-scale features allow arrays to be easily handled by surgeons for intramuscular or epimysial implantation. The polymer base of the array moves easily with muscles and electrodes have low impedances so that single motor units are recorded stably within a single multi-hour session and over weeks.

We present data to highlight different configurations during head-fixed and unrestrained behavior. Recordings during mouse locomotion show the quality of recordings over time, including single motor unit activity over weeks and bulk EMG over months. Single motor units recorded in songbirds show high-resolution EMG recordings can reveal coordination patterns of motor units across time scales from milliseconds to hours. Data recorded from monkey show how arrays can be used to record multiple single motor units during movement and reveal how single motor unit activity is modulated by task constraints. Finally, we demonstrate that our device can measure motor unit spike trains in human subjects, allowing us to probe human motor control at high resolution and creating new opportunities to examine how motor signals change following neural injury or rehabilitation. Together, we show how these novel flexible multielectrode arrays can be used to record single motor unit and bulk EMG activity over extended periods of time during restrained and unrestrained tasks in order to study how the nervous system produces complex motor behaviors.

Disclosures: B. Chung: None. **M. Zia:** None. **K.A. Thomas:** None. **J.A. Michaels:** None. **T. Oya:** None. **M. Kashefi:** None. **M. Williams:** None. **K. Nagapudi:** None. **A. Pruszynski:** None. **M. Bakir:** None. **S.J. Sober:** None.

Poster

392. Motor Unit Recordings, Kinematics, and EMG

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 392.07

Topic: E.09. Motor Neurons and Muscle

Title: Identification of Fasciculation Potentials using High-density Surface Electromyography

Authors: *S. CHANDRA[1], W. Z. RYMER[2];
[1]Northwestern Univ., Chicago, IL; [2]Shirley Ryan AbilityLab, Chicago, IL

Abstract: Accurate identification of muscle fasciculations arising from dying motor neurons is highly useful for early diagnosis of several motor neuron disorders (Amyotrophic lateral sclerosis, Spinal Muscular Atrophy, etc.) Fasciculation of individual muscle fibers is often the earliest sign of such degeneration There are existing diagnostic protocols including intramuscular electromyography to identify the fasciculation potential (FP), but these require IM needle recordings and are uncomfortable for the patient. There are currently very limited therapeutic options available. However, as new therapies are introduced, there is a compelling need to develop more targeted and sensitive tracking tools to characterize the illness severity, the rate of change in motoneuron survival or death, and the potential impact of novel therapies. Non-invasive techniques for accurate identification and classification of the FP are thus highly relevant in this scenario. There are recent techniques that employ High-density surface electromyography (HDsEMG) to identify the motor unit action potentials (MUAP) and the FP's. We demonstrate here accurate machine learning techniques that can identify fasciculation potentials, and separate those arising from motor neurons that appear to be dying. Three ALS subjects and two neurologically intact subjects participated in this study. The first dorsal interosseous (FDI) muscle was tested by placing HDsEMG electrode grids on the FDI during rest and during 20%MVC (index finger abduction task). All subjects signed informed consent via protocols approved by the IRB at Northwestern University (Chicago, USA). The recordings during the rest and during the contraction are separately decomposed through CKC based decomposition techniques to identify both normal MUAPs and the FPs. The MUAPs and the FPs across the grid were then used as input to a spatiotemporal convolution neural network. The convolution neural network incorporated the selected feature of each MU across the grid territory. The network architecture involves a convolutional layer and fully connected layers followed by a final feature classification layer. MUAPs of the Two ALS and two intact subjects were used to train the network while the rest of the subjects were used for validation. The convolution neural network was successfully able to classify the ALS subjects with an accuracy of>92%. In future, we plan to use this framework to track the disease progression in ALS; the possible application of long-term HDsEMG recording with a novel (skin like tattoo) electrode in future will be used along with this framework to facilitate accurate tracking of disease progression in ALS and SMA.

Disclosures: S. Chandra: None. **W.Z. Rymer:** None.

Poster

392. Motor Unit Recordings, Kinematics, and EMG

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 392.08

Topic: E.09. Motor Neurons and Muscle

Title: Sensing and decoding attempted movements utilizing deep learning in people with tetraplegia due to spinal cord injury

Authors: *N. CHETTY[1], N. VERMA[1], J. OH[5], A. G. STEELE[6], A. FARAJI[7], D. SAYENKO[5], D. WEBER[1,2,3,4];
[1]Dept. of Mechanical Engin., [2]Dept. of Biomed. Engin., [3]Neurosci. Inst., [4]Ctr. for Neural Basis of Cognition, Carnegie Mellon Univ., Pittsburgh, PA; [5]Dept. of Neurosurgery, Ctr. for Neuroregeneration, Houston Methodist Hosp., Houston, TX; [6]Dept. of Neurosurgery, Ctr. for Neuroregeneration,, [7]Dept. of Neurosurgery, Ctr. for Neuroregeneration, Houston Methodist Res. Inst., Houston, TX

Abstract: The human hand is an intricate biomechanical system and is vital for performing most activities of daily living, especially self-feeding, grooming, and many other tasks requiring dexterous manipulation. Thus, the loss of hand function greatly diminishes independence and quality of life for people with tetraplegia due to spinal cord injury (SCI). When asked, restoration of arm and hand function is a top priority for people with tetraplegia, inspiring many efforts to create technologies for assisting even basic hand functions, such as grasping. We and others have shown that motor attempts can be recorded and decoded from residual muscles using electromyography (EMG) in people with tetraplegia due to cervical SCI.
Studies utilizing only a few EMG channels have difficulty capturing the complex patterns of muscle activation associated with dexterous movements, especially in people with severe motor impairments. Due to the importance of both spatial and temporal relationships EMG, especially in the forearm, high-density EMG (HDEMG) is a promising tool. HDEMG, in conjunction with deep learning models, due to their ability to universally approximate even non-linear functions, have the potential to improve gesture classification and furthermore, inform on the underlying reorganization and recovery of spinal networks and muscle activation after injury.
Here, we demonstrate the use of HDEMG to record task-specific patterns of myoelectric activity associated with different movement intentions. We developed and implemented pattern classifiers using deep learning to discreetly classify gestures in an offline setting. Transfer learning was utilized by training the deep learning models on HDEMG measured from able-bodied individuals (n=3) and then training and evaluating on individuals with spinal cord injury (n=2) where the participants were not able to produce overt movement of their fingers. A total of three deep learning models were tested (Neural Net (NN), Convolutional Neural Net (CNN), and Convolutional-LSTM), with and without transfer learning. The best result achieved on the able-bodied dataset was obtained utilizing a NN and resulted in an overall accuracy of 98.8%. The best result achieved on the SCI dataset was utilizing a CNN with transfer learning with all weights released, which yielded a classification accuracy of 96.5%. All models were generalized

across all subjects within the given group and further work is required to identify if personalized models would further improve the model accuracy. Overall, this project provides evidence that it is possible to decode attempted hand gestures with high accuracy, even in people with severe tetraplegia.

Disclosures: **N. Chetty:** None. **N. Verma:** None. **J. Oh:** None. **A.G. Steele:** None. **A. Faraji:** None. **D. Sayenko:** None. **D. Weber:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bionic Power, Inc, BlackFynn, Inc, Iota Biosciences, Inc, Neuronoff, Inc, Neuroone, Inc, Reach Neuro, Inc.

Poster

392. Motor Unit Recordings, Kinematics, and EMG

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 392.09

Topic: E.09. Motor Neurons and Muscle

Support: NIH R01 HD084009
NIH K25HD096116
NIH R21HD099710
NIH R01HD039343
NIH R01NS105759

Title: Spasticity without stretch: A simple EMG-based hypertonia measure as a practical surrogate of motoneuron and associated stretch reflex excitability in individuals with hemiparetic stroke

Authors: *M. SOHN[1], N. GURARI[1,2], J. R. PATTERSON[1,3], J. M. DROGOS[1], J. P. A. DEWALD[1,4,3];
[1]Physical Therapy and Human Movement Sci., Northwestern Univ., Chicago, IL; [2]Mechanical Engin., Northwestern Univ., Evanston, IL; [3]Northwestern Univ. Interdepartmental Neurosci., Northwestern Univ., Chicago, IL; [4]Biomed. Engin., Northwestern Univ., Evanston, IL

Abstract: Cumulating evidence suggests that upregulation of the reticular formation following a stroke-induced loss of corticobulbar projections drives spinal motoneuron hyper-excitability via corticoreticulospinal pathways. In turn, individuals with stroke experience increased muscle tone (i.e., hypertonia) and associated hyperactive stretch reflexes (i.e., spasticity). Current assessments of spasticity rely on momentary examination of muscle/joint response to stretch at a joint while an individual with stroke is assumedly relaxed; such assessments include low-resolution manual movements (e.g., Modified Ashworth Scale) and high-resolution robotic device perturbations. A limitation of these assessments is that the findings do not readily translate to real-world scenarios because 1) paretic muscles can hardly relax; and 2) responses to manual/robotic stretch may not capture the impact of spasticity in a more functional context, e.g.,

during movement. Here, we propose a simple electromyography (EMG)-based measure of hypertonia as a surrogate for reflex-based quantification of spasticity; this approach avoids the need for stretching a muscle/joint. We postulated that hypertonia expressed in a paretic muscle prior to a stretch would be indicative of stretch reflex hyperexcitability, and thus, predictive of the reflex response. To test such predictive power, we determined whether a recently developed signal-processing method that sensitively detects and quantifies hypertonia can explain EMG and torque responses in 6 individuals with stroke who exhibited various levels of reflex responses to ramp stretch perturbations. Specifically, the stretch reflex was elicited by extending/flexing the elbow 40 times with a robotic device at 120°/s, with volitional elbow flexion/extension after the first 20 stretches to reinstate the hyperactive reflexes that attenuate over repetitions. Hypertonia was quantified from surface EMG (biceps, triceps, brachioradialis) measurements at 1s prior to each stretch. Stretch reflex activity was quantified from surface EMG and torque measurements at 20-120ms and 80-180ms windows post perturbation, respectively. Results demonstrate that our EMG-based hypertonia measure could explain the reflex EMG (R^2=0.63±0.15 across muscles, $p<0.05$ in all cases) and torque (R^2=0.95±0.04; $p<0.001$ in all cases), indicating that motoneuron hypertonia may drive the stretch reflex hyperexcitability. We conclude that our proposed approach for measuring hypertonia can be a simple and practical method to assess spasticity without a stretch, and, thus, can be applied in a functional context such as during activities of daily living.

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Poster

392. Motor Unit Recordings, Kinematics, and EMG

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 392.10

Topic: E.09. Motor Neurons and Muscle

Support: Onward Medical, Inc. (LIFT System)

Title: Reduction in abnormal intermuscular coherence in the biceps-triceps pair using cervical transcutaneous stimulation in a tetraplegic subject.

Authors: *N. DATTA[1], G. JIMSHELEISHVILI[1], D. CILIEN[1], J. D. GUEST[2];
[1]The department of Neurolog. Surgery, Miami Project to Cure Paralysis, Miller Sch. of Med., Univ. of Miami, Miami, FL; [2]Neurolog. Surgery/Miami Project to Cure Paralysis, Univ. of Miami Miller Sch. of Med., Miami, FL

Abstract: The commonest level of complete cervical spinal cord injury (cSCI) is C4/5 with recovery of biceps(B) function but frequent weakness in the triceps(T) disrupting agonist-antagonist (B/T) synergy. Aberrant motor control and neuroplasticity may restrict triceps range of motion (ROM) through B/T spasticity/co-contraction and compensatory dynamic coupling

between shoulder and elbow reducing the maximal forward and superior reach of the arm. Greater attempted voluntary effort can exacerbate co-contraction further reducing ROM. These changes significantly impact daily activities making triceps voluntary function a target of several interventions. Applied treatments such as oral Baclofen and intramuscular botulinum toxin may reduce spastic tone but also reduce voluntary muscle force and do not address abnormal intermuscular coherence. Cervical transcutaneous stimulation modulates sensory input to the spinal cord. We hypothesized that transcutaneous stimulation at 90 Hz could affect B/T co-activation and improve voluntary movement of the affected joints. We used surface electromyography (sEMG) as a tool to quantify B/T activation/coactivation in real-time and tested frequencies of 30, 60, and 90 Hz applied to the posterior C5 level (cathode) with anodes on iliac crests. Shoulder and elbow ROM and biceps/triceps co-contraction was measured clinically and using sEMG at rest and during attempted isometric, submaximal, and maximal isolated triceps contractions. EMG signals were sampled at 60Hz, amplified (10K), band pass filtered (10-10kHz), and analyzed offline to determine the frequency content contributing to signal power in biceps and triceps and changes in coactivation-co-contraction with and without neuromodulation. **Results.** The subject had difficulty inhibiting biceps-triceps co-contraction across all tasks prior to stimulation greatly limiting shoulder and elbow ROM. Strong coherence and coactivation in the biceps and triceps indicated a lack of independent motor control. Stimulation with 90 Hz allowed increased passive ROM, clinically improved voluntary function, and reduced effort for maximal contraction. These findings were supported by sEMG showing reduced co-contraction of B/T pair. **Conclusion.** Abnormal biceps-triceps motor control is a frequent consequence of cSCI lacking adequate therapies. High frequency cervical transcutaneous neuromodulation may improve motor control and help re-establish reciprocal inhibition of the biceps muscle during triceps contraction. This could potentially contribute to more effective motor rehabilitation when combined with physical and occupational therapy.

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Poster

392. Motor Unit Recordings, Kinematics, and EMG

Location: SDCC Halls B-H

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Program #/Poster #: 392.11

Topic: E.09. Motor Neurons and Muscle

Support: IITP 2017-0-00432

Title: Facial EMG noise rejection strategy in AR-based SSVEP-BCI system for the daily life application

Authors: *Y. HAN, J. HA, H. SEO, L. KIM;
Ctr. for Bionics, Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of

Abstract: There is increasing interest in the augmented reality brain-computer interface (AR-BCI) system due it is applicability in daily life as it provides a portable environment instead of the fixed visual stimulus presentation method of existing BCIs. However, facial electromyogram(fEMG) artifacts generated by the user's daily activities could not be avoided in the real environment and lead to reducing the EEG signal quality. In this study, we implemented an AR-based steady-state visual evoked potential (SSVEP)-BCI system and considered two noise conditions (jaw clenching/head shaking) similar to the real environment. We suggested fEMG noise rejection methods to improve the signal quality and to confirm whether the AR-BCI system can be applied to daily life. Ten subjects from both genders (M6, F4; age mean 26.6y±3.6) participated in this experiment, and the EEG signals were recorded using a 64-channel Easycap electrode cap. All the subjects wore an AR device (Microsoft Hololens) and performed SSVEPs every 4 seconds for a total of 20 times using flickering visual stimulation under 4 target frequencies (6.6, 7.5, 8.57, 10 Hz). When the visual stimulation was presented, the subjects were asked to generate jaw clenching or head shaking movements. The EEG signals acquired under these noise conditions were processed to remove facial noises by three methods; raw, artifact subspace reconstruction (ASR), and partial ASR(pASR). Subsequently, we evaluated the SSVEP accuracy in the parietal-occipital channels (Pz, Oz, O1, O2, PO3, POz, PO4, Iz) using an extension of the multivariate synchronization index (EMSI) algorithm (Zhang, Yangsong et al., 2017). pASR was applied to the fEMG section based on the signal envelope and integral extraction method (Wang, Mo, et al. 2019). For the EMG detection reference channel, we used Oz with 20Hz~54Hz bandpass filtering. As a result, in the jaw clench, pASR had the highest accuracy (mean 75%±14.72), followed by ASR (mean 72%±14.76) and raw (mean 66.5%±14.54). In the head shake, pASR had the highest accuracy (mean 78%±12.29), followed by ASR (mean 74%±13.5) and raw (mean 73.5%±14.35). We verified that the AR-based SSVEP-BCI system performance was improved using the noise rejection algorithm (ASR/pASR). Particularly, pASR indicated that the signal quality was affected by the fEMG section, and we confirmed that the noise rejection of the fEMG section can minimize data loss and improve the performance of the AR-BCI. The findings will encourage the use of the AR-BCI system as a portable system optimized for daily life without the environmental constraints.

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Poster

392. Motor Unit Recordings, Kinematics, and EMG

Location: SDCC Halls B-H

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Program #/Poster #: 392.12

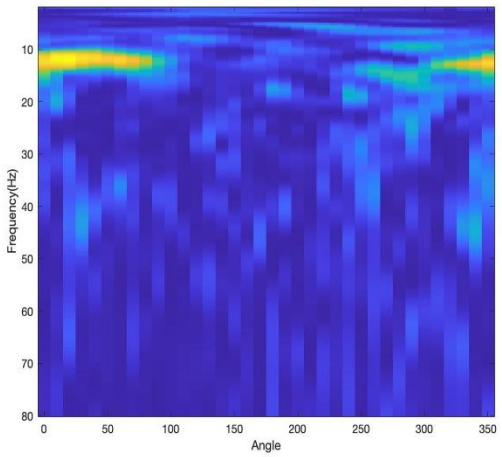
Topic: E.09. Motor Neurons and Muscle

Support: NSF CRCNS Japan-US 2113096
NIH R21-NS113613
DARPA W911NF1820264
Viterbi Graduate Fellowship

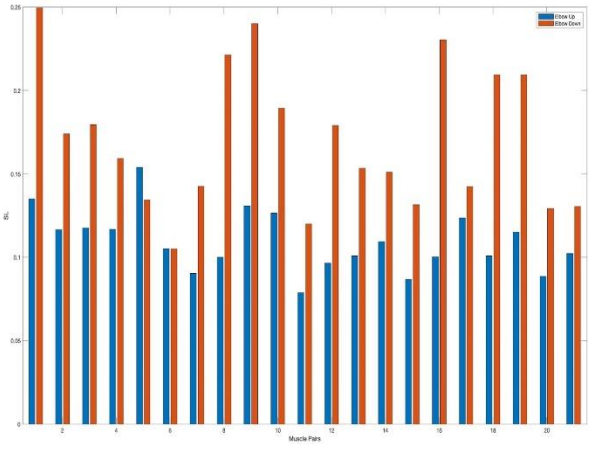
Title: Extensions to methods to quantify intermuscular and corticomuscular functional connectivity

Authors: *M. AZADJOU[1], F. J. VALERO-CUEVAS[2];
[1]USC, USC, Los Angeles, CA; [2]Biomed. Engin., USC, La Crescenta, CA

Abstract: Coherence analysis can capture the linear synchronization between two signals across the frequency spectrum, whereas correlation methods capture temporal relationships (Meyers et al. 2004). Motor unit pools show intermuscular coherence in the alpha band (Evans and Baker 2003), and corticomuscular coherence in the beta band (Gwin and Ferris 2012) in their EMG and EEG signals. This can be used to estimate common descending neural commands (Kutch and Valero-Cuevas 2012) as a feature of unimpaired motor control, or pathologic synergies and motor overflow in stroke patients (Chen et al. 2018). We extend coherence analysis by using a high-pass filter to reduce action potentials to spikes for a better temporal resolution (Boonstra and Breakspear 2012) to focus on the neural drive to motor unit pools. Moreover, we use Hann windows with different overlap rates for coherence computation to focus on specific frequency bands to compare the strength of neural input across them. Extending common wavelet-based methods for time-frequency analysis, we have also developed a novel way to produce the time-frequency representation of coherence based on time binning of the time series, which, with a low computational cost, enables us to determine how coherence changes during different temporal phases of the task. On the other hand, nonlinear synchronization may be crucial to understanding how motor unit pools are coactivated. We applied Synchronization Likelihood (SL) analysis to EMG, a novel signal analysis method that is capable of characterizing both linear and nonlinear interdependencies between time series (Stam and van Dijk, 2002). SL captures rapid fluctuations in synchronization and desynchronization because of its high temporal resolution (Betzel et al., 2012). Our preliminary results show that SL in EMG signals during voluntary unimanual rotation of a horizontal ergometer significantly varies by shoulder abduction, which promises to be a nonlinear tool to complement linear coherence for intermuscular synchronization analysis.



a. Time-Frequency Representation of Coherence.



b. Synchronization Likelihood for 21 Pairs of Muscles

Disclosures: M. Azadjou: None. **F.J. Valero-Cuevas:** None.

Poster

392. Motor Unit Recordings, Kinematics, and EMG

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 392.13

Topic: E.09. Motor Neurons and Muscle

Support: MEXT 19H05724

Title: Neural adaptation in response to tendon cross-union of an antagonistic muscle pair in the primate forearm.

Authors: *R. PHILIPP[1], N. UCHIDA[2], Y. HARA[3], T. FUNATO[4], K. SEKI[1];
[1]Natl. Inst. of Neuroscience, NCNP, Kodaira, Japan; [2]Dept. of Mechanical and Intelligent Systems Engin., Univ. of ElectroCommunications, Grad. Sch. of Informatics and Engin., Chofu, Japan; [3]Dept. of Orthopaedic Surgery, Natl. Ctr. of Neurol. and Psychiatry, Kodaira, Japan; [4]The Univ. of Electro-Communications, Chofu-shi, Japan

Abstract: The musculoskeletal system naturally changes over time (aging) or by injury (limb amputation or trauma). As a result, subcortical as well as somatosensory and motor cortical areas are subject to substantial structural changes. However, despite routine procedures in human (e.g. tendon transfer following radial nerve injury) only little information is available about the cortical and subcortical adaptations to this physically modified body and its underlying mechanisms. By using tendon cross-union between the Extensor Digitorum Communis and Flexor Digitorum Superficialis, an antagonistic muscle pair in the forearm controlling the fingers, we seek studying the monkey's potential to recover and perform in a simple grasping task. A tendon cross-union keeps the nervous system intact without triggering the usual response after injury and thus simulating adaptations which resemble those occurring during motor skill learning or development. This model may also give deeper insights into how muscle synergies develop and adapt to changes in the musculoskeletal system. Behavior, electromyographic (EMG) activity patterns and muscle synergies of two monkeys were analyzed. Both monkeys recovered from the surgery and successfully performed the task four weeks after tendon surgery (TS). For instance, contact times with the object were significantly longer immediately after TS compared to the first 5 sessions post TS (197.7 ±92.2 vs 660.6 ±221ms and 169.7 ±14 vs 316.3 ±31.9 ms for monkey A and B, respectively; $p<0.05$, two-sample t-test) and it took just over 10 days for both monkeys to recover ($p>0.05$; two-sample t-test). EMG (activity profile and cross-correlation analysis) and synergy analysis revealed typical patterns which changed over time during the monkey's recovery. We found a decrease in the correlation coefficients between pre and post-surgery in muscle synergies (from 1 to -0.4 and -0.3 in monkey A and B, respectively) as well as the relocated (-0.32 and 0.15) and non-relocated (-0.6 and -0.2) EMG activity patterns which became largest at the same time as behavior recovered. However, the cross-correlation coefficients of EMG activity and synergy of the repositioned muscles later increased again in both monkeys about 60 days after surgery and returned to their pre-operative values. Thus, the tendon transfer procedure revealed that the re-positioned muscles exhibit the temporal activity of

the original muscle activity/synergy at its new destination which was later reversed, hence indicating a two-stage adaptation of the central nervous system in which muscle activity/synergy are “replaced” in the first early phase and then “restored” in the second late phase.

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Poster

392. Motor Unit Recordings, Kinematics, and EMG

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Program #/Poster #: 392.14

Topic: E.09. Motor Neurons and Muscle

Support: NSF CRCNS Japan-US 211309
NIH R21-NS113613
DARPA W911NF1820264
Division of Biokinesiology and Physical Therapy Graduate Teaching Assistanships

Title: Muscle synergy residuals are necessary for accurate prediction of task progress

Authors: *A. BARTSCH-JIMENEZ[1], A. ERWIN[2], F. J. VALERO-CUEVAS[3];
[1]Biokinesiology, USC, Los Angeles, CA; [2]USC, Pasadena, CA; [3]Biomed. Engin., USC, La Crescenta, CA

Abstract: Introduction
Dimensionality reduction techniques are often applied to electromyographic (EMG) recordings to calculate basis functions that quantify muscle coordination strategies. Usually, muscle synergies are defined using these dimensionality reduction techniques from the basis functions that account for 90% of variance in the original signal. However, the residual activity (remaining 10%) is not entirely random and can inform the execution of motor tasks. To test the importance of residual activity to capture the fine features of upper-arm cyclical motion, we compared the task progress prediction error based on (I) EMG activity and (ii) after its dimensionality reduction.
Methods
Eleven participants (5 males, 7 females), with a mean age of 37.3 (± 14.2) years were evaluated using a horizontal ergometer (reaching-like cyclical motion), while electrodes were placed on seven muscles of their right upper extremity: Long and Short heads of biceps, Anterior, Middle and Posterior deltoid, Lateral head of triceps, Upper Trapezius. Each participant executed 30 repetitions at two speeds: self-selected and two seconds per cycle. EMG signals were filtered, then rectified and normalized to maximal contraction in each trial (over the 30 repetitions). Muscle activity was averaged over five degree intervals between 0 to 360º. To estimate task progress, a mixed effects model was fitted using cross validation (k=5), where task progress

(ergometer angle) is estimated based on EMG signals of the seven muscles and after dimensionality reduction using principal component analysis (PCA). Four PCs accounted for 91% of EMG signal variance, and were used as predictors.
Results
Root mean squared error (RMSE) of task progress prediction based on dimensionality reduction was 24º, while RMSE based on EMG signals was 9º. Statistical differences were found between observed and predicted ergometer angle (task progress) using PCA ($p < 0.01$), but none from EMG signals ($p = 0.21$).
Discussion
Our results suggest the remaining 9% of variance that was not considered after dimensionality reduction is necessary for accurate prediction of task progress. This raises questions about the utility of synergies for the accurate prediction of reaching kinematics.

Disclosures: A. Bartsch-Jimenez: None. **A. Erwin:** None. **F.J. Valero-Cuevas:** None.

Poster

392. Motor Unit Recordings, Kinematics, and EMG

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Program #/Poster #: 392.15

Topic: E.09. Motor Neurons and Muscle

Support: NIH R21-NS113613
NSF CRCNS Japan-US 2113096
DARPA W911NF1820264

Title: Effects of age on intermuscular coherence in a cyclical upper-extremity motor task

Authors: *A. ERWIN[1], A. BARTSCH-JIMENEZ[1], M. AZADJOU[2], L. I. ALMOFEEZ[2], G. NIYO[2], F. J. VALERO-CUEVAS[2];
[1]Kinesiology, [2]Biomed. Engin., USC, Los Angeles, CA

Abstract: Synchronous activity between electromyography (EMG) signals at high frequencies (>7 Hz), i.e., intermuscular coherence, is thought to reveal shared neural input to muscles. As such it may be able to quantify ‘synergies of neural origin’. As an essential step towards clinical utility of intermuscular coherence in neurorehabilitation, we extended Laine et al. (J Physiol, 2021) and contrasted alpha-band neural drive strength between four neurotypical young adults (23-29 years) and four neurotypical older adults (51-58 years—within the age range in the population of stroke survivors). Using their right arm, participants rotated an ergometer while surface EMG was measured from seven muscles including the upper trapezius, triceps (lateral head), biceps (short and long heads), and deltoid (anterior, middle, posterior). EMG data were high-pass filtered using a fourth-order Butterworth filter with a cutoff frequency of 250 Hz and then the signals were rectified. Magnitude squared coherence was estimated across all muscle pairs (21 comparisons), and a mean coherence per participant was estimated as the mean of the

maximum coherence in the alpha-band (8-16 Hz) per muscle pair. A corresponding grand mean was estimated for each group (young adults vs. older adults). These preliminary data show that the average coherence per participant for young adults vs. older adults was 0.0262 (± 0.0068) vs. 0.0363 (± 0.0195), which were not statistically different ($p = 0.14$). Each group mean was significantly above the coherence threshold of 0.006. This preliminary study of the previously unexplored age-dependent nature of coherence during cyclical reaching movements suggests that aging does not affect mean coherence across all muscles and phases of the cycle. These preliminary results encourage further study of this lack of age-dependent coherence up to 58 years of age, and beyond. If this result is further validated, it would provide a rigorous biomarker baseline against which to compare motor impairment in adults after neurological injuries, such as stroke.

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Poster

392. Motor Unit Recordings, Kinematics, and EMG

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Program #/Poster #: 392.16

Topic: E.09. Motor Neurons and Muscle

Title: Combined muscle EMG and finger kinematic signals for Task and Object Classification and Peak Force Prediction during Hand Gripping

Authors: *R. JIN[1], M. JAVIDI[2], D. J. WEBER[3];
[1]Biomed. Engin., [2]Mechanical Engin., [3]Mechanical Engin. and Neurosci. Inst., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Powered exoskeletons and assistive robots can be used to restore motion to paralyzed limbs and manipulate objects at a remote location. However, controlling such devices requires the operator to express motor intentions to the robot indicating the desired motion and force profiles. Motion commands can be derived readily from the human operator using joysticks, cameras, or inertial measurement units. However, force sensing is considerably more challenging, especially during tasks involving dynamic manipulation, requiring continuous monitoring of changes in grip force while the arm and hand are in motion. Myoelectric activity, measured through electromyography (EMG), may convey information about intended force output that could be used in combination with kinematic measures to simultaneously control motion and force. In this study, we performed experiments involving two types of gripping tasks and three load conditions to study how EMG and finger motion vary with the object size and force requirements for the different grasping conditions.
The experiments included 2 tasks, including static and dynamic gripping of 3 wooden blocks, varying in width (2, 6, and 10 cm). During the static gripping task, subjects grasp and hold each object in a stationary pose. During the dynamic gripping task, subjects grasp and lift each object.

The lifting force was also varied using springs, varying in stiffness to provide 10, 20, or 30 pounds of load force. Each subject repeated each task 3 times under each spring-brick combination under all conditions. Electromyography (EMG) from four forearm muscles extensor digitorum (ED), extensor pollicis longus (EPL), flexor digitorum superficialis (FDS), and flexor digitorum profundus (FDP), flexion from five fingers, and fingertip pressure from three fingers were recorded from the right arm of seven subjects.
Results showed that the EMG, finger flexion kinematics, and fingertip pressure differed significantly across tasks and objects ($p<0.001$). The Random Forest algorithm based machine learning classification model showed that finger kinematics can provide 90% and 83% maximum overall classification accuracy for tasks and objects. EMG signals were also used to predict peak gripping force across conditions with an RMS error of 0.11. In conclusion, individual and combined muscle EMG and finger flexion inputs contribute differently to the task and object classification. The combination of EMG and finger kinematics enable accurate classification of the task and object conditions, with EMG providing accurate estimates of grip force.

Disclosures: R. Jin: None. **M. Javidi:** None. **D.J. Weber:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bionic Power, Inc., BlackFynn, Inc., Iota Biosciences, Inc., Neuronoff, Inc., Neuroone, Inc., Reach Neuro, Inc..

Poster

392. Motor Unit Recordings, Kinematics, and EMG

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 392.17

Topic: E.09. Motor Neurons and Muscle

Title: Using Electromyography as a measure of perceived effort during manipulation tasks performed in physical and virtual environments.

Authors: *D. DESPRADEL[1], D. WEBER[2], N. POLLARD[3];
[1]Mechanical Engin., [2]Mechanical Engin. and Neurosci., [3]Robotics Inst. and Computer Sci., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Human perception of the environment and physical interactions is inherently multisensory. Virtual Reality (VR) is a technology intended to stimulate the user's senses in such a way that a computer-generated world is perceived as realistic. However, current VR applications typically provide rich visual and auditory experiences, while haptic feedback is generally limited to vibrotactile stimulation. Previous studies on the differences between physical and VR simulation show that haptic feedback (HF) is an essential feature that influences manipulation and grasping execution in a virtual environment (VE). Therefore, there is a clear need for haptic displays in VR that include force feedback to enhance the user's perception of physical interaction, such as grasping and manipulation. To support this claim, an experimental design was formulated for both physical and VE and fragmented into three parts (Physical, VR +

controller + leap, and VR + force feedback) to compare the user's muscle activity and perceived effort during a simple grasp and lift task. With this work, we propose an EMG-based framework to measure perceived effort in the real and the virtual world, as well as to evaluate how HF enhances the perception of physical interaction in VR. To do that, four participants completed the study. We measured EMG from 11 muscles (9 on the forearm and 2 on the upper arm) as the objective physiological measurement, and we used the Borg rating scale to measure perceived effort, as the subjective measurement, in both environments. We used a few physical objects and their corresponding meshed models gathered from the YCB (Yale-CMU-Berkely) benchmark for the users to manipulate. The specified 3D VE was created in Unity and was displayed to the participant via the Cosmos HTC head-mounted display (HMD). A Sigma.7 haptic robot was utilized in the experiment to provide the user with force feedback while manipulating virtual objects in a VE. The results demonstrate a direct correlation between EMG signal amplitudes and the user's perceived effort. In other words, the harder the user felt like they were working to grasp and lift an object, the stronger the muscle activation. There were also apparent EMG activation differences among participants, nevertheless, similar trends of EMG and perceived effort were detected. Noticeably, the EMG in the virtual tasks differed from the EMG in the physical tasks, but the differences were reduced significantly when force feedback was provided. In conclusion, our findings indicate that EMG signals can be used as a quantitative indicator of perceived effort when evaluated in different environments.

Disclosures: D. Despradel: None. **D. Weber:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bionic Power, INC, Blackfynn, INC, IOTA Biosciences, INC, Neuronoff, INC, Neuroone, INC, Reach Neuro, INC. **N. Pollard:** None.

Poster

392. Motor Unit Recordings, Kinematics, and EMG

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 392.18

Topic: E.09. Motor Neurons and Muscle

Support: NSERC 312038

Title: Enhancing Force Control and Motor Unit Behavior with Contralateral Tendon Vibration in Parkinson's Disease

Authors: *C. KIM, K. A. LAROCQUE, J. M. JAKOBI;
Univ. of British Columbia, Kelowna, BC, Canada

Abstract: Unilateral functional electrical stimulation (FES) improves contralateral motor performance in persons with Parkinson's Disease (PD)(Popa et al., 2012, 2013). Force steadiness is impaired in persons with PD, and there is evidence to suggest that the fluctuation in force output arises from alterations in motor unit activity. Tendon vibration is an effective way of

enhancing motor performance by altering Ia afferent feedback (DeForest et al., 2020). In this study, we aim to determine the effects of acute contralateral tendon vibration of the distal biceps brachii tendon on force steadiness and motor unit activity in persons with PD.Six males and four females with mild to moderate PD severity performed a ramp, hold and deramp isometric elbow flexion at 5% of maximum voluntary contraction (MVC) with the more-affected arm while vibration (100Hz, ± 3.5-4.0mm) was applied to the distal biceps brachii tendon on the contralateral, less-affected arm. Using intramuscular fine wire electrodes, 35 MUs in the short and long head of the biceps brachii on the more-affected arm were recorded and tracked across three vibration conditions (pre-vibration, vibration, and post-vibration). Motor unit recruitment threshold, derecruitment threshold, discharge rates (MUDR), discharge rate variability (MUDRV), and elbow flexion force steadiness were compared across the three vibration conditions. CV of force and MUDRV decreased in the post-vibration compared with pre-vibration and vibration conditions, and MUDR did not differ between these conditions. MU recruitment thresholds were higher than derecruitment thresholds regardless of vibration; however, the total number of MUs that were recorded during the de-ramp were fewer in the post-vibration condition. These results suggest that contralateral tendon vibration could be an important neurophysiological path in improving force control in PD; characterized by a decrease in MUDRV and enhancement in force steadiness.

Disclosures: C. Kim: None. **K.A. Larocque:** None. **J.M. Jakobi:** None.

Poster

392. Motor Unit Recordings, Kinematics, and EMG

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Program #/Poster #: 392.19

Topic: E.09. Motor Neurons and Muscle

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T32 HD071845
R01NS099375
R01NS084844
R01NS109237
NNF17OC0028928

Title: Millisecond-scale differences in muscle stimulation patterns modify motor output

Authors: *A. PACK[1], I. ADAM[3], C. P. ELEMANS[3], S. J. SOBER[2];
[2]Biol., [1]Emory Univ., Atlanta, GA; [3]Biol., Univ. of Southern Denmark, Odense M, Denmark

Abstract: Motor control requires the brain to rapidly process sensory signals and coordinate precise patterns of muscle activity. A central objective in neuroscience is to establish how the brain controls muscle activity and modifies muscle output during motor skill learning. Our prior work in songbirds quantified the timescale at which patterns of neural and muscle activity

control vocal and respiratory behavior and demonstrated that millisecond-scale variations in spike patterning in cortical neurons and respiratory muscle fibers are correlated with the upcoming motor output (Tang, et al., 2014; Srivastava, et al., 2017). However, it is still unknown whether and how muscle fibers transform these precisely-timed spikes patterns into variations in behavior. To answer this question, we quantified and compared the effects of spike timing patterns on vocal muscles in songbirds *in vitro* to decipher how spike pattern-based strategies can modulate vocal motor output. We experimentally induced three-pulse stimulation patterns in fiber bundles from a vocal muscle and measured the corresponding changes in force output. The three-pulse stimulation pattern allowed for a change in pulse timing without a change in overall pulse number. These experiments demonstrated that songbird vocal muscles exhibit strong timing-based nonlinear force output during short inter-spike intervals (i.e., inter-spike intervals < 20 ms), and suggest that these nonlinearities, along with nonlinearities we have previously described in songbird respiratory muscles, are a crucial feature of vocal behavior. Upcoming experiments will evaluate how three-pulse stimulation patterns delivered to vocal organ muscles *in vivo* change singing behavior (e.g., pitch). These results will guide future studies to examine how muscle activity is organized across time and space, offering new insights into how the coordination of activity within and across co-active muscles develop as a skilled behavior is learned.

Disclosures: **A. Pack:** None. **I. Adam:** None. **C.P. Elemans:** None. **S.J. Sober:** None.

Poster

392. Motor Unit Recordings, Kinematics, and EMG

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 392.20

Topic: E.09. Motor Neurons and Muscle

Title: Analysis of lower limb muscular latency during perturbation tasks

Authors: *B. YASIN, I. B. OMOFUMA, S. K. AGRAWAL;
Mechanical Engin., Columbia Univ., New York, NY

Abstract: Maintaining balance requires the integration of motor and sensory information that generates appropriate muscle responses to achieve stability. This study aims to characterize the pattern of muscle responses during different perturbations using EMG latency and determine whether this pattern exhibits plasticity with repeated trials. Establishing this will lead to improved rehabilitation strategies for the lower limb. Here, we applied perturbations to healthy subjects to displace them either laterally or forward-backward. We used a cable-driven robotic system that applies perturbation forces at the trunk to displace the subject in different directions. Surface EMGs were measured from lower limb muscles bilaterally. The perturbation directions and magnitude were randomized and occurred with random timing. Our results show that EMG latency was significantly longer on the contralateral muscles compared with the ipsilateral, suggesting a voluntary response component controlled by the cerebral cortex. Furthermore,

following repeated perturbations, the EMG latency got significantly shorter which indicated an improved response after repetition. This latency reduction was more significant in ipsilateral as opposed to contralateral muscles, relative to the perturbation direction, and was most significant in forwards-backward perturbation. It indicates increased plasticity and induced adaptation to perturbation. Given that contralateral muscle responses are mostly mediated by cortical origin, supports the notion that repetition-induced plasticity seen in ipsilateral might be regulated subcortically at the brainstem level. This result is inconsistent with other studies which found no significant change in latency following repeated perturbations. It might be because our experiments involve more repetitions and perturbation directions, inducing more plasticity. Finally, we looked at the sequencing of muscle responses in different perturbation directions. A robust onset of distal muscles in the ipsilateral and forward-backward perturbations, as opposed to contralateral, suggests an ankle strategy aimed to increase stiffness and maintain balance. We assume that increased load of body weight towards the perturbed direction triggers load-sensitive sensory afferents that integrate with synapses at the spinal level. This ultimately modifies the stretch reflex and induces fast responses and muscle co-activation. To sum up, the EMG latency during the perturbation tasks is a powerful tool to teach us about the plasticity, muscle groups, and neural pathways essential to maintaining balance.

Disclosures: B. Yasin: None. **I.B. Omofuma:** None. **S.K. Agrawal:** None.

Poster

392. Motor Unit Recordings, Kinematics, and EMG

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 392.21

Topic: E.09. Motor Neurons and Muscle

Support: NS119849

Title: Coherent modulation of reaction time and motor response intra-individual variability occurs below 0.05 Hz

Authors: *J. HUBBARD, J. KIM, S. DELMAS, Y. CHOI, E. A. CHRISTOU, B. YACOUBI KEYHANI;
Applied Physiol. and Kinesiology, Univ. of Florida, Gainesville, FL

Abstract: Modulation of physiological signals at frequencies below 0.4 Hz have been associated with sympathetic (SNS) and parasympathetic (PNS) nervous system inputs. For example, the modulation of heart rate variability is modulated by the SNS (thermoregulation; 0 - 0.04 Hz) and PNS (respiration; 0.15 - 0.4 Hz). Similarly, the modulation of reaction time (RT) intra-individual variability, which also occurs at very low frequencies (<0.25 Hz), has been used as proxy to neurobiological deficits in various populations. However, the modulation of the peak force and muscle activity intra-individual variability that occurs across discrete ballistic contractions remains unknown. The RT and motor response are distinct neural processes and signify temporal

variability in information processing (RT) and motor plan variability (peak force and EMG). Thus, our purpose was to determine if there is a coherent modulation of intra-individual variability for RT and motor response, which would suggest a common underlying mechanism (e.g., inputs from the SNS and PNS). Sixteen healthy participants (21.5 ± 2.6 years) performed 200 ankle dorsiflexion trials. A visual stimulus (every 3 s) prompted subjects to dorsiflex their ankle as fast as possible and match a force target equal to 10% of their maximum. Participants received visual feedback for the first 50 trials to allow for learning the motor response. They performed the remaining 150 trials without visual feedback, which we used for data analysis. We converted the 150 discrete outcomes into a continuous time signal to determine the modulation of the intra-individual variability for the RT, peak force, and EMG peak burst. We quantified the common frequency modulation between the RT and peak EMG using wavelet coherence. In both RT and motor response, the largest coherence occurred from 0 - 0.05 Hz ($r = 0.5$) suggesting that the two signals are co-modulated by SNS inputs. The speed of the RT was associated with a significant coherence from 0.1 - 0.2 Hz between RT and peak EMG ($r^2 = 0.46$). These findings suggest that both information processing time and motor planning are perturbed by a <0.05 Hz oscillation, which has been associated with the activity of the SNS. These results could have clinical implications as they suggest that modulation of the SNS and PNS could influence RT and motor response performance.

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Poster

392. Motor Unit Recordings, Kinematics, and EMG

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Program #/Poster #: 392.22

Topic: E.09. Motor Neurons and Muscle

Support: GSK Japan Research Grant 2018

Title: Analyses of respiration and swallowing changes in Chronic Obstructive Pulmonary Disease (COPD) model rats

Authors: *K. NAGOYA, T. TSUJIMURA, Y. NAKAJIMA, Y. TSUTSUI, J. MAGARA, M. INOUE;
Niigata Univ., Niigata City / Niigata, Japan

Abstract: <META NAME="author" CONTENT="那小屋公太">Chronic obstructive pulmonary disease (COPD) is a respiratory illness, of which the number of patients is expected to increase with age. Dyspnea, chronic cough and sputum are known as the main symptom of COPD, often accompanied by swallowing disorder. Although many clinical reports dealing with swallowing disorder due to COPD have been published, physiological mechanism of swallowing changes due to COPD still remains unclear. So, we analyzed how COPD contributes to

swallowing responses using COPD rats.COPD rats were produced by intratracheal administration of elastase (28 U/body weight 100g) and LPS (5 mg/mL) for SD rats. Electromyographic (EMG) activities were recorded from control and COPD rats anesthetized with urethane (1.3 g/kg, ip). EMG electrodes were implanted at digastric (Dig) and thyrohyoid (TH) muscles and diaphragm (Dia). Swallowing reflex was evoked by superior laryngeal nerve (SLN) electrical stimulation. The stimulation threshold for evoking swallow was determined as the minimum stimulus intensity needed to evoke a swallow at least once during the SLN stimulation for 10 s. The current intensity was determined as 1.2 or 2 times the stimulation threshold. COPD condition was confirmed by micro-CT scanning and HE staining of lung. First, we analyzed respiration changes using Dia EMG waveform. The respiratory rate of COPD rats did not demonstrate significant difference compared with control rats. The duty cycle (a ratio of the inspiratory duration to the total respiratory duration) of COPD rats was significantly higher than that of control rats. Next, we analyzed swallowing changes. The onset-to-onset interval between Dia and Dig activity did not show significant difference between control and COPD rats. The frequency of swallowing reflex during inspiratory phase was higher in COPD model rats. At 12th week after elastase administration, falling time and duration of Dig and TH EMG activities were significantly longer compared with control rats. Finally, to clear the cause of the time dependent change of swallowing related muscles activity in COPD rats, we stained swallowing related muscles by HE methods. There was no difference in the mean muscle cell number of Dig and TH between control and COPD rats. Either Dig and TH muscle images did not show pathological changes.This study suggested that COPD condition caused the respiratory discoordination. Furthermore, long term COPD affected the swallow-related muscle activity but not pathological changes of muscles. So, Further studies are necessary to clarify which mechanisms contribute to functional changes of respiration and swallowing in COPD rats.

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Poster

392. Motor Unit Recordings, Kinematics, and EMG

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Program #/Poster #: 392.23

Topic: E.09. Motor Neurons and Muscle

Support: NIH 5U01EB021921-04
NIH F31NS124347

Title: New tools to interpret the role of spinal interneurons in motor modularity

Authors: *T. S. SMITH[1], T. KIM[1], T. D. SANGER[2], S. F. GISZTER[1];
[1]Neurobio. & Anat., Drexel Univ. Col. of Med., Philadelphia, PA; [2]Electrical Engin., CHOC, Redondo Beach, CA

Abstract: Motor behaviors operate through the spinal cord to controls the musculature quickly and efficiently. In the motor modularity model of movement, dynamic ‘building blocks’ are combined to construct most routine motor commands. We define a motor ‘module’ as a neural element evoking stereotyped motor activity, extracted from kinetic or biological features (e.g. muscle EMG). In the spinal frog, free of the influence of higher centers, spinal modularity has been studied in various contexts. The protective inter-hindlimb wiping reflex is an inducible behavior composed of three motor modules. Alternatively, focal stimulation to the intermediate spinal gray, such as via intraspinal microstimulation (ISMS), directly evokes multi-muscle motor behavior with dual-site stimulation usually summing independently (i.e. behaving modularly). Because such ISMS recruits motoneurons in a naturalistic recruitment order, its use has been proposed for neuroprostheses. During wiping, the spinal cord must control when modules are recruited and how modules coordinate the motor pools to evoke muscle synergies, while also retaining flexiblity to permit corrections to external perturbations. Previous research has suggested that such corrections are gated by spinal state. Integration of ISMS-evoked responses into spinal motor plans remains an area of active research. If ISMS responses are independent throughout wipe, ISMS-based manipulations may be implemented as a simple linear sum of existing motor components, while phase dependence may reveal intrinsic motor constraints and clarify granularity of motor recruitment. Beyond evaluating the linearity of the evoked force and muscles responses, one method of elucidating this relationship is to investigate patterns of motoneuron recruitment within and across motor pools. We can record activity from both single motor units and interneurons with our fine-wire braided electrodes, and have found that single motor units may be strongly tuned to activity of a synergist muscle, suggesting fixed patterns of muscle coactivations. Because the role of individual spiking units in the motor pattern may be small and spinal-state dependent, we utilize the stochastic dynamic operator (SDO) framework to evaluate relationships between spike times and continuous signals (e.g. EMG). We demonstrate here that the SDO improves upon the classical spike-triggered average for predicting post-spike signal behavior. As we continue to explore interpret this data, we expect to further elucidate the neural underpinnings of spinal modularity. Furthermore, we anticipate our innovations to be broadly applicable to other realms of neuroscience.

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Poster

392. Motor Unit Recordings, Kinematics, and EMG

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 392.24

Topic: E.09. Motor Neurons and Muscle

Support: NINDS
VA

Title: Corticospinal Excitability Across Lower Limb Muscles in Humans

Authors: *I. EISNER-JANOWICZ[1], M. A. PEREZ[1,2,3];
[1]Shirley Ryan AbilityLab, Chicago, IL; [2]Dept. of Physical Med. and Rehabilitation, Northwestern Univ., Chicago, IL; [3]Edward Hines Jr., VA Hosp., Hines, IL

Abstract: Anatomical and physiological studies in non-human primates reported the existence of strong corticospinal projections to intrinsic foot muscles compared with other hindlimb muscles. However, the extent to which corticospinal excitability differs across muscles in the lower limb in humans remains largely unknown. Here, we constructed recruitment curves of motor evoked potentials (MEPs), elicited by transcranial magnetic stimulation over the leg representation of the primary motor cortex, in the quadriceps femoris, hamstrings, tibialis anterior, soleus, and intrinsic foot muscles including the abductor hallucis, extensor digitorum brevis, and flexor digitorum brevis in seventeen control volunteers to measure the resting motor threshold (RMT) and the maximum MEP size (MEP-max) and slope. We found that the MEP-max and slope were larger in intrinsic foot muscles compared to the other leg muscles tested. In addition, the MEP-max and slope were larger in the quadriceps femoris and tibialis anterior compared with the hamstrings and soleus muscles, respectively, suggesting larger corticospinal excitability in muscles in the anterior compared with the posterior compartment of the leg. To further understand the mechanisms contributing to changes in corticospinal excitability, we tested motor neuron excitability (measured by F-waves) in distal leg muscles. We found that the F-wave amplitude was larger in the abductor hallucis compared with the tibialis anterior and soleus muscle. These results provide evidence for the existence of a non-uniform distribution of corticospinal responses to lower limb muscles in intact humans, highlighting an increased corticospinal excitability to intrinsic foot muscles related, at least in part, to spinal mechanisms.

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Poster

392. Motor Unit Recordings, Kinematics, and EMG

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Topic: E.09. Motor Neurons and Muscle

Support: Conacyt grant (2019-000006-01NACV-00352)
Universidad de Guadalajara

Title: Locomotion outcome improvement in mouse with glioblastoma multiforme after treatment with anastrozole

Authors: *S. H. DUENAS JIMENEZ[1], I. AGUILAR[2], G. MENDIZABAL RUIZ[2], J. ALPIRES NARANJO[2], J. DUEÑAS JIMENEZ[2], C. GUTIÉRREZ ALMEIDA[2], L. OSUNA CARRASCO[2], V. RAMÍREZ ABUNDIS[2], I. JIMÉNEZ ESTRADA[3];
[1]Univ. De Guadalajara, Univ. de Guadalajara, Zapopan, Mexico; [2]Univ. de Guadalajara, Guadalajara, Mexico; [3]Univ. de Guadalajara, Ciudad de México, Mexico

Abstract: Glioblastoma Multiforme (GBM) infiltrates several brain structures, and estrogen receptors participate in tumor growth. GBM is associated with abnormal motor activities resulting in impaired mobility and loss of functional motor independence. We used a GBM xenograft implanted in the striatum to analyze the changes, before and after anastrozole treatment, in Y (vertical) and X (horizontal) axis displacement of the metatarsus, ankle, and knee. The dissimilarity factor between control and GBM mice steps was also analyzed. The body weight of the untreated animals decreased compared to treated rats. Anastrozole reduced tumor growth and decremented GPR30 and ERa receptor expression. In addition, we observed a partial recovery in left metatarsus and knee joint displacement (dissimilarity factor). The GBM+anastrozole group showed a minor difference in the right metatarsus, right knee, and left ankle vertical axis displacement compared to GBM. Furthermore, in the right metatarsus, ankle, and knee horizontal axis displacement of GBM+anastrozole, a difference at the end of the step cycle was observed compared to GBM. Thus, anastrozole partially modified kinematics joint displacement. The dissimilarity factor and the vertical and horizontal displacements should be studied in patients with GBM presenting locomotion alterations. Hindlimb displacement and gait locomotion analysis could be a valuable methodological tool in experimental and clinical studies to help diagnose locomotive deficits.

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Poster

392. Motor Unit Recordings, Kinematics, and EMG

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 392.26

Topic: E.09. Motor Neurons and Muscle

Support: CIHR Grant
NSERC Grant
Ontario Graduate Scholarship

Title: Elucidating the mechanisms of transcranial ultrasound for modulation of human motor cortex.

Authors: *Y. SHAMLI OGHLI, T. CORTEZ-GRIPPE, T. ARORA, T. HOQUE, G. DARMANI, R. CHEN;
Toronto Western Res. Inst., Toronto Western Res. Inst., Toronto, ON, Canada

Abstract: Transcranial ultrasound stimulation (TUS) is a non-invasive brain stimulation technique, capable of immediate (“online”) inhibitory effects, and prolonged (“offline”) excitatory effects with theta burst TUS in the human motor cortex. Compared to other non-invasive techniques such as transcranial magnetic stimulation (TMS), it is capable of stimulating

with a greater depth penetration and spatial resolution. However, the *in vivo* mechanisms of action are unclear. By administering brain-active drugs with known mechanisms together with TUS, we aimed to determine the mechanisms of action of TUS. Nine healthy subjects participated in a within-subject randomized, double-blinded, cross-over study with five visits. At each visit, one of four study drugs (carbamazepine, nimodipine, lorazepam, dextromethorphan) or placebo was administered. Online inhibitory TUS effects on motor evoked potentials (MEPs) from TMS were reduced following administration of lorazepam. Offline excitatory TUS effects were reduced by all study drugs compared to placebo. The online inhibitory effect of TUS may involve GABAA receptors, but the effect may have been confounded by auditory activation. The offline results suggests that theta burst TUS induces synaptic plasticity mediated by NMDA receptors, involves Ca2+/Na+ channels and is modulated by GABAA receptors. These results provide the first assessment of the pharmacological mechanisms of TUS brain stimulation in human and will be conducive to the development of TUS as a potential treatment of neurological and psychiatric disorders, as well as a tool for the study of the brain. Data collection is ongoing and more subjects are being recruited, increasing sample size in time for the conference.

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Poster

392. Motor Unit Recordings, Kinematics, and EMG

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 392.27

Topic: E.09. Motor Neurons and Muscle

Title: Predicting the amount of food swallowed during Aplysia californica feeding from neural recordings

Authors: *M. BERAMENDI CABALLERO[1], J. P. GILL[1], H. J. CHIEL[2];
[1]Biol., [2]Biology, Neurosciences, Biomed. Engin., Case Western Reserve Univ., Cleveland, OH

Abstract: To understand adaptive behavior, it would be useful to use neural recordings to predict the movements or forces that an animal generates. A model system in which this problem can be addressed is the marine mollusk *Aplysia californica*. The collection of nerve cells that control feeding behavior, the buccal ganglia, has about 2,000 neurons, many of which are identified as individuals. Moreover, the feeding behavior of the animal has been intensively studied: biting, which is a failed attempt to grasp food; swallowing, which is a successful attempt to ingest food; and rejection, which is the expulsion of food or inedible material from the buccal cavity. Furthermore, many of the specific muscles that control these movements and the motor neurons that direct the muscles' activity have been intensively studied. Thus, it is possible to create a quantitative model that takes neural activity as input and predicts movements or forces as output. We focus on swallowing, whose power stroke is retraction, during which the animal draws food into the buccal cavity. The key muscle responsible for retraction is I3, which is

innervated by neurons B6/B9, B3, and B10. Since the I3 muscle, like smooth muscle, generates excitatory junction potentials in response to motoneuronal activity, we modeled neural inputs using alpha functions. When a spike occurs, an alpha function is generated so that, in response to a spike train, alpha functions summate. To predict the amount of food pulled in, we use simulated annealing to fit the parameters of the model: relative amplitudes and time constants of the alpha functions. Since feeding is quasi-static, neural activity determines muscular contraction, and thus inward movement, so the net amount of food drawn in can be predicted. Spike timings were measured from extracellular recordings of buccal nerve 2 in intact, freely behaving animals (Cullins & Chiel, 2010). Spike timings for B3, B6/B9, and B10 were based on spike amplitude and behavioral phase. Animals were fed on uniform seaweed strips marked at 1-cm intervals and were videoed to determine swallow amounts. Preliminary results suggest that it is possible to use this simple model to predict the amount of inward seaweed movement. In one animal, whose swallows were recorded on two successive days, the R-squared values between the amount predicted and the actual inward movement were 0.57 (n=15 swallows) and 0.65 (n=12 swallows). In a second animal, the R-squared value was 0.35 (n=53 swallows). A sensitivity analysis showed that the model was relatively robust to measurement perturbation. These results suggest that incorporating feeding apparatus biomechanics may be important for more accurate predictions.

Disclosures: **M. Beramendi caballero:** None. **J.P. Gill:** None. **H.J. Chiel:** None.

Poster

392. Motor Unit Recordings, Kinematics, and EMG

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 392.28

Topic: E.09. Motor Neurons and Muscle

Support: DARPA N65236-19-C-8017

Title: Non-uniform pulse trains for efficient transcranial motor stimulation in rodents

Authors: *M. FORSSELL, V. JAIN, C. GOSWAMI, D. Z. TANSEL, G. K. FEDDER, M. CHAMANZAR, P. GROVER;
Electrical and Computer Engin., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: *Objective:* Traditional neural stimulation uses uniform trains of identical rectangular pulses, where pulse widths, amplitudes, and frequencies can be tuned to meet experimental needs. Some recent research has focused on changing the shape of individual pulses in order to improve stimulation, primarily by reducing the energy required to produce spikes, but little attention has been devoted to trains with non-identical pulses. Optimizing amplitudes of individual pulses in a train reduces the total energy required to stimulate, which could alleviate scalp heating resulting from transcranial stimulation, and might also reduce the risk of tissue damage. An optimized temporal waveform could ultimately allow transcranial neural stimulation

without activating the scalp nociceptors, which are known to cause discomfort with typical current waveforms.
Methods: We investigated varying amplitudes of individual pulses in pulse trains used for both intracortical and transcranial stimulation of mouse motor cortex, while measuring motor evoked potentials (MEPs) in forelimbs.
Results: We found that, for transcranial stimulation, the electric charge required to generate an MEP is reduced by 39 ± 14 % on average for a train of 4 pulses (biphasic 1ms/phase, repeated at 200 Hz) with linearly increasing amplitude (25% - 50% - 75% - 100% of the final amplitude) compared to a train of 4 uniform pulses. This effect is not observed for intracortical stimulation, where the threshold increases by 16 ± 13 %. Other train conditions were investigated, showing some improvements when transcranially stimulating using trains with fewer pulses. A simple model of motor activation is proposed which explains these results. Assuming a sea of identical neurons within a specific volume are excited by either a transcranial or an intracortical electric field, the total number of neural spikes in this volume that result from the defined stimulation waveform shows a trend similar to that of the experimental motor output.
Conclusions: Varying amplitude pulse trains can lower the required charge to generate the same MEP. Conversely, for the same required charge, in some cases, the generated MEP amplitude can be two or more orders of magnitude higher.

Disclosures: **M. Forssell:** None. **V. Jain:** None. **C. Goswami:** None. **D.Z. Tansel:** None. **G.K. Fedder:** None. **M. Chamanzar:** None. **P. Grover:** None.

Poster

392. Motor Unit Recordings, Kinematics, and EMG

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 392.29

Topic: E.09. Motor Neurons and Muscle

Support: CONACyT Grant 628536 (CHB)
CONACyT Grant 732830 (DAZL)
NIH Grant 1R01DK20307-01

Title: Neuromodulation of the pelvic floor muscles increase urethral and vaginal pressure in the female rabbit

Authors: *C. HERNÁNDEZ BONILLA[1], D. A. ZACAPA[1], R. ZEMPOALTECA[2], D. L. CORONA QUINTANILLA[2], F. CASTELÁN[3], M. I. ROMERO-ORTEGA[4], M. MARTÍNEZ GÓMEZ[3];
[1]Univ. Autónoma de Tlaxcala, Doctorado en Ciencias Biológicas, Tlaxcala, Mexico; [2]Ctr. Tlaxcala de Biología de la Conducta, Univ. Autónoma de Tlaxcala, Tlaxcala, Mexico; [3]Dept. de Biología Celular y Fisiología, Inst. de Investigaciones Biomédicas, Univ. Nacional Autónoma de México, México, México, Mexico; [4]Bioengineering and Biomed. Sci., Univ. of Houston, Houston, TX

Abstract: Urinary incontinence (UI) is a major public health condition that negatively affects the quality of life of millions of people globally, and with great cost to society. In Overactive Bladder or Urge Urinary Incontinence Neuromodulation of the sacral S2-4 nerve branches, or the tibial nerve are considered as viable therapies. However, these treatments are not effective for stress urinary incontinence (SUI), a condition associated with partial nerve damage and muscle weakness in the pelvic and perineal muscles. We have previously reported that acute pelvic floor muscle neuromodulation (PFMN) increases the bladder capacity in old multiparous rabbits, an accepted SUI model. As these muscles serve as secondary sphincters, we reasoned that will increase the urethral and vaginal pressure of female rabbits. This hypothesis was tested in 18 nulliparous Chinchilla-breed female rabbits randomly distributed into three groups of six animals each for electrical stimulation of the following targets: 1) bulbospongiosus nerve (Bsn), isquiocavernosus nerve (Isn) and the clitoral nerve as non-specific control (Cln). Silver hook electrodes were used to stimulate the exposed target nerves and the muscle contraction threshold current was determined. Electromyograms, muscle contractile force, and urethral and vaginal pressure were recorded. Neuromodulation was applied as trains of bipolar electrical pulses ranging from 1-80 Hz with a duration of 4 seconds each. At low frequencies (20-30 Hz) muscle contraction achieved activation without fatigue. At 40-60 Hz a maximum increase in urethra and vaginal pressure was observed (0.33 ± 0.11 and 3.24±1.84 mmHg, respectively). Stimulation of the Isn resulted mild increase in urethral (0.40 ± 0.04 mmHg) but significant increase in vaginal (2.56 ± 0.29 mmHg) pressure. The data supports the notion that pelvic floor neuromodulation assist in urethral closure and improves continence in SUI and may be beneficial in other pelvic floor disorders.

Disclosures: C. Hernández Bonilla: None. **D.A. Zacapa:** None. **R. Zempoalteca:** None. **D.L. Corona Quintanilla:** None. **F. Castelán:** None. **M.I. Romero-Ortega:** Other; Shareholder of Regenerative Bioelectronics Inc. (RBI Medical), a company that has commercial interest in neuromodulation of the PFM. **M. Martínez Gómez:** None.

Poster

392. Motor Unit Recordings, Kinematics, and EMG

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 392.30

Topic: E.09. Motor Neurons and Muscle

Support: Conacyt CF1311312
Conacyt ZFL 1001233

Title: Effect L6-S2 root avulsion on the morphophysyological characteristics of the urinary bladder in the female rabbit

Authors: M. MERCADO[1], A. FLORES-HERNÁNDEZ[2], Z. FLORES-LOZADA[2], O. SÁNCHEZ-GARCÍA[1], R. ZEMPOALTECA[3], F. CASTELÁN[4], M. MARTÍNEZ-GÓMEZ[4], D. CORONA-QUINTANILLA[3];

[1]Licenciatura en Química Clínica, [2]Posgrado en Ciencias Biológicas, Univ. Autónoma de Tlaxcala, Tlaxcala, Mexico; [3]Ctr. Tlaxcala de Biología de la Conducta, Univ. Autónoma Tlaxcala, Tlaxcala, Mexico; [4]Inst. de Investigaciones Biomédicas, UNAM, Tlaxcala, Mexico

Abstract: The spinal cord is a center specialized in the reception and sending of stimuli. At the lumbosacral level (L6-S2) the excitatory-inhibitory reflexes are integrated to regulate different functions of the lower urinary tract and pelvic floor musculature. The nerve roots that emerge from the ventral horn of the spinal cord have a certain fragility and the axons carry information to effectors, such as viscera and pelvic floor muscles. However, ventral roots are susceptible to a variety of mechanical damage. For example, its stretching or rupture (avulsion) interrupts the transmission of bioelectrical signals between the central nervous system and effectors. The aim was to determine if the physiological alterations of the urinary bladder are derived from its hypertrophy, caused by the L6-S2 ventral root avulsion (VRA) in female rabbit. For this purpose, virgin rabbits were randomly assigned into two groups (n= 16): A) Sham and B) L6-S2 VRA. Once the rabbits were anesthetized, group A) Sham was performed VRA simulation; and B) L6-S2 VRA, the ventral roots of the lumbosacral plexus (L6-S2) were avulsed unilaterally. On the 15th day after the lesion, simultaneous cystometrograms and urethral profile were recorded. Subsequently, they were perfused with paraformaldehyde, the urinary bladder was located and obtained, to continue with histological processing and the performance of the sections. The results obtained demonstrated that the rabbits at 15 days with L6-S2 VRA show urodynamic affectations, such as a decrease in bladder efficiency and bladder contraction, due to the morphological changes shown in the histological sections of the urinary bladder, as well as an increase in its weight and in the thickness of the lamina propria and serous layers, in addition to the thinning of the smooth muscle layer. These findings provide insight into the level of damage that is in the urinary bladder due to VRA.

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Poster

393. Social Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 393.01

Title: WITHDRAWN

Poster

393. Social Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 393.02

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: JSPS 18K10830

Title: Effects of voluntary physical activity enhanced by multistory enriched environment on physical functions

Authors: *S. YANAGITA[1], N. KUBOTA[2], N. UBA[1], N. KASAI[1], T. YAMAMOTO[1];
[1]Tokyo Univ. of Sci., Tokyo Univ. of Sci., Chiba, Japan; [2]Tokyo Intl. Univsesity, Saitama, Japan

Abstract: Numerous studies have shown that enriched environments (EE) could be effective for experimental rodents to improve some brain functions related to stress response and anxiolytic effect, and speculating that playfulness in EE might influence it. On the other hand, it is well known that increasing levels of physical activity could have beneficial effects as well as EE. Taken together with this evidence, the question arises: Which is effective for the improvement of brain function between playing or physical activity? To answer the question, we made a multistory enriched environment (Multi-EE) that can increase physical activity in rats. However, it is difficult to measure physical activity by existing analysis methods, such as infrared ray systems, and video tracking. Recently, a mobile accelerometer has been developed to be able to analyze the amount of physical activity without limitation. In this study, we performed the behavioral analysis of voluntary physical activity using the mobile accelerometer in our original enriched environments. We originally made Multi-EE, which are consisted of three stories. The male Wistar rats housed the Multi-EE or normal- EE for 4weeks in group housing conditions (2 rats per cage). The rats housed in Multi-EE allow access to the three stories freely by ladders. Daily physical activity was recorded using a mobile accelerometer and compared Multi-EE and Normal-EE. Following 4weeks, brain monoamine levels, which are involved with increasing physical activity-induced- psychological effects, were measured by High-Performance Liquid Chromatography (HPLC) in several brain regions. Muscle and fat volume were also measured. In this study, we have been successful to analyze voluntary physical activity in both normal-EE and Multi-EE. The Multi-EE significantly changed physical activity compared to normal-EE. The voluntary physical activity in Multi-EE significantly increased the volume of soleus muscle compared to normal-EE, indicating that Multi-EE might be effective to increase the physical load. Furthermore, the Multi-EE housing was able to change the brain monoamine levels, such as serotonin and dopamine. The changing levels of these monoamines are known to have some beneficial effects on brain health. Therefore, the results of the present study suggest that increasing levels of physical activity by Multi-EE could influence some brain and physical functions.

Disclosures: S. Yanagita: None. **N. Kubota:** None. **N. Uba:** None. **N. Kasai:** None. **T. Yamamoto:** None.

Poster

393. Social Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 393.03

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH grant
NIH grant
Center for behavior neuroscience

Title: V1ar knockdown in the ventral pallidum area alters male social behavior

Authors: *A. SELKE, I. AMMAVAJJALA, G. DEVRIES, A. PETRULIS;
Neurosci., Georgia State Univ., Atlanta, GA

Abstract: The neuropeptide arginine-vasopressin (AVP) has long been implicated in the regulation of diverse social behaviors, including maternal care, pair bonding, aggression, and social communication, often in a sexually differentiated way. One source of sexually differentiated AVP pathways is the bed nucleus of the stria terminalis (BNST) and manipulations of these BNST AVP cells strongly alters social behavior and communication. The BNST AVP cells project to various brain regions that contain cells expressing vasopressin 1a receptors (V1aR), the main AVP receptors in the brain. Consequently, AVP may be altering social behavior by acting on cells expressing V1aR. Here we examine the behavioral effects of reducing V1aR expression in the ventral pallidum (VPal), an V1aR-rich area. Previously, the VPal has been implicated in V1aR effects on social behavior as well as reward-related processing more generally. As past work in male prairie voles and male and female rats has demonstrated that V1aR in the VPal alters social preferences differently between sexes, we hypothesize that V1aR-expressing VPal cells support different social behaviors for males and females. To test this hypothesis, we injected a viral vector encoding shRNA targeting V1aR (or control scrambled sequence) into the VPal of adult male and female C57BL/6J mice and examined their social investigation and social communication (urine marking and ultrasonic vocalizations) behaviors. Preliminary data indicate that knockdown of V1aR in the VPal of males reduced their urine marking specifically toward females. No effect of V1aR knockdown was observed on male social investigation, ultrasonic vocalizations, or on any female social behavior. These results suggest that V1aR in VPal is needed for appropriately-directed male social communication.

Disclosures: **A. Selke:** None. **I. Ammavajjala:** None. **G. Devries:** None. **A. Petrulis:** None.

Poster

393. Social Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 393.04

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: Phencyclidine-induced social withdrawal is not dependent on a functional oxytocin receptor

Authors: *C. SAPP, A. LOSCO, A. ZUPANCIC, H. K. CALDWELL;
Dept. of Biol. Sci., Kent State Univ., Kent, OH

Abstract: Schizophrenia is a debilitating multi-etiological neurodevelopmental disorder whose neural underpinnings are most often linked to dysregulations in the dopaminergic and glutamatergic systems. However, there is also evidence that the oxytocin (Oxt) system may contribute to some of the negative symptoms, such as impaired social interactions and social motivation. Here, we wanted to explore how the presence or absence of oxytocin receptor signaling would affect behaviors in a phencyclidine (PCP)-withdrawal mouse model of schizophrenia. We hypothesized that mice lacking functional Oxt receptors (Oxtr) would have impaired social approach behaviors following withdrawal from subchronic PCP treatment up and above that observed in control mice. To test this hypothesis, Oxtr wildtype (+/+) and Oxtr knockout (-/-) mice, generated from heterozygous breeding pairs, were administered subchronic PCP (5mg/kg) intraperitoneally, twice daily for seven days. Control animals were injected with saline on the same schedule. Following the week of injections, experimental animals were given another seven days to withdrawal; this has been shown to induce schizophrenia-like symptoms. On the first day following withdrawal, mice were tested for locomotor activity. The following day, mice were tested for sociability using a 3-chamber social interaction test, utilizing ovariectomized female C57BL/6J mice as stimulus animals. On the final day, mice were tested for depression-like behaviors using a forced swim test. When analyzed, the data did not support our hypothesis, as we found no genotypic-dependent effects of PCP on any of the behaviors measured. However, as would be expected, PCP-injected mice spent less time in the social chamber than their saline injected counterparts. These data suggest that the social withdrawal induced in this schizophrenia model is not Oxtr-dependent.

Disclosures: C. Sapp: None. **A. Losco:** None. **A. Zupancic:** None. **H.K. Caldwell:** None.

Poster

393. Social Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 393.05

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: The role of striatum in social hierarchy formation in mice.

Authors: *M.-T. HSU[1,2], K. Z. TANAKA[2], J. R. WICKENS[1];
[1]Neurobio. Res. Unit, [2]Memory Res. Unit, Okinawa Inst. of Sci. and Technol. Grad. Univ., Onna-son, Kunigami-gun, Okinawa, Japan

Abstract: A social hierarchy is a ranked social group. The existence of a social hierarchy reduces needless competition for space and resources within a social group. Within a social

hierarchy, a dominant individual has reproductive and food access privileges and engages in aggressive behavior as needed to protect them. Conversely, a subordinate individual has less access to resources and follows the rules created by a dominant. Previous studies indicate that the thalamocortical projection and the ventral tegmental projection to the nucleus accumbens are involved in regulating social dominance during social hierarchy formation, but the underlying neural mechanisms are not yet fully understood. Behavioral flexibility is involved in constructing a social hierarchy because it requires strategy switching from winner to loser during a competition. Recent evidence indicates that the cholinergic interneurons of the striatum may play a role in behavioral flexibility. Thus, we hypothesize that the striatum is involved in social hierarchy formation. To test this hypothesis, we are using the tube dominance test to identify the existing social status in the same cage of mice and forcibly construct a new social hierarchy in the pairs of mice with the same social ranking. We are then applying optogenetics to manipulate the neuronal activity in the striatum during the tube dominance test to understand the causal role of the striatum in social hierarchy formation. We have found that a forced outcome of competition in the tube dominance test causes stable social hierarchy formation that persists in the home cage setting. Therefore it is possible to examine the effects of striatal manipulations on social hierarchy formation. Ongoing work is examining the effects of optogenetic manipulation during the forced tube dominance test. In summary, we have developed a reliable behavioral protocol to construct a new social hierarchy in mice, and the winner/loser consequence during competitions may affect the existing social status in the same cage of mice. In addition, we are testing the hypothesis that the striatum may play an essential role in the strategy switching from winner to loser, which affects a new social hierarchy construction in mice.

Disclosures: **M. Hsu:** None. **K.Z. Tanaka:** None. **J.R. Wickens:** None.

Poster

393. Social Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 393.06

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NSF IOS 1946613

Title: Perineuronal net presence and pair bond status in monogamous mice

Authors: *C. L. MALONE[1], C. A. MARLER[2];
[1]Psychology, Univ. of Wisconsin- Madison, Madison, WI; [2]Univ. of Wisconsin-Madison, Madison, WI

Abstract: Behavioral transitions are important for adult context-dependent behaviors.The monogamous California mouse exhibits a stark behavioral change during pair bond establishment, namely a shift from territorial aggression to affiliative monogamy. We found a reduction in aggression within 24 hours and a slow increase in classical affiliative behaviors over

72 hours. In addition, using newly-trained machine learning and neural networks, we counted and categorized ultrasonic vocalizations (USVs) that were concurrently recorded with behavioral observations. Results indicated a decrease in sweep USVs after the first day of pair establishment. We predicted that the biological mechanisms underlying this behavioral transition would be reflected in restructuring of the extracellular matrices of cells in brain areas important for social learning and adaptation. One such extracellular change that supports social learning is the number and density of perineuronal nets (PNN). PNN are structural supports that surround neuron bodies to solidify new synaptic connections and support dendritic changes associated with learning. Such changes occur with developmental and seasonal plasticity in neural tissue underlying avian social song. The structural changes governing the shift from aggressive to affiliative behavior in mammals have not been readily studied. In order to address the hypothesis that changes in PNN may correlate with changes in pair-bonding behavior across this establishment period, cell counts of parvalbumin+ (PV+) and PNN+ were completed in the anterior cingulate cortex and the lateral septum, two areas highly associated with affiliative and aggressive behavior, in both paired and unpaired mice. Preliminary results indicate that a difference in the number of PNN+ and PV+ cells were not found between unpaired and paired animals. Other brain regions are being investigated as well.

Disclosures: C.L. Malone: None. **C.A. Marler:** None.

Poster

393. Social Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 393.07

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NSERC

Title: Estradiol Rapid Facilitation of Social Recognition in the Medial Amygdala of Female Mice Depends upon Oxytocin Receptors

Authors: *C. SEXTON, S. MCGUINNESS, E. CHOLERIS;
Univ. of Guelph, Guelph, ON, Canada

Abstract: Social recognition (SR) is an adaptive cognitive skill critical for animals' successful participation in their social group. Estrogens and their better-known receptors, ERα, ERβ, and GPER1 have been shown to rapidly facilitate SR. Estrogens interact with other neurochemicals such as oxytocin (OT). Previous research has shown infusions of a subeffective dose of an oxytocin receptor antagonist (OTRA) into the medial amygdala (MeA) prior to an infusion of 17-β estradiol (E2) into the PVN prevents E2 rapid facilitating effects on SR. E2 and agonists for all 3 ERs rapidly facilitate SR even when infused directly into the MeA. Whether an E2/OTR interaction occurs also within the MeA, is unknown. If such interactions exist, then the OTRA will block the enhancing effects of E2 in the MeA, at a dose that does not block the natural

occurrence of SR. Ovariectomies were conducted to reduce circulating estrogen levels to those of diestrus, and cannulae were surgically implanted into the MeA. The test mouse was initially administered either 75nM OTRA, previously shown to be a subeffective dose, or a vehicle of artificial cerebrospinal fluid (aCSF) into the MeA. After two minutes the test mice then received either aCSF or 25nM or 10nM of E2 into the MeA, both E2 doses having shown to facilitate SR in MeA. Next, the test mouse underwent a "difficult" social recognition paradigm that ovariectomized mice cannot perform. Additionally, a difficult object recognition (OR) paradigm was employed to determine whether facilitating effects of 25nM E2 in the MeA were specific for SR. It was predicted that the infusion of the OTRA would prevent the enhancing effects of E2 in the SR paradigm, but these results would be social specific. Preliminary results indicate 10nM and 25nM of E2 when paired with aCSF facilitates SR, but when paired with the OTRA does not, supporting our hypothesis that E2 rapid facilitation of SR in MeA requires OTRs. Additionally, no significant results were found in the OR paradigm, indicating social specificity in the effects of 25nM E2 in the MeA. Combined, this research program elucidates the mechanisms of estrogens, specifically those of E2, rapid enhancing effects on female SR in the MeA, which is a key component of the social brain.

Disclosures: **C. Sexton:** None. **S. McGuinness:** None. **E. Choleris:** None.

Poster

393. Social Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 393.08

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: Are subordinate rats more susceptible to stress?

Authors: *D. SRINIVASAN, S. CHATTARJI;
Natl. Ctr. for Biol. Sci., Natl. Ctr. for Biol Sci., Bangalore, India

Abstract: Establishing social dominance hierarchy is important for access to resources such as food, space, reproductive success in social organisms. Studies in humans and other species show that social rank can affect physiology, behaviour, and health of individuals (Adler et al., 1994). Despite growing evidence for individual differences in vulnerability to stress, how social hierarchy affects an animal's ability to cope with stress remains less explored. Hence, we examined how hierarchical status of an animal (i.e., dominant vs. subordinate) influences its response to stress, and how stress exposure alters pre-existing social hierarchy. Specifically, we analysed (a) the animal's ability to form and express a social hierarchy, (b) social dominance behavior when animals are confronted by another rat living in the same cage and (c) social dominance when confronted by a stranger rat from another cage. To this end, we subjected male Sprague Dawley rats (55-60 days old), to the social dominance tube test (Fan et al., 2019). Once the status of the animals within a cage was determined, rats were subjected to either a single episode of 2-hour of immobilisation stress (acute) or chronic immobilisation stress (2 h/day for

10 days). Next, cage-mates were compared in the tube test one day after acute and chronic stress. Surprisingly, pre-determined hierarchical rank amongst rats housed in the same cage was unaffected one day after either acute or chronic stress. However, the same acute stress had a significant impact on social rank when stressed rats competed with stranger rats from a different cage. Notably, stressed subordinates avoided conflicts regardless of the social status of the novel conspecifics. Thus, individual differences, as evidenced by their social ranks, shape how they respond to stress. This underscores the importance of considering the sub-group structures that exist within cages while assessing the impact of stress on social behaviours in rodents. These findings add a new dimension to animal models of stress used for exploring facets of social withdrawal and social anxiety in stress-related psychiatric disorders.

Disclosures: D. Srinivasan: None. **S. Chattarji:** None.

Poster

393. Social Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 393.09

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: Disruption of vasopressin 1a signaling on embryonic day 16.5 does not affect anxiety-like or depression-like behaviors in adult mice

Authors: *K. REESE[1], H. K. CALDWELL[2];
[1]Biol. Sci., [2]Dept. of Biol. Sci., Kent State Univ., Kent, OH

Abstract: Disruption of vasopressin 1a signaling on embryonic day 16.5 does not affect anxiety-like or depression-like behaviors in adult mice
Katlynd Reese, Heather K. Caldwell
Vasopressin (Avp) has been shown to play an important role in the sex specific neural regulation of social behavior. Additionally, there is growing evidence that Avp, and the closely related nonapeptide oxytocin (Oxt), modulate aspects of neural development and that alterations in signaling through their receptors in a developing embryo can impact social behaviors in adulthood. Previous work from our lab found that transient disruption of Oxt receptor signaling on embryonic day (E) 16.5 results in sex-specific behavioral effects in adulthood. Specifically, male Oxtr antagonist-treated mice have increases in agonistic behavior and social investigation as well as increases in depressive-like behavior. Female, Oxtr antagonist-treated mice have impaired social recognition memory. Given that Avp and Oxt are closely related, and that crosstalk often occurs between their receptors, it is reasonable to speculate that disrupting Avp signaling at this critical timepoint is likely to alter the trajectory of brain development such that adult behavior is affected. Thus, in this experiment we tested the hypothesis that transient disruption of Avpr1a signaling at E16.5 would impact adult behavior. To test this hypothesis, at E16.5, 2µL of either 0.05 ng/µL of an Avpr1a antagonist or vehicle was injected into the lateral ventricle. Dams were allowed to give birth normally and behavioral testing was performed when

the offspring were at least 2 months old. Male and female experimental animals underwent open field, elevated plus, forced swim, and 2-trial social discrimination testing. Male offspring were also used in resident-intruder aggression testing. No significant effects of Avpr1a disruption were observed in the open field, elevated plus, or forced swim test. These preliminary data suggest that disrupting Avpr1a signaling at E16.5 does not have a significant effect on anxiety-like or depressive-like behaviors in adulthood. The impact of disrupted Avpr1a signaling on social behavior is still being investigated. Given the presence of functional Avpr1a during embryonic brain development, future studies will continue to investigate the contribution of embryonic Avpr1a signaling on brain development and behavioral endpoints.

Disclosures: K. Reese: None. **H.K. Caldwell:** None.

Poster

393. Social Behavior

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Program #/Poster #: 393.10

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: ANII-Uruguay to DEO

Title: Description and comparison of oxytocin receptors brain distribution in Rhabdomys pumillio and Rhabdomys dilectus

Authors: *D. E. OLAZABAL[1], N. PILLAY[2], N. SANDBERG[1];
[1]Fisiología, Facultad de Medicina, Udelar, Montevideo, Uruguay; [2]Sch. of Animal, Plant, and Envrn. Sci., University of the Witwatersrand, South Africa

Abstract: Oxytocin receptor (OXTR) distribution in the brain has been associated with different reproductive and social strategies of the species. *Rhabdomys pumilio* (R.pumilio) and *Rhabdomys dilectus* (R.dilectus) are two related species that live in large (but flexible) and small social groups respectively. In this study we describe and compare for the first time the distribution of OXTR in these two species. OXTR binding in the brain of 26 R.pumilio (8 females and 5 males) and R.dilectus (8 females and 5 males) adults was determined using autoradiography. Our results revealed significant differences in the olfactory bulb, nucleus accumbens, diagonal band, medial preoptic area, lateral habenula, superior colliculus, periaqueductal area, and anterior paraventricular nucleus (higher in R.dilectus), and the dorsal lateral septum and anterior bed nucleus of the stria terminalis (higher in R.pumilio). OXTR density in other brain regions such as the amygdala nuclei, and hippocampus did not differ between these two species. Sex differences were found in the medial preoptic area, and ventral region of the lateral septum in R.pumilio (higher OXTR density in males) and in the anterior paraventricular nucleus, ventromedial nucleus of the hypothalamus, and basolateral amygdala of R.dilectus (higher OXTR density in females). A sex difference was also found in the posterior region of the bed nucleus of the stria terminalis, higher in males of these two species. This study

shows species specific brain distribution of OXTR in R.pumilio and R.dilectus that are unique, but with similarities with other promiscuous species that live in large or small groups such as R.norvergicus, C.sociabilis, S.teguina and M.musculus.

Disclosures: D.E. Olazabal: None. **N. Pillay:** None. **N. Sandberg:** None.

Poster

393. Social Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 393.11

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: Home-cage behaviors correlated with synchronization of fear in mice.

Authors: *N. TRIVEDI[1], A. MOROZOV[2], W. ITO[3];
[1]Virginia Tech. Carilion Sch. of Med., Virginia Tech. Carilion SOM, Roanoke, VA; [3]Virginia Tech. Carilion Res. Inst., [2]Virginia Tech. Carilion Res. Inst., Roanoke, VA

Abstract: Among humans, coordination depicts normal social behaviors, and these are often disrupted in mental/developmental disorders. Other social animals, such as mice, are shown to coordinate certain behaviors, like fear responses and can be used to model human behavior. In our lab, synchronization of fear responses was demonstrated in mice, however, there was variability of synchrony between pairs. Previous literature has identified neural structures necessary for synchrony and characterized social behaviors in mice. Despite this data, there is no established paradigm that can predict which dyads will demonstrate greater synchronization. The aim of this project is to determine if homecage social behaviors in mice can predict the synchrony of conditioned fear responses. We hypothesize that social homecage behaviors will be correlated significantly with synchronization of freezing. In our study, thirteen dyads of adult male mice (age ≥ p60) were housed in a homecage and were continuously monitored. Four one hour time periods were chosen for analysis and seven behaviors were identified: out of nest, immobility, eating, following, nose touching, self-grooming, and allo-grooming. The duration of each behavior was determined and, subsequently, the mice were individually conditioned to elicit the fear response (freezing) and tested in dyads. Levels of synchrony were calculated for every pair and graphed against each behavior duration. A t-test for linear regression was performed. The correlation data is as follows: nose touching showed a positive correlation (slope = 0.094, p=0.001), following had a lesser positive trend (slope = 0.0023, p=0.46), and total immobility had a negative correlation (slope = -0.00026, p=0.06). The other behaviors all had p-values above 0.05. Of the behaviors, nose touching was the only behavior that was significantly positively correlated with greater levels of synchrony (95% CI with $p<0.05$). The other behaviors showed no significance. The data shows that longer durations of nose touching are correlated positively with greater levels of fear synchronization. It can be posited that since nose touching is a social behavior, mice that engage in greater levels would show greater coordination in other social responses such as fear synchrony. Thus, nose touching duration can potentially be used to

predict levels of synchronization in mice. Future work includes similar studies on juvenile mice, developmental studies to determine when these behaviors emerge, and experiments to determine if the same circuits underlie synchrony and home cage behaviors.

Disclosures: **N. Trivedi:** None. **A. Morozov:** None. **W. Ito:** None.

Poster

393. Social Behavior

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Program #/Poster #: 393.12

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: Young Scholars Grant Undergraduate Research Opportunities Program, University of St. Thomas
Other Support, Neuroscience Department of the University of St. Thomas
Other Support, Biology Department of the University of St. Thomas

Title: Dopamine related gene expression in the social decision-making network in response to an infidelity challenge in the monogamous zebra finch

Authors: M. ERPELDING[1], M. MERRICKS[2], J. WESTBERRY[3], *S. HEIMOVICS[1]; [1]Biol., [2]Biochem., [3]Neurosci., Univ. of St. Thomas, St. Paul, MN

Abstract: Understanding the behavioral neuroendocrinology of pair-bond maintenance is critical to understanding the evolution of monogamy. While much is already known about how the brain regulates courtship and pair-bond formation, much less is known about how the neuroendocrine system maintains bonds once they are formed. In the current study, we used the zebra finch (ZEFI) (Taeniopygia guttata) model to explore the relationship between male motivation to maintain monogamous pair bonds during a social challenge and dopamine (DA)-related gene expression in the social decision-making network (SDMN). To quantify male motivation to maintain pair bonds, we performed an "infidelity challenge (IF)" which consisted of presenting a pair-bonded male with a novel, unpaired female. Control (CON) males were presented with their pair-bonded female partner. IF and CON males were observed for 30 min, and sociosexual interactions -- including directed and undirected song, courtship dancing (beak wipe, 'turn-around,' and hop), clumping, allopreening, billing, copulation solicitation, mounting, and copulation -- were recorded. Immediately following behavioral observations, brains were collected, frozen, and the Palkovits punch technique was used to microdissect nuclei of the SDMN. We then used RT-qPCR to quantify relative levels of mRNA for DA-associated genes including tyrosine hydroxylase (TH), D1-like receptors (D1R), and D2-like receptors (D2R). Pearson correlation analysis was used to explore the relationship between TH, D1R, and D2R mRNA levels and within-pair versus extra-pair behavior. Remarkably, we observed a significant negative correlation between D1R mRNA in the nucleus accumbens (AC) and the number of directed songs sung by IF, but not CON, males. An identical relationship was observed in the

ratio of D1R:D2R mRNA and number of directed songs. These data are consistent with findings in prairie voles, and further suggest that individual variation in the motivation to maintain monogamous pair bonds can be explained by individual variation in the level of D1R in AC. Additionally, t-tests revealed significantly lower relative levels of D1R and D2R mRNA in the preoptic area and TH mRNA in the bed nucleus of the stria terminalis in IF males compared to controls. Taken together, these data add to converging lines of evidence that implicate dopaminergic neurotransmission within the SDMN in monogamous pair bond maintenance. The potential for a role of opioidergic and nonpeptidergic gene expression in the SDMN in long-term pair bond maintenance will also be discussed.

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Poster

393. Social Behavior

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Topic: F.02. Neuroendrocrine Processes and Behavior

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Japan Agency for Medical Research and Development (AMED) Grant Numbers JP21wm0425005, 21ek0109490h0002

Title: Targeting neurons with functional oxytocin receptors: A novel set of simple knock-in mouse lines for oxytocin receptor visualization and manipulation

Authors: *Y. U. INOUE[1], H. MIWA[2], K. HORI[1], R. KANEKO[3], Y. MORIMOTO[1], E. KOIKE[1], J. ASAMI[1], S. KAMIJO[2], M. YAMADA[2], M. HOSHINO[1], T. INOUE[1];
[1]Dept. of Biochem. and Cell. Biol., Natl. Inst. of Neuroscience, NCNP, Kodaira, Tokyo, Japan; [2]Dept. of Neuropsychopharm., Natl. Inst. of Mental Health, NCNP, Kodaira, Tokyo, Japan; [3]KOKORO-Biology Group, Labs. for Integrated Biology, Grad. Sch. of Frontier Biosciences, Osaka Univ., Suita, Osaka, Japan

Abstract: The neuropeptide oxytocin (Oxt) plays important roles in modulating social behaviors. Oxytocin receptor (Oxtr) is abundantly expressed in the brain and its relationship to socio-behavioral controls has been extensively studied using mouse brains. Several genetic tools to visualize and/or manipulate Oxtr-expressing cells, such as fluorescent reporters and Cre recombinase drivers, have been generated by ES-cell based gene targeting or bacterial artificial chromosome (BAC) transgenesis. However, these mouse lines displayed some differences in

their Oxtr expression profiles probably due to the complex context and integrity of their genomic configurations in each line. Here we apply our sophisticated genome-editing techniques to the *Oxtr* locus, systematically generating a series of knock-in mouse lines, in which its endogenous transcriptional regulations are intactly preserved and evaluate their expression profiles to ensure the reliability of our new tools. We employ the epitope tagging strategy, with which C-terminally fused tags can be detected by highly specific antibodies, to successfully visualize the Oxtr protein distribution on the neural membrane with super-resolution imaging for the first time. By utilizing T2A self-cleaving peptide sequences, we also induce proper expressions of tdTomato reporter, codon-improved Cre recombinase, and spatiotemporally inducible Cre-ERT2 in Oxtr-expressing neurons. Electrophysiological recordings from tdTomato-positive cells in the reporter mice support the validity of our tool design. Retro-orbital injections of AAV-PHP.eB vector into the Cre line further enable visualization of recombinase activities in the appropriate brain regions. Moreover, the first-time Cre-ERT2 line drives Cre-mediated recombination in a spatiotemporally controlled manner upon tamoxifen administration. These tools thus provide excellent resource for future functional studies in Oxt-responsive neurons and should prove of broad interest in the field.

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Poster

393. Social Behavior

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Topic: F.02. Neuroendrocrine Processes and Behavior

Support: FCT grant PTDC/BIA-COM/30627/2017

Title: Oxytocin regulation of social buffering and social transmission of fear in zebrafish reveals its evolutionary conserved role in social information use

Authors: I. AKINRINADE[1], K. KAREKLAS[1], M. TELES[1], M. GLIKSBERG[2], G. PETRI[3], G. LEVKOWITZ[2], ***R. F. OLIVEIRA**[4,1];
[1]Gulbenkian Inst. of Sci., Oeiras, Portugal; [2]Weizmann Inst. of Sci., Rohovot, Israel; [3]ISI Fndn., Torino, Italy; [4]ISPA, Lisboa, Portugal

Abstract: It is adaptive for group-living animals to use social cues/ signals emitted by others in order to detect threats in the environment (e.g. predator detection). This can be hypothesized as an evolutionary origin for the transmission of emotional-like states between individuals. Here we tested to what extent the neurocircuitry for social threat detection is evolutionary conserved by assessing the role of oxytocin, known to regulate it in mammals, in social buffering and social fear contagion in zebrafish. Using mutants for the ligand of fish oxytocin (OT) and both of its

receptors (OTRs) present in zebrafish we showed that OT is necessary for observers to copy the distressed behavior of demonstrators (social fear transmission) as well as for dampening the alarm response of focal fish exposed to alarm substance in the presence of non-alarmed conspecifics (social buffering). OT exogeneous administration to the ligand mutant rescued the ability of observers to express both social fear transmission and social buffering, indicating that oxytocin both necessary and sufficient for both effects. Using the phosphorylation of the ribosomal protein S6 as a marker of neuronal activation, we have mapped the brain response to social buffering and contagion. In social contagion the activity of the central (Vc) and ventral (Vv) areas of the ventral telencephalon decrease suggesting the presence of a disinhibition mechanism, which we subsequently confirmed to be GABAergic. In contrast in social buffering the activity of Vc, Vv, Vd (dorsal area of the ventral telencephalon) and the pre-optic area increase. Moreover, the analysis of functional connectivity networks from neuronal co-activity data allowed us to identify different putative networks associated with buffering and contagion, which are disrupted in OT and OTR mutants. Together our results support an evolutionary conserved role for oxytocin as a key regulator of basic empathic behaviors across vertebrates.

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Poster

393. Social Behavior

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Program #/Poster #: 393.15

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIMH R00MH109674
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Title: Temporally selective inhibitory gates for aggressive motivation and action

Authors: *T. MINAKUCHI, S. N. OLINE, E. GUTHMAN, A. L. FALKNER;
Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ

Abstract: Social behaviors, like other motivated seeking behaviors, are generated through a temporal evolution from a motivated-seeking state to social action, and then potentially a reinitiation of this sequence. Deficits in social behavior can be primarily motivational, or can be in the execution of the behavior itself, suggesting that these processes can be independently regulated. However, it is unclear how the brain generates and gates this motivation-to-action sequence. Here, we develop a novel social operant task, “Social Operant task with Actuator-mediated Reward” (SOAR) to separate the motivation and interaction phases, and we record populations of neurons during the motivation and action phases of aggression in this task and

during free aggressive interactions. We find that neurons in the ventromedial hypothalamus ventrolateral area (VMHvl) and surrounding region represent the temporal evolution of aggressive motivation to aggressive interaction in the SOAR task, and also during aggression during free social interaction, with neurons exhibiting activity aligned to distinct phases of this transition. Using *ex vivo* physiology, we find that VMHvl neurons receive inhibitory input from both local sources (the VMHvl "shell") and also from long-range inputs, with nearly every neuron receiving input. Population recordings show that the VMHvl shell $Vgat^{+}$ neurons respond primarily during aggressive action, and MPO-VMHvl $Vgat^{+}$ neurons have their peak response at the end of the interaction. Using closed-loop optogenetic stimulation timed to specific phases of the social operant task, we find that activation of inhibitory inputs to the VMHvl from the MPO produces primarily motivational deficits, delaying the initiation of the next trial, while activation of the VMHvl shell produces primarily action-related deficits, reducing the likelihood of attack within a trial. Lastly, we find that optogenetic manipulation of local and long-range inhibitory neurons while recording from downstream VMHvl neurons modulates cells with a wide range of activity signatures, suggesting that the timing of activation of these inputs provides a temporally specific braking mechanism. Overall, this suggests a mechanism whereby sequential inhibition and disinhibition from long-range and local inhibitory inputs may facilitate the transition from motivation to action.

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Poster

393. Social Behavior

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Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH R01 GM134732
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Title: Characterization of social interaction in juvenile and adult zebrafish following genetic manipulation of stress modulators

Authors: *S. SHAMS, H. B. LEE, K. J. CLARK;
Biochem. and Mol. Biol., Mayo Clin., Rochester, MN

Abstract: Socialization can both relieve and induce stress. This complex and reciprocal interaction can influence onset and exacerbation of psychiatric disorders, such as depression, anxiety, and PTSD. Zebrafish (*Danio rerio*) are a valuable tool for studying socialization and stress biology due to their highly social nature, conservation of the HPA-axis across vertebrates, and availability of extraordinary sophistication in genetic tools available for this species. Previous studies showed that when 5-days-old zebrafish were subjected to acute environmental

stress (light and salinity changes), behavioral responses to these stressors were dependent on a functional HPA-axis, particularly adrenocortiocotropic hormone receptor (ACTH-R, *mc2r* gene) and glucocorticoid receptor (GR, *nr3c1* gene) but not mineralocorticoid receptor (MR, *nr3c2* gene). It is unknown how mutations in these key HPA-axis regulators, namely ACTH-R, GR, and MR, affect social behavior and stress responses of older fish. Thus, we hypothesized that juvenile and adult zebrafish lacking functional GR, MR, and ACTH-R would have altered social behavior and stress responses and these behavioral effects would resemble locomotor effects previously seen in younger fish, i.e., requiring functional ACTH-R and GR, but not MR. Groups of fish (both males and females) would be observed as juveniles (21-days) and adults (90-days) and characteristics of schooling (coordinated movement with similar direction) and shoaling (maintenance of stable distances between fish with no specific direction) would be quantified in mutant fish and wild-type sibling controls. Additionally, we are currently in the process of using targeted integration of gene-breaking cassettes flanked by loxP sites to make Cre-recombinase-revertible conditional alleles for *mc2r* and *nr3c1* genes. The conditional knock-in and knock-out mutants would provide temporal specificity to establish whether these HPA-axis regulators must be present during development or at the time of the behavioral test. This study would expand and confirm earlier findings with larval fish and provide understanding of behavior of juvenile and adult mutants. This would also be the first study of behavior in adult conditional mutant zebrafish and pave way for genetic dissection of specific brain areas. This understanding would provide excellent basic insight into regulation of vertebrate socialization by stress and into onset and aggravation of a wide range of illnesses, including mood, anxiety- and stress- and trauma-related disorders.

Disclosures: S. Shams: None. **H.B. Lee:** None. **K.J. Clark:** None.

Poster

393. Social Behavior

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Program #/Poster #: 393.17

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: DeepLabCut AI Residency
UMass Boston RTF Fund

Title: Utilizing DeepLabCut to uncover steroid-independent sexual phenotypes in orchidectomized B6D2F1 hybrid male mice

Authors: *V.-C. CHIANG, J. PARK;
Developmental and Brain Sciences, Dept. of Psychology, Univ. of Massachusetts, Boston, Boston, MA

Abstract: Background: Sexual behavior is traditionally viewed to involve sex steroid hormones. Additionally, several lines of evidence also suggest sexual behavior can be

independent of sex steroid hormones, which unfortunately, is a line of investigation that is largely neglected. One mouse model of steroid-independent sexual behavior is the B6D2F1 hybrid male mice. Approximately 30% exhibit the full repertoire of male sexual behavior months after orchidectomy. In previous studies, the sexual phenotype of orchidectomized B6D2F1 male mice has been binarized into those that continue to demonstrate male sexual behavior and those that cease (maters and non-maters, respectively). However, there was a substantial proportion of mice that did not fit into either category, and these 'intermediates' have been largely ignored in those previous studies. Therefore, we anticipate by using a data-driven approach that this will garner a new and more accurate classification system of sexual phenotypes in orchidectomized B6D2F1 male mice. We hypothesize that by considering a comprehensive profile of sexual parameters, we will uncover distinct data-driven sexual phenotypes. **Methods:** Adult B6D2F1 male mice were orchidectomized followed by 11 sexual behavior tests spread out across 62 weeks. Video recordings of the sexual behavior tests will be analyzed by the pose estimation algorithm, DeepLabCut, to obtain coordinates of each body part across time. These pose coordinates will be used to train behavioral classifiers in Simple Behavioral Analysis to automatically identify mounting, intromission and ejaculation. A Python program will be coded to automatically obtain the latency and number of each behavior, as well as the intromission ratio, inter-intromission interval, and copulatory rate. These parameters will be used as input into clustering algorithms to find distinct clusters of sexual phenotypes. **Significance:** More meaningful neuroscience studies could be conducted by having more accurate experimental groups from data-driven approaches, which provides a high resolution of spatiotemporal behavioral dynamics. Given that the neural space capable of being measured also exhibits high spatiotemporal resolution, future studies in sexual behavior would allow more accurate mapping between the two. Another implication of this approach is improvement in animal ethics because a much greater amount of data can be obtained from the animal before it is sacrificed. Finally, this approach has significance in biodiversity, due to its ease of application in recording behavior in the wild, as it does not require animal recordings in a lab environment.

Disclosures: V. Chiang: None. **J. Park:** None.

Poster

393. Social Behavior

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Program #/Poster #: 393.18

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: PAPIIT-DGAPA IN206521

Title: Motivated behaviors and the effects of experience on brain plasticity in female rats evaluated by manganese-enhanced magnetic resonance imaging (MEMRI)

Authors: *J. AGUILAR MORENO[1], M. F. BARRERA TENORIO[2], L. MENDOZA[3], R. G. PAREDES[4];

[1]Conducta sexual y plasticidad cerebral, Inst. De Neurobiología, Querétaro, Mexico; [2]Conducta sexual y plasticidad cerebral, Inst. de Neurobiología, Querétaro, Mexico; [3]Conducta sexual y Plasticidad Cerebral, Inst. De Neurobiología, UNAM Inst. De Neurobiologia, Queretaro, Mexico; [4]Escuela Nacional de Estudios Superiores, Querétaro, QRO, Mexico

Abstract: Motivated behaviors such as pacing and running wheel can induce a reward state and activate different neural circuits. Several studies have shown that the frequent display of these behaviors produce biological and structural changes in different brain regions. Manganese-enhanced magnetic resonance imaging (MEMRI) allows obtaining anatomical and functional information of the brain focusing on behavior-activated brain regions. We aimed to identify and compare brain regions activated by paced mating and running wheel performed separately or simultaneously in the same session and determine possible changes by experience. To achieve that, ovariectomized females supplemented with estradiol and progesterone were divided into the following groups: open field test (OFT), running wheel test (RWT); paced sexual behavior (SBT); the fourth group could perform both RW and SB; a fifth group (control) was not evaluated in any test. All tests lasted one hour, and subjects were tested once weekly for 10 weeks. On weeks 1, 5 and 10, a dose of 16 mg/kg of chloride manganese ($MnCl_2$) was administrated subcutaneously 24 hours before the scanning session. Once the test ended, females were taken to be scanned in a Bruker Pharmascan 7-Tesla MR unit. T1 images were used to measure the changes in signal intensity due to $MnCl_2$. Results shows that females that mate and run display less activity in the RWT than the females that only run. No differences were found between the groups that mated. MEMRI results shows changes in signal intensity across time in the groups that mated, in the RW group and in the group that could mate and use the RW in the same session. Areas and circuits that regulate socio-sexual behavior and the reward circuitry were activated. Pacing induced greater activation in both circuits than pacing alternated with wheel running exercise. The study has shown that MEMRI is useful to evaluate brain plastic changes in the same subject as result of experience.

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Poster

393. Social Behavior

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Program #/Poster #: 393.19

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH G-RISE T32

Title: The Effects of Pair Bonding and Monogamy on Gonadal Characteristics and Cauda Epididymal Sperm Count in Prairie Voles (Microtus ochrogaster)

Authors: *J. HURD[1], S. NGUYEN[1], C. SERGOTT[1], E. FOWLER[1], D. KELLEY[2], E. MCCULLAGH[1];
[1]Integrative Biol., [2]Col. of Vet. Med., Oklahoma State Univ., Stillwater, OK

Abstract: Prairie voles (*Microtus ochrogaster*) are a biparental, pair bonding rodent species that have gained traction as a model organism in the field of neurobiology. Monogamy and biparental behavior are rare in mammals, thus prairie voles offer a promising opportunity to study the neuroscience and neuroendocrinology underlying these behaviors as well as the associated changes in neuronal and reproductive physiology. The differing effects of promiscuity, monogamy, and perceived sperm competition on the mammalian male reproductive system between species have been well documented. Associated plasticity in sperm production and gonadal characteristics within males of the same species remains poorly understood. While male exposure and pair bonding are known to have effects on the female reproductive system in prairie voles, it remains unknown if/how female exposure and pair bonding affect the male reproductive system. Here, we examine the relationship between monogamous pair bonding and changes in gonadal characteristics and cauda epidydimal sperm count in male prairie voles. All voles (20 unmated and 20 mated) were kept in a climate controlled, light regulated (14:10) facility and housed in groups of 2-3 same-sex individuals from weaning until mating or harvest. All were harvested at a minimum of 95 days of age. The mated prairie voles were allowed to complete a minimum of one spermatogenesis cycle post mating, prior to harvesting. Following euthanasia, the gonads were removed, separated, and weighed and the amount of sperm in each cauda epididymis suspended and quantified. The mated prairie voles exhibited significantly higher amounts of epididymal sperm (116% increase), larger testicles (44% increase), and larger cauda epididymides (150% increase) than their unmated counterparts. This indicates a possibility that pair bonding induces changes in male prairie vole reproductive physiology via the hypothalamic-pituitary-gonadal axis. Further work is being done to investigate the neuronal mechanisms involved in these changes.

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Poster

393. Social Behavior

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Program #/Poster #: 393.20

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: UNAM GI200121

Title: Osmoresponsive supraoptic neurons encode prosocial drive via cortico-amygdalar complex connections: a vasopressin-PACAP pathway for behavioral adaptation under homeostatic challenge

Authors: *L. ZHANG[1], O. R. HERNÁNDEZ-PÉREZ[2], V. S. HERNÁNDEZ[1], M. A. ZETTER[1], R. P. MILLAR[3], L. E. EIDEN[4];
[1]Natl. Autonomous Univ. of Mexico, [2]Natl. Autonomous Univ. of Mexico, Mexico City, Mexico; [3]Ctr. for Neuroendocrinology, Dept. of Immunol., Univ. of Pretoria, Pretoria, South Africa; [4]Natl. Autonomous Univ. of Mexico, Natl. Inst. of Hlth. (NIH) NIMH -, Bethesda, MD

Abstract: Homeostatic challenges have been observed to increase the drive for social interaction. How neural activity prompts this motivation remains poorly understood. Hypothalamic supraoptic nucleus (SON) contains a major population of arginine vasopressin magnocellular neurosecretory neurons (AVP-MNNs) responsible for body water balance control. However, SON's ascending projections and its roles in behavioral adaptation when organism is under homeostatic challenge has been generally overlooked. By analyzing Golgi-Cox staining, we observed direct projections from SON to the nucleus of lateral olfactory tract (NLOT) of cortico-amygdalar complex. Fluoro-Gold injection into the NLOT revealed retrogradely labelled AVP-positive somata in SON. We previously reported that main neuron population of the NLOT co-express neuropeptide PACAP as well as vesicular glutamate transport 1 and 2 (VGLUT1 and VGLUT2). Here, using the dual in situ hybridization method we demonstrate the principal cell population PACAP/VGLUT1/VGLUT2 co-express vasopressin receptors V1a and V1b. Considering these observations, we devised a behavioral experiment using 24 hours water deprivation (WD24) and three-chamber social interaction test (3CSI) to assess the sociability comparing with euhydrated subjects. WD48 significantly increased the sociability parameters. Fos expression assessment showed the downstream-regions of AVPMNNs and PACAP-NLOT had further increases in rats subjected to WD48+3CSI. The downstream regions of PACAP-NLOT strongly expressed PAC1 mRNA. AVP and NLOT involvement in this increased sociability and Fos expression was further demonstrated though microinjections of AVP, AVP+V1a or V1b antagonists, targeting NLOT, that AVP microinjection alone produced similar increase produced by WD24 but if applied together with V1a or V1b antagonists, the AVP-stimulated increases were ablated. This result suggest that under situations where homeostasis is compromised by osmotic challenge, there is a recruitment of a glutamatergic-multi-peptidergic circuit that is able to promote social behavior.

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Poster

393. Social Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 393.21

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: JSPS KAKENHI 20H03160

Title: Existence of ingroup favoritism based on strain in male rats

Authors: *Y. KIYOKAWA, K. NAKAMURA, H. KOGO, N. KURODA, N. MAEDA, Y. TAKEUCHI;
Lab. of Vet. Ethology, The Univ. of Tokyo, Bunkyo-ku, Japan

Abstract: Humans show distinct social behavior when we evaluate an individual as being a member of the same group and recognize social similarity to the individual. For example, even people who explicitly endorse egalitarian values are typically not free of biases related to the race or ethnicity of the individual. However, relatively little attention has been paid to the role of social similarity in non-human animals. Here we performed a series of experiments to assess the role of social similarity in affiliative interactions among male rats. In Experiment 1, we assessed whether the strain of unfamiliar conspecifics affected the efficacy of social buffering of conditioned fear responses. We found that the presence of a Wistar, Sprague-Dawley (SD), or Long-Evans (LE) rat, but not a Fischer 344 (F344) rat, ameliorated conditioned fear responses of the Wistar subjects. The same classification was observed in the social preference for unfamiliar rats. In Experiment 2, we found that the Wistar subjects showed a preference for Wistar, SD, or LE rats over F344 rats. However, the Wistar subjects did not show a preference between Wistar and SD rats or between Wistar and LE rats. We further confirmed that the Wistar subjects showed a preference for all strains of rats over being alone. In Experiment 3, we assessed the ability of Wistar subjects to discriminate between unfamiliar rats based on stress status. We found that the Wistar subjects could discriminate between naïve and foot-shocked Wistar and SD rats. However, the subjects not only could not identify stress in foot-shocked F344 rats, but also in foot-shocked LE rats. Finally, we explored neurotransmitters that enabled rats to recognize social similarity to unfamiliar rats based on their strain in Experiment 4. We found that a pretreatment with an opioid or dopamine antagonist, but not an oxytocin nor vasopressin antagonist, blocked the preference for Wistar or SD rats over F344 rats. Taken together, these results propose that opioid and/or dopamine drives ingroup favoritism based on strain in male rats.

Disclosures: Y. Kiyokawa: None. **K. Nakamura:** None. **H. Kogo:** None. **N. Kuroda:** None. **N. Maeda:** None. **Y. Takeuchi:** None.

Poster

393. Social Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 393.22

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NSF grant No. 1449440
NIMH R01-108527

Title: Lipopolysaccharide-induced behavioral alterations in the socially monogamous prairie vole

Authors: *E. K. CHUN, Y. LIU, Z. WANG;
Florida State Univ., Tallahassee, FL

Abstract: The socially monogamous prairie vole (*Microtus* ochrogaster) is a unique animal model that is utilized to study the neurobiological systems underlying a wide variety of social behaviors. Data from our recent studies have demonstrated that after various stressors, such as immobilization stress or social isolation, prairie voles exhibit altered anxiety-like and social behaviors, which are correlated with changes in microglial expression in select brain regions including the nucleus accumbens (NAcc) and amygdala. To further understand the role of neuroimmune modulation in these behavioral changes, we treated prairie voles with lipopolysaccharide (LPS) - a commonly used bacterial endotoxin to induce inflammation - to examine its effects on anxiety-like and social affiliation behaviors as well as microglial expression in the brain. Male and female voles received an i.p. injection of either 2.0 mg/kg LPS or saline for two consecutive days. On the first day, two hours after injections, subjects were tested for anxiety-like behaviors using a 5-min elevated plus maze (EPM) test. On the second day, two hours after injections, subjects were tested for social affiliation by undergoing a 30-min, two-chamber social affiliation (SA) test. The two chambers were connected by a hollow tube, where one contained a tethered same-sex novel stimulus vole while the other remained empty. Our data indicate that LPS treatment differentially affected male and female voles on the EPM, where females show reduced locomotor activity and males spent increased time on the open arm of the EPM. In the SA test, LPS treated animals spent more time in the cage containing the stimulus and spent more time engaging with the stimulus compared to the saline-treated control animals. We are currently in the process of adding more subjects and will process the brain tissues for immunoreactive labeling of microglial cells in selected brain regions, such as the NAcc, paraventricular nucleus of the hypothalamus, and amygdala that are important for anxiety-like and social behaviors.

Disclosures: E.K. Chun: None. **Y. Liu:** None. **Z. Wang:** None.

Poster

393. Social Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 393.23

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NRF-2021R1F1A1064658
KBRI, 22-BR-02-01

Title: Differential encoding of social fear memory along with development

Authors: J. YEO, S. SHIN, ***S. LEE**;
Korea Brain Res. Inst. (KBRI), Daegu, Korea, Republic of

Abstract: Social anxiety disorder (SAD), also known as social phobia, is one of the most prevalent psychiatric disorders. The major behavioral symptom of patients with SAD is social fear, which is the fear of social activity and avoidance of social situations. During the adolescent period, children easily suffer from unwanted social situations, and SAD is evoked. However, the therapeutic method for treatment of SAD in adults shows limited effect on the child patient. It may result from the differential encoding of social fear along with development. However, the study of the neural mechanisms for this differential encoding is unclear. In these experiments, we used socially fear-conditioned mice with social fear conditioning protocol which can induce social fear without disturbing other psychiatric components, such as general anxiety and depression. We also develop a Matlab-based application that can utilize output from DeepLabCut analysis.We found that there was no difference in the level of the acquisition of social fear between adolescent and adult mice. However, there was a significant difference in the extinction level. The number of c-fos positive neurons is increased in the amygdala of socially fear-conditioned adult mice. However, the neuronal excitability of mPFC-projecting BLA neurons is increased in adolescent mice. These results indicate that the differential neural mechanisms for social fear exist in adolescent mice.

Disclosures: J. Yeo: None. **S. Shin:** None. **S. Lee:** None.

Poster

393. Social Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 393.24

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant R01DA039062 to MMM
Simons Foundation pilot award to MMM
University of MD Visiting Fellows in Neuroscience award to SEA

Title: Characterization of social and cognitive behaviors across the lifespan in a “two-hit” rat model of neuropsychiatric developmental disorders

Authors: *S. ASHTON[1], P. SHARALLA[3], N. KANG[3], A. T. BROCKETT[3], M. R. ROESCH[3], M. M. MCCARTHY[2];
[1]Program in Neurosci., [2]Dept. of Pharmacol., Univ. of Maryland Sch. of Med., Baltimore, MD; [3]Dept. of Psychology, Univ. of Maryland, College Park, MD

Abstract: Neuropsychiatric developmental disorders (NDDs), including autism spectrum disorder, schizophrenia and attention-deficit/hyperactivity disorder, are a class of complex disorders whose origins likely involve genetic, physiological and environmental factors. Symptoms of NDDs, marked by characteristic core deficits in social cognition, appear in childhood and often last throughout life. Our lab is exploiting a “two-hit” rat model of NDDs by combining two established risk factors: haploinsufficiency of the candidate gene Nrxn1α

(Sprague Dawley-Nrxn1tm1sage) and early life inflammation, as modeled by poly(I:C) injection on postnatal days 8 (P8) and 10, which correlates to birth and early infancy in humans. This model capitalizes on the rich social repertoire of the laboratory rat, resulting in a more translationally-relevant model in which previous work in the lab has identified social deficits in prepubertal males but not females. Here we hypothesize that the combination of genetic vulnerability and inflammation produces sex-specific alterations in social behavior that endure beyond puberty into adulthood. To test this hypothesis, we performed a longitudinal battery of behavioral tasks to track differences across the lifespan in WT and Het Nrxn1a KOs with and without early life inflammation. We tested maternal isolation-induced ultrasonic vocalizations (P12; analyzed using DeepSqueak), social motivation (P25-26), juvenile play (P27), open field behavior (distance traveled, center time and wall time; P28), and social recognition (time spent with a novel vs familiar stimulus animal; P30 and P52). The animals are currently undergoing three additional tasks in adulthood to assess their social cognition (Pavlovian social outcome task), inhibitory control (stop-signal task) and flexible decision-making (reward-guided decision-making). Together, this battery assesses a variety of social and cognitive skills that reflect the symptomology often present in children with NDDs. Importantly, using the same animals in all tests will allow us to employ factor analysis to reveal any latent variables underlying group and individual differences that may drive performance across the lifespan. As the COVID-19 pandemic continues, it becomes increasingly urgent that we understand how early life viral challenges interact with genetic factors to induce lifelong changes in brain function and behavior.

Disclosures: S. Ashton: None. **P. Sharalla:** None. **N. Kang:** None. **A.T. Brockett:** None. **M.R. Roesch:** None. **M.M. McCarthy:** None.

Poster

393. Social Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 393.25

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: JSPS KAKENHI 21J20176
JSPS KAKENHI 15H05724
JSPS KAKENHI 22H02941

Title: Neuronal projections of estrogen receptor α and β expressing neurons in the lateral septum of male mice

Authors: *K. HASUNUMA[1], K. MITSUI[2], S. TAKENAWA[1], K. SANO[1], A. TAKAHASHI[2], M. NAKATA[1], S. OGAWA[1];
[1]Lab. of behavioral neuroendocrinology, [2]Univ. of Tsukuba, Tsukuba, Japan

Abstract: Testosterone is known to modulate male social behavior by acting on two types of estrogen receptors (ER), ERα and ERβ, after being converted to estradiol in the brain. One of the

potential target sites of action is the lateral septum (LS), where two types of ERs may be differently involved in the regulation of social behavior. Although it is known that both ERα and ERβ are expressed in the LS, we have recently reported that their levels and cellular distribution patterns are greatly different, with the use of newly developed ERβ-RFP transgenic mice. Importantly, ERβ expressed in the rostro-medial LS was about 2.5 times more abundant than ERα which is distributed mainly in the latero-caudal LS. In the present study, we further investigated neuronal projection patterns of ERα and ERβ expressing neurons in the LS. For this purpose, adeno-associated virus which cre-dependently expresses GFP-tagged synaptophysin in synaptic terminals was injected into the LS in ERα-cre and ERβ-cre male mice. Strong synaptophysin-GFP signals were found in ERβ-cre mice but not in ERα-cre mice in a number of areas such as the ventrolateral preoptic nucleus, anterior hypothalamus and latero-anterior hypothalamic nucleus. In the ventrolateral nucleus of hypothalamus (VMH), synaptophysin-GFP expression patterns in various subregions were markedly different between ERα- and ERβ-cre mice. Synaptophysin-GFP positive signals were mainly found in the ventrolateral part of the VMH (VMHvl) in ERα-cre. On the other hand, in ERβ-cre mice, positive signals were found in the dorsomedial part of the VMH (VMHdm) and VMH shell. In the medial preoptic area and anteroventral periventricular nucleus, synaptophysin-GFP signals were detected in both ERα- and ERβ-cre mice. These results demonstrate that not only the distribution of ERα and ERβ expressing neurons in the LS but also their neuronal projection patterns are different. The present findings provide a neuroanatomical basis for differential contribution of ERα and ERβ expressing LS neurons in the estrogenic regulation of social behavior. Supported by JSPS 21J20176 to KH, 15H05724 and 22H02941 to SO.

Disclosures: **K. Hasunuma:** None. **K. Mitsui:** None. **S. Takenawa:** None. **K. Sano:** None. **A. Takahashi:** None. **M. Nakata:** None. **S. Ogawa:** None.

Poster

393. Social Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 393.26

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant DK125480

Title: Estrogen receptor α in leptin receptor-expressing neurons regulates sexual function

Authors: *M. WANG, Y. XU;
Pediatrics, Baylor Col. of Med., Houston, TX

Abstract: Sexual dysfunction is a common, burdensome, and costly health condition. Individuals with sexual dysfunction suffer from significant psychological distress, physical and mental disorders. The increasing prevalence of overweight/obesity put individuals at higher risks of sexual dysfunction. A more comprehensive understanding of how nutrition affects sexual

function may provide novel treatment options to manage sexual function. To understand how estrogen and leptin signaling interact and integrate to regulate sexual function, we generated mice with deletion of estrogen receptor α (ERα) specifically in leptin receptor (LepRb)-expressing neurons ($ERα^{ΔLepRb}$ mice). We found that male $ERα^{ΔLepRb}$ mice spent longer time and more effort in anogenital sniffing and mounting compared to controls. These extra efforts, accompanied by significantly decreased mean intromission time and impaired ejaculation, indicate that ERα in LepRb neurons is required for maintaining normal ejaculation and its efficiency. Female $ERα^{ΔLepRb}$ mice displayed strikingly sexual rejection, indicated by significantly increased number of rejecting behavior (fleeing, boxing, kicking, rearing) and longer mean and total rejecting behavior time. Our findings imply that ERα in LepRb neurons is required for maintaining normal sexual function in mice of both sexes. Human and animal studies emphasizes the importance of healthy dietary pattern in maintaining sexual health. To test if ERα in LepR neurons plays an important role in this scenario, we challenged mice with 16-week high-fat diet (HFD) feeding, followed with chow diet for 4 weeks, and tested sexual function. $ERα^{ΔLepRb}$ and control mice of both sexes demonstrated HFD-induced obesity and sexual dysfunction, and subsequent chow diet-induced body weight loss which was more profound in males compared to females. Along with the harder body weight loss, the chow diet did not improve the sexual dysfunction in either female control or $ERα^{ΔLepRb}$ mice. Notably, chow diet largely rescued the mean intromission time in male controls but not $ERα^{ΔLepRb}$ mice, indicating that this effect was dependent on the ERα in LepR neurons. Our results highlight the important role of ERα in LepR neurons in the interaction between nutrition and sexual function and in a sex-specific way. In summary, our data show that ERα in LepRb neurons is required for maintaining normal sexual function and involved in the interaction between nutrition and sexual function.

Disclosures: **M. Wang:** None. **Y. Xu:** None.

Poster

393. Social Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 393.27

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: JSPS 21J10590 to TM
JSPS 15H05724 and 22H02941 to SO

Title: Neuroendocrinological mechanisms controlling the expression of sexual behavior in female mice on the day after ovulation.

Authors: *T. MURAKAWA, K. HATA, L. KOGURE, S. TAKENAWA, K. SANO, S. OGAWA;
Lab. Behavioral Neuroendocrinology, Univ. of Tsukuba, Tsukuba, Japan

Abstract: Female mice are sexually receptive during the fertile ovulation period (i.e., behavioral estrus) but not during the non-ovulation period. Induction and levels of sexually receptive behavior (i.e., lordosis), is mainly regulated by estradiol (E2) in female mice. E2 is known to act through two types of estrogen receptors (ER), ERα and ERβ. We have previously reported that lordosis is completely abolished in ERα knockout (KO) mice, whereas ERβ-KO mice not only show normal levels of lordosis on the day of ovulation but also are highly receptive even on the day after ovulation. Our study with site-specific ERβ knockdown in the dorsal raphe nucleus (DRN), where ERβ is abundantly distributed, has demonstrated that a lack of ERβ in this brain site prevents a decline of lordosis on the day after ovulation. In the present study, we further investigated whether manipulation of neuronal activity of ERβ expressing cells in the DRN ($DRN^{ER\beta}$ cells) could affect the levels of lordosis on the day after ovulation, with the use of chemogenetic methods. Ovariectomized female mice of newly developed ERβ-iCre line were injected with adeno-associated virus either containing a sequence which cre-dependently transduced chemogenetic receptors for inhibition (hM4Di) or facilitation (hM3Dq) of neuronal activity, or control virus in the DRN. They were then primed with estradiol benzoate (48hr and 24hr before) and tested on two consecutive days; 4hr after progesterone injection mimicking the day of ovulation (Day 1) and 24hr later as the day after ovulation (Day 2) with either clozapine N-oxide (CNO) or saline injection. All groups of mice showed high levels of lordosis behavior on Day1 which markedly decreased on Day2 when they were tested with saline injection. On the other hand, CNO injection to hM4Di treated mice before the tests on Day 2 prevented a decline of lordosis. Their levels of lordosis were comparable to those on Day 1. CNO injection on Day 1 had no effect in hM4Di treated mice, whereas hM3Dq-induced activation of the neuronal activity of $DRN^{ER\beta}$ cells reduced the levels of lordosis on Day 1. These results suggest that: (1) activation of $DRN^{ER\beta}$ cells may be involved in a decline of lordosis expression normally observed on the day after ovulation (Day 2); and (2) over excitation of $DRN^{ER\beta}$ cells may induce an inhibitory impact on lordosis neural circuitry, which is mainly regulated by estrogenic action on ERα.

Disclosures: **T. Murakawa:** None. **K. Hata:** None. **L. Kogure:** None. **S. Takenawa:** None. **K. Sano:** None. **S. Ogawa:** None.

Poster

393. Social Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 393.28

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: Exploring neural connectivity and representation of reward in the lateral septum

Authors: *S. KARKARE[1], J. ISAAC[2], M. MURUGAN[3];
[1]Emory Univ. Neurosci. and Behavioral Biol., Emory Univ. Neurosci. and Behavioral Biol., Atlanta, GA; [2]Emory Univ., Atlanta, GA; [3]Princeton Neurosci. Inst., Emory Univ., Decatur, GA

Abstract: The lateral septum (LS) is a brain region that has been implicated in behaviors ranging from kinship recognition to aggression. In addition, early self-stimulation and lesion studies suggest a role for the LS in reward-related behaviors. These studies are complicated by evidence of molecularly distinct subregions within the LS that may serve different and even antagonistic functions. In this study, we test the possibility that the functional heterogeneity observed with LS manipulations may be attributed to distinct projection populations in the LS. We focused on LS projections to five downstream target brain regions implicated in various reward-related behaviors: the nucleus accumbens (NAc), ventral tegmental area (VTA), bed nucleus of the stria terminalis (BNST), basolateral amygdala (BLA), and ventromedial hypothalamus (vmH). Using retrogradely transporting viruses, preliminary data shows that the various LS projection populations occupy distinct compartments of the LS. For example, while the LS-NAc neurons are largely limited to the dorsal region of the LS, LS-vmH neurons are located in the ventral region of the LS. Furthermore, combining monosynaptic rabies tracing technology and whole brain mapping software, we have identified that the various LS populations receive largely distinct patterns of monosynaptic inputs (LS-NAc n = 3 mice, LS-VTA n = 2 mice, LS-BNST n = 3 mice, LS-BLA n = 2 mice, LS-vmH n = 3 mice). The LS-NAc and LS-VTA populations show similar inputs from the hippocampus but strongly varying levels of input from distinct compartments of the thalamus. The LS-vmH, LS-BNST, and LS-BLA populations receive inputs from distinct hippocampal compartments; in contrast to the dorsal cornu ammonis 1 (CA1) and ventral subiculum inputs to the LS-BNST population, the LS-vmH population held a higher proportion of inputs from the ventral CA1 region, and the LS-BLA population had sparse inputs from both the dorsal and ventral subiculum, yet dense CA1 and cornu ammonis 3 (CA3) inputs. Next, to determine the role of the LS in reward-related behaviors, we imaged the activity of individual LS neurons using cellular resolution calcium imaging methods while mice engaged in a series of increasingly difficult operant conditioning tasks (n = 6 mice). We found that LS neurons had an increased anticipatory choice response as well as an increased general inhibitory response with training (n = 312 neurons). In the future, we hope to use viral intersectional strategies to characterize the reward responses of the above described LS projections, thus allowing us to better understand how reward processing may be shaped by the internal organization of the LS and its inputs/outputs.

Disclosures: **S. Karkare:** None. **J. Isaac:** None. **M. Murugan:** None.

Poster

393. Social Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 393.29

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: Emory Start Up Funds

Title: Identifying the Neural Circuits That Underlie Social Recognition Behavior

Authors: *M. RASHID, M. MURUGAN;
Biol., Emory Univ., Atlanta, GA

Abstract: The ability to recognize familiar conspecifics is crucial for the survival of most mammalian species. The key role social recognition plays in shaping animal behavior and the neural circuits underlying social recognition remain poorly understood. Recent evidence has determined the ventral hippocampus (vHPC) as necessary for social recognition. The lateral septum is a major output of the vHPC and has been implicated in kin recognition. The exact contribution of vHPC projections to the lateral septum remains unknown. In this study, we used a combination of chemogenetic, optogenetic, and monosynaptic tracing methods to determine the role of the vHPC-LS neurons and its downstream targets in mediating social recognition. To evaluate if vHPC-LS was neurons are necessary for social recognition we used a viral intersectional strategy to chemogenetically inhibit vHPC-LS neurons in the social discrimination assay. In contrast, to control mice, inhibition of this pathway disrupted the ability of mice to discriminate between familiar and novel conspecifics (n=49; p=0.034). To determine if vHPC-LS inhibition caused increased investigation of a familiar mouse, we optogenetically inhibited vHPC-LS neurons in a spatially and temporally specific manner in the proximity of either a novel or a familiar mouse in a social discrimination assay. In contrast to control mice that demonstrate an increased preference for the novel mouse, optogenetic inhibition of vHPC-LS neurons around the familiar mouse caused an increased investigation of the familiar mouse. (n=19; p=0.031). Furthermore, we found that spatially and temporally restricted optogenetic inhibition of vHPC-LS neurons in the presence of two novel mice increased the investigation of the mouse paired with inhibition. Since LS projections are largely GABAergic, we hypothesized that inhibiting vHPC-LS neurons disinhibits a downstream region that in turn promotes social investigation. Monosynaptic rabies tracing technology, we found that 1) LS neurons projecting to the ventral tegmental area (VTA), a region known to promote social investigation, 2) LS-VTA neurons receive monosynaptic inputs from the vHPC. These findings suggest that disinhibition of LS-VTA neurons might be involved in the increased social investigation observed with silencing vHPC-LS neurons. We chemogenetically inhibited LS-VTA during the SDA. Initial data shows that LS-VTA neurons are not necessary for social discrimination, rather they play a broader role in discrimination behavior (n=15; p=0.094). Thus, by combining multimodal techniques this study provides the first insight into the role of vHPC-LS-VTA circuit in social recognition

Disclosures: **M. Rashid:** None. **M. Murugan:** None.

Poster

394. Effects of Early Life Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 394.01

Topic: F.03. Stress and the Brain

Support: F32HD101303
ES028202
HD097093
MH104184
MH108286

Title: Preconception stress reprogramming of circulating extracellular vesicle proteome and uterine transcriptome is unmasked by pregnancy

Authors: *Y. CISSE[1], W. HUANG[2], M. A. KANE[2], T. L. BALE[1];
[1]Pharmacol., [2]Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Lifetime adversity is a strong predictor of adverse gestational outcomes and offspring neurodevelopment. However, little is known about the biological mechanisms involved in perpetuating prior stress experience across generations. In our mouse model of maternal preconception stress (MPS), chronic stress prior to conception programs adult offspring sex-specific sensitivity to stress and metabolic insults. We hypothesize that preconception stress interacts with the incredible energetic demands of pregnancy, unmasking latent programming affecting highly metabolic tissues such as the placenta, ultimately affecting offspring development. Using the proteomic cargo of circulating extracellular vesicles (EVs) as a stress-responsive indicator of whole body signaling, we examined the effects of pregnancy and MPS. The EV proteome of mid-gestation MPS dams closely resembled that of their non-pregnant counterparts and non-pregnant controls, suggesting a diminished effect of pregnancy on whole body signaling in MPS. In addition to its role in sex-specific offspring development, the placenta is a major contributor to the increased concentration of circulating EVs during pregnancy. We therefore examined the placental transcriptome at mid-gestation and determined that placentas developing in MPS dams increased cellular metabolic gene expression, and decreased trophoblast differentiation genes, suggesting a disruption placental development. To better understand interactions at the maternal:fetal interface, we examined the transcriptome of the uterine segments apposing these placentas. Relative to controls, MPS uterine tissue apposing male placentas decreased cellular metabolic gene expression, but tissue apposing female placentas increased innate immune gene expression. These data suggest a novel robust offspring sex-specific change in the uterine response and adaptation to pregnancy in dams with prior stress experience, resulting in changes in the rate of brain development at this timepoint suggested by a reduction in neuronal gene expression in the fetal brain. Finally, to dissect the role of the maternal environment vs the oocyte itself in driving the transmission of this programming, we performed embryo transfer of control or MPS embryos into control or MPS dams. Preliminary results suggest that MPS females exhibit reduced receptivity to both control and MPS embryos. Together, these data suggest that pregnancy, a pervasive physiological/metabolic challenge, unmasks the lasting effects of preconception stress on maternal somatic and reproductive tissues to shape both maternal and offspring long-term health outcomes.

Disclosures: Y. Cisse: None. **W. Huang:** None. **M.A. Kane:** None. **T.L. Bale:** None.

Poster

394. Effects of Early Life Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 394.02

Topic: F.03. Stress and the Brain

Support: NIH R00 Grant MH115096 (Peña)

Title: Overexpression of monomethyltransferase Setd7 in the VTA primes chromatin and sensitizes mice to future stress

Authors: *J.-A. BALOUEK, A. S. CHEN, M. TANG, C. J. PENA;
Princeton Neurosci. Inst., Princeton, NJ

Abstract: In humans, early-life stress (ELS) raises the risk of developing psychiatric diseases such as depression. There is evidence that early life stress makes individuals more vulnerable to future stress. We use a stress paradigm in mice to investigate how epigenetic mechanisms may govern maladaptive behavioral outcomes following ELS and their impact on neurodevelopment. In the ventral tegmental area (VTA), we found an increase in the proportions of chromatin modifications associated with a more open or permissive state. We confirmed by Western blot analysis in male mice, that ELS increases the proportion of monomethylation of histone 3 lysine 4 (H3K4me1), an epigenetic marker linked to transcriptional priming. We noticed a trend for increased mRNA levels of Setd7, a mono-methyltransferase specific to H3K4 in published RNA-seq data in VTA of male mice who experienced ELS. We postulate that this open chromatin state, which is characterized by a high level of H3K4me1, allows for more reactive transcription in response to additional stress throughout the lifespan. We altered the epigenome in vivo to causally test this hypothesis: we created a custom viral vector tool to over-express Setd7 at an ELS-specific time-sensitive timeframe. We performed stereotaxic surgery on 14-day-old male and female pups to target viral over-expression of Setd7 in the VTA, a deep brain structure. When compared to the endogenous levels of SETD7 in control GFP-injected animals, our vector increases the protein concentration by 6 folds. We also observed a 1.35-fold increase in H3K4me1 in SETD7-injected mice compared to controls, like what is found after ELS. We also tested depression- and anxiety-like behaviors, before and after adult chronic social defeat stress (CSDS). In the social interaction test, we found a modified avoidance behavior in male mice, revealing an interaction between juvenile Setd7 overexpression and adult stress exposure. To test the consequences of heightened H3K4me1 levels, we performed an RNA-Seq analysis on male and female mice injected with either SETD7 or a control GFP vector, and who experienced CSDS or not. We identified differentially expressed genes associated with Setd7 overexpression, social defeat, or both. This suggests that overexpression of Setd7 alone, or Setd7 paired with CSDS, causes transcriptional alterations that reflect latent stress vulnerability. We observed that Setd7 overexpression combined with adult social defeat results in a distinct transcriptional response compared to GFP. Together, this establishes a causal relationship between early life stress and epigenetic changes in reward circuitry, which mediate lifelong stress sensitivity.

Disclosures: **J. Balouek:** None. **A.S. Chen:** None. **M. Tang:** None. **C.J. Pena:** None.

Poster

394. Effects of Early Life Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 394.03

Topic: F.03. Stress and the Brain

Support: R01MH129643 to CJP

Title: Early life stress alters thyroid hormone signaling and ventral tegmental area

Authors: *S. N. BENNETT, A. B. CHANG, C. J. PENA;
Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ

Abstract: A healthy functioning thyroid is essential to the developing brain. Despite the prevalence of thyroid disease, whether and how stress during development alters thyroid function and downstream brain development and behavior is understudied. Here we explored if early life stress (ELS) altered thyroid function, and how such changes altered genes related to development of dopamine neurons in the ventral tegmental area (VTA) known to be under developmental control of thyroid signaling. We also sought to understand if rescuing thyroid levels through administration of levothyroxine (LT4), a synthetic hormone, during juvenile development, could rescue the impact of ELS on gene expression and behavior. We performed an ELISA which revealed main effects of early life stress and sex on plasma thyroid stimulating hormone (TSH) levels at P21, but this effect is transient and does not last into adulthood. We then asked whether juvenile LT4 treatment would reverse the impact of ELS on expression of genes related to dopamine neuron development or thyroid signaling in VTA, with or without a second hit of stress in adulthood. LT4 rescued ELS-altered expression of genes related to thyroid signaling or developmental plasticity in females (PVALB, THRA, and THRB) and protected against other genes against adult social defeat stress-induced changes (males: OTX2; females: NURR1, DIO2). Juvenile LT4 treatment after ELS also rescued some aspects of social and exploratory behavior among male mice. These findings suggest that thyroid signaling mediates the deleterious impact of ELS on VTA development, which may be restored by transient administration of synthetic thyroid given to juvenile animals after a sensitive period of development.

Disclosures: S.N. Bennett: None. **A.B. Chang:** None. **C.J. Pena:** None.

Poster

394. Effects of Early Life Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 394.04

Topic: F.03. Stress and the Brain

Title: Changes in Chromatin State as an Epigenetic Response Mechanism to Early Life Stress

Authors: *R. RASHFORD[1], M. OKE[1], C. J. PENA[2];
[2]Princeton Neurosci. Inst., [1]Princeton Neurosci. Inst., Princeton, NJ

Abstract: Early life stress (ELS) is one of the strongest predictors for the risk of developing depression and/or anxiety disorders. ELS includes trauma (neglect, and/or physical, sexual, and/or emotional abuse) and other highly negative childhood experiences. However, the specific role ELS plays in increasing this risk for mood disorders in adulthood is not well understood. Previous work in a mouse model of stress across the lifespan has shown unique gene expression patterns after both ELS and adult stress in the nucleus accumbens (NAc), a key region of the reward pathway implicated in stress response. Here, we test the hypothesis that ELS changes the three-dimensional architecture of chromatin within stress-responsive cells in the NAc, such that the chromatin state adopts a more open conformation, making transcription more reactive to future stress. To examine chromatin accessibility in ELS-responsive cells, we bred a double-transgenic mouse (Arc-Cre-ERT2 x Sun1-sfGFP-myc) to label and immunopurify experience-responsive nuclei. We then performed ATAC-sequencing (assay for transpose-accessible chromatin followed by sequencing) in labeled and non-labeled cells to capture the regions of open chromatin within these labeled nuclei. Computational analyses of the chromatin accessibility profiles showed that there are more instances of open chromatin observed in neurons activated by ELS compared to the experience of control mice, and that this effect lasts into adulthood. Interestingly, the majority of these differentially open regions are found at potential genomic enhancer sites. This provides evidence that ELS primes enhancers to be in a more open, reactive state, which may modulate gene expression responses upon future stress experiences. Together, this research provides a biological mechanism as to how ELS increases sensitivity to future stress.

Disclosures: R. Rashford: None. **M. Oke:** None. **C.J. Pena:** None.

Poster

394. Effects of Early Life Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 394.05

Topic: F.03. Stress and the Brain

Support: NARSAD Young Investigator Award
R01MH129643

Title: Transcriptomic prediction of antidepressant treatment response after early-life stress

Authors: *S. T. PAREL[1], G. TURECKI[3], C. J. PENA[2];
[1]Neurosci., [2]Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ; [3]Psychiatry, McGill Univ., Montreal, QC, Canada

Abstract: Each year nearly one billion children across the world experience early-life stress (ELS). ELS increases the risk for depression while also predicting poor response to first-line antidepressant treatments. How ELS can alter the efficacy of antidepressant treatments in the brain remains unclear. Previous studies implicate the nucleus accumbens (NAc) of the brain in both depression and response to antidepressant treatment. In the NAc of mice, ELS can sensitize individuals to future stress, and these changes are observable at the level of gene expression. We hypothesize that ELS produces lasting transcriptional changes in the NAc that predict non-response to antidepressant treatment. To test this hypothesis, we have performed cross-species integrated transcriptomic analyses on three independent RNA-sequencing datasets: from the NAc of a mouse model for ELS; NAc of a mouse model for non-/response to the antidepressants imipramine and ketamine; and leukocytes of human patients assessed for non-/response to antidepressant treatment. In the NAc of female-assigned mice, we found that gene expression patterns after ELS overlap with response to imipramine and ketamine treatment. However, female mice that experienced ELS before adult stress predicted non-response to both antidepressants. In the NAc of male-assigned mice, adult stress regardless of ELS exposure produced transcriptional patterns similar to efficacious antidepressant response. These findings are highly relevant to known differences in depression rates and treatment response across sexes. Despite species and tissue differences, we also observed overlap between signatures of mouse ELS and human SSRI response. To test our predictions through behavior and pharmacology experiments, we implemented ELS involving maternal separation and limited nesting, social defeat stress in adulthood, and treatment with the antidepressants escitalopram and ketamine. The results suggest that ELS is associated with altered behavioral response to antidepressant treatment. Overall, our integrated analyses of genome-wide patterns and pharmacology experiments provide biological insights linking ELS with antidepressant response, laying the foundation for more targeted antidepressant treatments for this vulnerable population.

Disclosures: S.T. Parel: None. **G. Turecki:** None. **C.J. Pena:** None.

Poster

394. Effects of Early Life Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 394.06

Topic: F.03. Stress and the Brain

Support: Department of Psychology, Radford University
Office of Undergraduate Research and Scholarship, Radford University

Title: Investigation of long-term alterations in Corticotropin Releasing Factor in rat hypothalamus following early-life maternal separation

Authors: Z. M. HANNABASS, B. MULHOLLAND, C. PANIAGUA-UGARTE, L. D. GRIFFITH, P. A. JACKSON, *D. M. HAYES;
Psychology, Radford Univ., Radford, VA

Abstract: Rodent pups separated from their mothers for prolonged periods have shown alterations in social interaction, appetite, and stress reactions among others though the neurobiological mechanisms of these changes are not well understood (Nishi et al., 2014; Zimmerberg and Sageser, 2011). Importantly, the primary stress response system is the hypothalamic-pituitary-adrenal (HPA) axis whereby corticotropin-releasing factor (CRF), adrenocorticotropic hormone (ACTH), and corticosterone (CORT) function through intricate feedback loops (Lee et al., 2015). Activation of this system should occur during times of stress but should then deactivate and return to baseline following the removal of the stressor. However, stress experienced during certain developmental phases (i.e. early life) can lead to long-term disruptions of this system and detrimental impacts to the mental and physical health of the offspring (Nishi, 2020; Veenema, 2009). In the present study, male and female, Long-Evans pups were separated from their mothers for 3-hours a day during days 1-14 after birth. Some pups were separated from mom via a perforated metal barrier allowing for limited interaction with mom via certain sensory system modalities (vision, audition, olfaction) while other pup/mom pairs were separated in completely different rooms. Additionally, some pups were allowed to interact with siblings during the separation phase while others were completely isolated. Following this separation and a behavioral test battery, brains were extracted following transcardial perfusion then sliced at 40 microns with a vibrating microtome. Tissue sections were exposed to CRF primary antibodies following standard immunohistochemical procedures, mounted on slides, blinded, then quantified for CRF expression in the paraventricular nucleus of the hypothalamus via Image J software. A 2 (sex) X 2 (maternal location) X 2 (sibling presence) factorial ANOVA failed to reveal any significant differences based on maternal location or sibling presence but indicated a trend towards sex differences with females showing higher levels of CRF expression in the PVN. Future research will investigate short-term changes to CRF expression and expand to analyze CRF levels in the amygdala.

Disclosures: Z.M. Hannabass: None. **B. Mulholland:** None. **C. Paniagua-Ugarte:** None. **L.D. Griffith:** None. **P.A. Jackson:** None. **D.M. Hayes:** None.

Poster

394. Effects of Early Life Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 394.07

Topic: F.03. Stress and the Brain

Support: MH 096889
George E. Hewitt Foundation for Medical Research (AFS)

Title: Setting a double TRAP to reveal the effects of early life adversity on the translatome of the paraventricular nucleus of the thalamus (PVT)

Authors: *A. FLORIOU-SERVOU[1], M. GANTUZ[2], H. Y. LIANG[2], T. Z. BARAM[3], A. MORTAZAVI[2];

[1]Dept. of Anat. & Neurobio., [2]Dept. of Developmental and Cell Biol., [3]Dept. of Anat. & Neurobiology, Dept. of Pediatrics, Univ. of California, Irvine, Irvine, CA

Abstract: Background: Early-life adversity (ELA) is associated with cognitive and mental health problems later in life. Evidence in rodents points to a causal role of ELA, with structural and functional changes in the brain's reward circuitry. However, the mechanisms through which ELA might change the maturation and function of the brain's reward circuit remain poorly understood. One emerging key node of the reward circuit is the paraventricular nucleus of the thalamus (PVT). Most importantly, according to data from our lab, the PVT is strongly and almost exclusively activated in the mouse brain early in life. This raises the possibility that the PVT encodes the ELA experience and influences reward-seeking behaviors later in life. Here, we explore how the PVT is itself affected by ELA. To this end, we explore the translatome of PVT cells activated early in life, and test the hypothesis that ELA causes enduring changes in their translational profiles. Methods: We combined activity-dependent genetic labeling (TRAP2) with translating ribosome affinity purification (TRAP), to isolate actively translated mRNA from cells that are activated during P6-P8, when mice are raised in either typical (control group) or ELA conditions. Then we collected the midline thalamus containing the whole PVT from 60-70 days old mice, we isolated the RNA bound to tagged ribosomes and performed next generation RNA sequencing. Mice from ELA (11 males and 8 females) and control group (9 males, 9 females) were randomized during tissue collection and processing, and the experimenter was blinded throughout the experiment. Results: After the purification our samples show a strong enrichment in genes that are highly expressed in the PVT such as Snca and Calb2, and a reduction in genes that are less expressed in the PVT such as Slc17a7 and Fras1, confirming that the majority of the isolated RNA is from PVT cells. In addition, we detect dozens of differentially expressed genes between control male and female mice, as well as genes that are differentially expressed after ELA in both sexes. Conclusions: We have established a methodology that allows us to isolate actively translated RNA from the mouse PVT. Furthermore, our results indicate that the PVT is highly sexually dimorphic, and that its translatomic profile is influenced by early life experience.

Disclosures: A. Floriou-Servou: None. **M. Gantuz:** None. **H.Y. Liang:** None. **T.Z. Baram:** None. **A. Mortazavi:** None.

Poster

394. Effects of Early Life Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 394.08

Topic: F.03. Stress and the Brain

Support: NIH grant HD091376

Title: Gene expression reprogramming in the paraventricular nucleus following pubertal stress and adult hormone exposure

Authors: *K. GAUTIER[1], S. HIGLEY[1], P. J. KANE[2], T. L. BALE[2], K. E. MORRISON[1]; [1]West Virginia Univ., Morgantown, WV; [2]Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Women who undergo adverse childhood experiences are at risk for lasting biological consequences, including affective disorders and stress dysregulation. However, the mechanisms underlying this relationship are unclear. We have previously shown that pubertal adversity is associated with a blunted glucocorticoid response within the hypothalamic-pituitary-adrenal (HPA) axis in both peripartum humans and mice. In mice, we examined puberty-stress reprogramming in the paraventricular nucleus (PVN) of the hypothalamus, which initiates the HPA axis response. We found that pubertal stress led to an upregulation of six immediate early genes (IEGs) in the PVN of adult, pregnant mice. IEGs are stimulus-dependent transcription factors that have many important downstream targets. Separately, we showed that the pregnancy-associated hormone allopregnanolone is necessary and sufficient to produce the blunted stress response phenotype in pubertally stressed adult female and male mice. Here, we assessed allopregnanolone as the potential mechanism underlying pubertal stress-induced IEG upregulation originally seen in pregnant females. We hypothesized that administration of allopregnanolone would increase IEG expression in the PVN of pubertally stressed mice. Male and female mice underwent 14 days of chronic variable stress beginning on postnatal day (PN) 21. Pharmacological treatment and brain collection occurred in adulthood. Mice were given either allopregnanolone or vehicle via two separate subcutaneous injections (peripheral) or intra-PVN cannulae (direct) before brain collection. RNA from PVNs was isolated and gene expression was measured using quantitative real-time PCR. Peripheral allopregnanolone interacted with pubertal stress to alter gene expression in the PVN; however, these effects were minimal compared to direct allopregnanolone administration. IEGs were upregulated at a greater magnitude following direct administration of allopregnanolone, representing the data previously seen in pregnant females. As the two families underlying AP-1 complex formation, *Fos and Jun*, were seen significantly upregulated by allopregnanolone, we examined downstream targets of this complex, such as Vgat and other GABA-related genes, to further understand the system of change occurring after pubertal stress. These studies provide novel insight into the mechanisms underlying female-relevant risk for stress dysregulation, a central endophenotype of affective disorders.

Disclosures: K. Gautier: None. **S. Higley:** None. **P.J. Kane:** None. **T.L. Bale:** None. **K.E. Morrison:** None.

Poster

394. Effects of Early Life Stress

Location: SDCC Halls B-H

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Program #/Poster #: 394.09

Topic: F.03. Stress and the Brain

Support: NIH Grant HD091376

Title: Pubertal stress dynamically alters the transcriptional response of the paraventricular nucleus of the hypothalamus

Authors: *S. L. HIGLEY, J. M. MENDOZA, K. N. GAUTIER, K. E. MORRISON;
West Virginia Univ., Morgantown, WV

Abstract: Chronic stress, especially when experienced during key times of development, can have lasting negative outcomes, such as the emergence of mood disorders in adulthood. Puberty is a dynamic period during which brain maturation confers sensitivity to the negative effects of stress. We previously found that pubertal stress in female humans and mice resulted in a blunted hypothalamic-pituitary-adrenal (HPA) axis response, an endophenotype of mood disorders. Further work in mice showed that pubertal stress led to altered gene expression in the paraventricular nucleus (PVN) of the hypothalamus, the brain region of HPA axis initiation. We found an increased expression of six immediate early genes (IEGs) in the PVN of pregnant adult mice that had previously undergone pubertal stress. IEGs are stimulus-responsive genes that initiate downstream cellular cascades. However, these genes were permissively expressed in pregnant, pubertally stressed females in baseline conditions, suggesting a potential mechanistic role of IEGs in the blunted HPA axis phenotype. Although we have examined the adult PVN transcriptome, when and how these changes manifest after pubertal stress is unknown. Here, we examined gene expression in the PVN before, during, and shortly after pubertal stress exposure. Male and female mice were exposed to chronic variable stress or left undisturbed from postnatal day (PN) 21-34. Brains were collected either at baseline (PN21), 24h following one week of stress (PN28), or 24h following two weeks of stress (PN35). RNA was isolated from the PVN and expression of six previously identified IEGs (Fos, Fosb, Junb, Jund, Egr1, Arc) was quantified via qRT-PCR. We predicted that if pubertal stress induces immediate reprogramming of the PVN transcriptome, increased IEG expression in pubertally stressed mice would be observed in adolescence. However, reduced IEG expression would provide evidence that the adult phenotype is not an immediate consequence of pubertal stress. We found a significant decrease in expression of Fos in the PVN of PN35 females, with no impact of pubertal stress on the remaining IEGs. These findings demonstrate that pubertal stress alters IEGs and provide evidence for a potential mechanistic role for Fos in the lasting reprogramming of the PVN. With this understanding of the trajectory of gene expression, further experiments were conducted to gain a deeper understanding of the molecular mechanisms driving IEG expression. This translationally-relevant mouse model provides the opportunity to understand the molecular underpinnings of risk for stress dysregulation, a central endophenotype of affective disorders

Disclosures: S.L. Higley: None. **J.M. Mendoza:** None. **K.N. Gautier:** None. **K.E. Morrison:** None.

Poster

394. Effects of Early Life Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 394.10

Topic: F.03. Stress and the Brain

Support: NIH Grant HD091376

Title: Pubertal stress produces deficits in the formation of maternal memory and alters gene expression in the medial preoptic area

Authors: *G. C. PIFER, S. L. HIGLEY, B. M. KAREM, K. E. MORRISON;
West Virginia Univ., Morgantown, WV

Abstract: Early life stress has a negative impact on adaptive and efficacious parenting in humans. Effective maternal care is necessary for physical and emotional development in offspring. Previous studies in mice have established that induced maternal behavior by exposure to pups is enduring. We have previously shown that stress during puberty alters the hypothalamic-pituitary-adrenal (HPA) stress axis response in adult female mice only during pregnancy and postpartum. Pubertal adversity led to a blunted HPA response during pregnancy in humans, which was associated with increased postnatal depression scores. We also observed mild alterations of pup-directed behaviors in pubertally stressed dams. It is possible that pubertal stress creates a disorganization of maternal responsiveness and vulnerability to affective dysfunction. We hypothesized that pubertally stressed, virgin females would have a deficit in the ability to form maternal memory in adulthood. Female mice were exposed to chronic variable stress from postnatal day 21-34, during which they received one stressor (olfactory, tactile, or auditory) per day for 2h each day. In adulthood, virgin females were either left undisturbed (no exposure) or were exposed to novel pups for 2h per day for 4 days (maternal exposure). All females were tested for pup retrieval in a maze 24h following the last maternal exposure, after which they were left undisturbed for two weeks. The pup retrieval task was repeated and brains were collected. Pubertally stressed females required an increased number of exposures to novel pups to show pup-directed behavior. Additionally, pubertally stressed females with no exposure showed no capacity for pup retrieval, showing that pubertal stress had detrimental effects on maternal behavior. To examine molecular consequences, the medial preoptic area (mPOA) and the paraventricular nucleus (PVN) of the hypothalamus were extracted from the brains, RNA was isolated, and RT-qPCR was used to measure gene expression. In the mPOA, there was a significant upregulation of *Crebbp* in pubertally stressed females with no exposure compared to controls with no exposure. This upregulation was associated with poor performance on the memory task, indicating that pubertal stress is disrupting how *Crebbp* canonically influences memory. We investigated gene expression within the PVN and examined the up- down-stream targets of CREB binding protein in the mPOA. Stress during puberty could have a lifelong impact on continual parental responding, which is supported by maternal memory. These results provide novel insight into the impact of adversity during puberty on lifelong risk for altered maternal behavior.

Disclosures: G.C. Pifer: None. **S.L. Higley:** None. **B.M. Karem:** None. **K.E. Morrison:** None.

Poster

394. Effects of Early Life Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 394.11

Topic: F.03. Stress and the Brain

Support: NIH Grant HD091376

Title: Pubertal stress increases sensitivity to the negative impact of subthreshold limited resources during pregnancy on maternal behavior and brain

Authors: *B. M. KAREM, B. D. ELLIOTT, G. C. PIFER, K. E. MORRISON;
West Virginia Univ., Morgantown, WV

Abstract: Stress during puberty is associated with adult stress dysregulation and dysfunctional parental behaviors in humans. We have previously shown that stress during puberty resulted in an altered hypothalamic-pituitary-adrenal (HPA) axis stress response in adult humans and mice. This adverse HPA response was only observed during the peripartum period and produced limited impacts on maternal behavior. Other work has shown that limiting resources during pregnancy negatively impacts maternal behavior in both humans and mice. We hypothesized that the combination of stress during puberty and a subthreshold exposure to limited resources during pregnancy would have a negative impact on maternal behavior, offspring health outcomes, and the underlying molecular and endocrine systems within the brain. We conducted chronic variable stress (CVS) during puberty, postnatal days (PN) 21-34, using a variety of auditory, olfactory, and tactile stressors. As adults, all females were bred with naive males. Upon confirmation of pregnancy, females were put into a subthreshold limited nesting condition or in standard bedding. At17.5 days post conception they were placed back into standard conditions for birth. On PN3, we measured behavior during a home cage pup retrieval task in order to assess early naturalistic maternal behaviors. On PN7, we measured maternal behavior during a separation task in which 4 of the dam's pups were placed in a mesh box in the center of an open field. Additionally, we collected blood at several time points to assess the HPA axis response to the separation task. On PN28, when pups were weaned, all dams were exposed to a 15 min restraint stress to assess HPA axis response. To assess the impact of having a mother with varying pubertal and pregnancy experiences, adult offspring experienced a 15 min restraint stress and blood collection. In pup retrieval, pubertally stressed females with limited nesting sniffed pups significantly more than puberal stress alone females. Pubertal stress and limited resources during pregnancy interacted to alter the corticosterone response to pup separation. Due to its known role in stress regulation, we examined the paraventricular nucleus of the hypothalamus for alterations to gene expression. Overall, these data show that pubertal stress results in increased sensitivity to even subthreshold limited resources during pregnancy, with the combination producing an altered response to stress and maternal behavior. These studies provide insight into the complex risk factors that interact in the lifespan to produce negative outcomes for females and their offspring.

Disclosures: B.M. Karem: None. **B.D. Elliott:** None. **G.C. Pifer:** None. **K.E. Morrison:** None.

Poster

394. Effects of Early Life Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 394.12

Topic: F.03. Stress and the Brain

Support: NIH Grant HD091376

Title: Dysfunctional hypothalamic-pituitary-adrenal axis reactivity following pubertal stress is developmentally dependent

Authors: *B. D. ELLIOTT, G. C. PIFER, R. B. GANDEE, K. E. MORRISON;
West Virginia Univ., Morgantown, WV

Abstract: Two potent risk factors for the development of affective disorders in women are adversity experienced during puberty and later becoming pregnant. A key endophenotype observed across affective disorders is a disrupted hypothalamic-pituitary-adrenal (HPA) axis. We have previously shown that adult female mice that experienced pubertal stress displayed a blunted corticosterone response to an acute stressor. We have only observed this effect in the peripartum window, which suggests that the mechanisms driving the uncovering of HPA disruption may be pregnancy dependent. Until now, we have only examined this interaction between pubertal stress and later pregnancy in adult dams. However, many individuals become pregnant in adolescence, a time during which the HPA axis is still maturing. The trajectory by which pubertal stress alters HPA reactivity throughout development is unknown. This study aimed to address whether the blunted HPA phenotype was apparent prior to complete HPA axis maturation. We hypothesized there would be an interaction between pubertal stress and age at the time of pregnancy in producing the blunted HPA axis response. Female mice were either exposed to chronic variable stress for two weeks from postnatal day (PN) 21-34 or were undisturbed. All females were bred with a naïve male either in adolescence (PN42) or in adulthood (PN70). In late pregnancy (17.5 post conception), all females were exposed to a 15 min restraint stress and blood was collected at baseline, rise, peak, and recovery timepoints. Dam and fetal tissues were immediately collected. Blood plasma was assessed via radioimmunoassay for corticosterone quantity. As predicted, we found that pregnant, adolescent females had an increased corticosterone response compared to pregnant, adult females. Neither pubertal stress nor age of pregnancy had an impact on fecundity, weight gained, litter size, or fetal weight. Key tissues of the HPA axis, including the paraventricular nucleus of the hypothalamus, pituitary gland, and adrenal gland, were assessed for relevant gene expression. Given the known impact of maternal early life stress on offspring fetal outcomes, we also examined fetal tissue. These findings provide insight into the impact of pubertal stress on the developmental trajectory of the HPA axis, including whether pubertal stress is likely to facilitate or decelerate maturation. Understanding the relationship between development and the emergence of stress-related dysregulation is important in determining when in the lifespan risk or resilience is likely to be evident.

Disclosures: **B.D. Elliott:** None. **G.C. Pifer:** None. **R.B. Gandee:** None. **K.E. Morrison:** None.

Poster

394. Effects of Early Life Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 394.13

Topic: F.03. Stress and the Brain

Support: NIH grant HD091376

Title: Increased coexpression of CB1 receptors and corticotropin releasing factor cells within the central amygdala following pubertal stress and pregnancy

Authors: *R. B. GANDEE[1], T. L. BALE[2], K. E. MORRISON[1];
[1]West Virginia Univ., Morgantown, WV; [2]Psychiatry, Univ. of Maryland Sch. of Med., Aurora, MD

Abstract: Chronic stress during puberty, a time of rapid brain development and neural plasticity, can induce lasting changes in the adult stress response. Previously, we showed that pubertal stress leads to blunted activity in the hypothalamic-pituitary-adrenal (HPA) stress axis, but only in pregnant females. The endocannabinoid system (ECS) has a role in stress regulation, particularly in the amygdala where it is heavily implicated in processing stressful stimuli and regulating the HPA axis. In the central amygdala (CeA) are corticotropin releasing factor (CRF) cells and CB1 receptors (CB1r), which are colocalized and active during the stress response. The ECS is sensitive to changes after pubertal stress, but the exact outcome of this interaction is unknown, as studies report mixed findings. Further, little is known about the relationship between the ECS and pregnancy. We examined whether exposure to pubertal stress and pregnancy later in life would interact to impact the ECS. We predicted that pubertally stressed female mice would show increased CB1r expression in the CeA. Mice with transgenically labeled CRF cells (tdTomato) were exposed to pubertal stress from postnatal day 21-34, during which they received olfactory, auditory, or tactile stressors. After pubertal stress, mice were left undisturbed until breeding at 10-12 weeks of age. On 17.5 days post conception, females underwent a 15 min restraint stress and subsequent cardiac perfusion. Brains were collected, cryosectioned, and labeled with Hoechst stain and anti-CB1r fluorescent antibodies. Fluorescent microscopy was used to capture the distribution of CB1r on CRF cells. FIJI intensity analyses were used to interpret optical density of CRF cells, CB1r, and cell nuclei. The analyses revealed no difference in optical density of CRF or CB1r as a result of the conditions. However, a colocalization analysis showed increased colocalization of CB1r with CRF cells in nonpregnant, pubertally stressed females compared to nonpregnant controls. Pregnancy produced opposing effects based on pubertal stress, such that there were no longer differences in colocalization. Ongoing studies utilizing confocal microscopy will determine the distribution of CB1r on CRF cells by dimensional separation of cells and receptors. Future studies will examine CB1r

expression at different time points during puberty to understand the development of the ECS in this time frame. These studies provide insight into the complex relationship between the ECS and stress regulation in the amygdala, and how this relationship is altered by exposure to developmental stress, the experience of pregnancy, and the combination of both experiences.

Disclosures: R.B. Gandee: None. **T.L. Bale:** None. **K.E. Morrison:** None.

Poster

394. Effects of Early Life Stress

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 394.14

Topic: F.03. Stress and the Brain

Support: NIH Grant HD091376

Title: Pubertal stress programs lasting changes in the prefrontal cortex of male and female mice that are mitigated by social interaction

Authors: *B. RODRIGUEZ, J. M. MENDOZA, L. A. M. LUTHER, R. A. EWANYK, K. E. MORRISON;
West Virginia Univ., Morgantown, WV

Abstract: Chronic stress experienced during puberty poses a significant risk for the development of neuropsychiatric disorders in adulthood. Disruption of the prefrontal cortex (PFC) during puberty has been shown to induce behavioral deficits in executive planning, working memory, and learning. In humans and mice, social interaction has been shown to mitigate these negative consequences. We have shown that pubertal stress with concomitant social interaction produced resilience to age-related decline in behavior and altered gene expression in the PFC of aged female mice. Here, we sought to assess both how pubertal stress and stress with concomitant social interaction would impact performance on PFC-related tasks and gene expression during adolescence and adulthood. We hypothesized that pubertal stress would disrupt behavior and gene expression, while social interaction during stress would attenuate behavioral deficits and produce unique gene expression patterns. Male and female mice underwent two weeks of pubertal stress or were left undisturbed between postnatal days (PN) 21-34. Mice were either singly housed for the duration of pubertal stress (CVS), or were returned to dam and littermates for social interaction between stressors (CVS+SI). After pubertal stress, all mice were pair-housed and left undisturbed until testing. Half of the mice were administered a battery of PFC-related behavioral tasks in adolescence (starting PN40-43), while the other half was tested in adulthood (starting PN70-74), including the Open Field, Light Dark Box, Y-Maze Spatial Alternation, and Barnes Maze. Brains were collected 24h after the final test. RNA was isolated from the medial prefrontal cortex (mPFC) and qRT-PCR was performed to assess gene expression. Body weight was reduced in adolescent and adult CVS mice and normalized in CVS+SI mice. In adulthood, male and female control mice performed similarly on the open

field, light dark box, and spatial alternation. Initial evidence suggests that CVS was producing sex-specific effects on PFC-related behavioral tasks, such that CVS facilitated performance in males but diminished performanced in females. In both sexes, exposure to social interaction reversed the impact of pubertal stress. To better understand the mechanisms by which these experiences produce risk or resilience, we examined gene expression of previously identified microRNAs and myelin-related genes. These studies provide evidence in support of dynamic cortical maturation during puberty that are affected by stress experience and offer valuable insight into risk and resilience for negative outcomes following stress during puberty.

Disclosures: B. Rodriguez: None. **J.M. Mendoza:** None. **L.A.M. Luther:** None. **R.A. Ewanyk:** None. **K.E. Morrison:** None.

Poster

394. Effects of Early Life Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 394.15

Topic: F.03. Stress and the Brain

Support: NIH SR107118

Title: Early Life Stress Obstruct Axonal Pathfinding and Myelination in the Perforant Pathway

Authors: *R. ISLAM[1], J. D. WHITE[1], B. POLIS[1], S. AHMED[1], T. M. AREFIN[2], J. ZHANG[2], A. KAFFMAN[1];
[1]Dept. of Psychiatry, Yale Univ., New Haven, CT; [2]New York Univ., Bernard Irene Schwartz Ctr. for Biomed. Imaging, New York, NY

Abstract: Early life stress (ELS) impairs hippocampal function and connectivity across diverse mammalian species including humans and rodents. Despite some progress in our understanding of the mechanisms by which ELS disrupts normal hippocampal function in rodents, most of the work has focused on deficits that take place in adult or aging animals and additional work is needed to clarify the effects of ELS on hippocampal development and its contribution to hippocampal-dependent cognitive deficits in peri-pubescent juvenile mice. To address this issue, we used RNA-seq to assess the effects of limited bedding (LB) on gene expression in the hippocampus of 17-day old pups (P17, n= 16 mice per condition, half of which were males). RNA-seq identified 747 differentially regulated genes (FDR< 0.05) with abnormal myelination as the most significant pathway impaired by LB (FDR= $3.83e^{-11}$). LB reduced the expression of genes necessary for oligodendrocyte differentiation but not oligodendrocyte progenitor cells proliferation. Follow-up Immunohistochemistry studies showed that the perforant pathway is one of the most myelinated structures in the developing hippocampus and confirmed reduced oligodendrocyte maturation in the perforant pathway of LB mice ($F(1, 16) = 35.56$ $P< 0.0001$, $\eta p^2 = 0.69$). Abnormal myelination was associated with reduced axonal markers ($F(1, 16) = 9.88$, $P< 0.0063$, $\eta p^2 = 0.38$) suggesting that LB impaired axonal pathfinding and connectivity

between the entorhinal cortex and the dorsal hippocampus. This assertion was further confirmed using diffusion MRI tractography and retrograde tracing and was correlated with abnormal contextual freezing in P33 juvenile mice. These studies highlight abnormal axonal pathfinding in the perforant pathway as a potentially novel mechanism for abnormal myelination and hippocampal dependent deficits in juvenile mice exposed to ELS. The use of high resolution dMRI in mouse enables for direct comparison with findings in humans exposed to early adversity.

Disclosures: **R. Islam:** None. **J.D. White:** None. **B. Polis:** None. **S. Ahmed:** None. **T.M. Arefin:** None. **J. Zhang:** None. **A. Kaffman:** None.

Poster

394. Effects of Early Life Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 394.16

Topic: F.03. Stress and the Brain

Support: NIMH RO1MH096093
Harvey Family Endowment

Title: Early life adversity reconfigures the adult brain: A longitudinal MEMRI study

Authors: *T. W. USELMAN[1], R. E. JACOBS[2], E. L. BEARER[1,3];
[1]Pathology, Univ. of New Mexico Hlth. Sci. Ctr., Albuquerque, NM; [2]Zilkha Neurogenetic Inst., USC Keck Sch. of Med., Los Angeles, CA; [3]Div. of Biol. and Biol. Engin., Caltech, Pasadena, CA

Abstract: Early life adversity (ELA) is correlated with later life vulnerabilities to stress reactions and substance use disorders. In non-human primates, maternal care is critical for normal brain development and later life outcomes. How fragmented maternal care affects brain structure-function leading to mental illness is an area of intense investigation. Manganese-enhanced magnetic resonance imaging (MEMRI) offers an opportunity to define brain function unique to ELA. Mn(II) delivered systemically diffuses throughout brain parenchyma, accumulating in active neurons of awake-behaving mice via voltage-gated calcium channels. T_1-weighted MRI detects Mn(II) due to a hyperintense signal captured retrospectively. Combined with data-driven analytics MEMRI reveals whole-brain regional activity and networks. To model ELA dams were deprived of adequate bedding from P2-9 (n=12). Pups were then aged under normal housing conditions for 10 weeks. Adult pups received IP $MnCl_2$ (0.3 mmol/kg) and returned to their home cage. Mn(II) accumulation was imaged by MR 24h later, labeling active neurons during basal state. Then mice were exposed to neutral odor followed by predator stress (PS) (TMT, 2,3,5-Trimethyl-3-thiazoline) under video monitoring. Responses to PS were as expected. Mice were MR scanned 1h after PS. At 9 days the Mn(II)-MRI sequence was repeated without PS to test for residual Mn(II) and sustained brain activity. MR images were skull-stripped and normalized.

Rich information about ELA's impact on the brain was calculated using a suite of data-driven voxel- and segment-wise analyses: statistical parametric mapping, regional intensity measurements, inter-subject cross-correlations of segment-wise intensities, graph theory, and independent component analysis. Cross-validation and permutation testing validated results. Compared to normal rearing, ELA increased signal in stress- and reward-related regions: ventral striatum, ventromedial hypothalamus, amygdala, ventral tegmental area, locus coeruleus, and raphe nuclei; and decreased in executive control and stress-mediating regions: medial prefrontal cortex and medial thalamus, regions influenced by cocaine and other psychotropic drugs. Cross-correlations of 90 regions revealed a global decrease in basal connectivity and restructured networks at all time points after ELA. Disrupted connectivity and decreased prefrontal cortical inhibition of deeper structures may explain ELA's effect on vulnerability to mood disorders and propensity towards drug use. Future work will study medial prefrontal cortical projections in adult mice after ELA using *in vivo* MEMRI tract-tracing.

Disclosures: T.W. Uselman: None. **R.E. Jacobs:** None. **E.L. Bearer:** None.

Poster

394. Effects of Early Life Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 394.17

Topic: F.03. Stress and the Brain

Title: Characterization of astrocytic morphology following early life adversity

Authors: *C. DECKERS[1], E. A. WITT[2], E. HARDER[3], K. J. REISSNER[4], D. A. BANGASSER[5];
[1]Temple Univ. Grad. Neurosci. Program, Temple Univ. Grad. Neurosci. Program, Philadelphia, PA; [2]UNC Chapel Hill, UNC Chapel Hill, Chapel Hill, NC; [3]Univ. of North Carolina at Chapel Hill, Chapel Hill, NC; [4]UNC-CH, UNC-CH, Chapel Hill, NC; [5]Temple Univ., Temple Univ., Philadelphia, PA

Abstract: Undergoing stressful experiences early in life has been tied to structural and cellular level changes in the brain, which may contribute to behavioral alterations in adulthood. Importantly, there is evidence showing that early life stress that is not overwhelming, such as some forms of mild resource scarcity, can actually promote resilience to later development of psychiatric disorders. Resource scarcity can be modeled by using a limited bedding and nesting model (LBN), in which the dam's access to nesting materials is restricted from pup's postnatal days 2-9. Previous work has demonstrated that the experience of LBN reduces impulsive choice in male subjects alone. Impulsivity is thought to be mediated by brain regions including the medial orbitofrontal cortex (mOFC) and medial prefrontal cortex (mPFC). Therefore, we sought to elucidate the functional underpinnings of the sex-specific inoculating effect of LBN within these regions. RNA sequencing data suggest that the experience of LBN is associated with an upregulation of the astrocytic marker glial fibrillary acidic protein (GFAP) in the mOFC of male

subjects. Conversely, GFAP expression is slightly downregulated in the mOFC of female subjects. These data suggest early life stress-induced changes in astrocyte gene expression. In addition to gene and protein expression, changes in astrocyte function can be revealed through morphological analyses. Prior work examining the effects of various forms of stress has found that undergoing either acute or chronic paradigms changes astrocytic morphology. Here we will extend this work to determine if LBN has a lasting impact on the morphological characteristics of astrocytes. To this end, we injected AAV-GfaABC1D-Lck-GFP, an adeno-associated virus (AAV) that selectively labels astrocytes into the mOFC and mPFC of adult rats. Using this tissue, immunohistochemical labeling of GFAP and postsynaptic density protein 95 (PSD-95) was performed, in addition to the application of a 4′,6-diamidino-2-phenylindole (DAPI) stain. Three dimensional reconstructions of individual astrocytes were generated using Imaris and analyzed for morphological parameters including surface area, volume, and relative synaptic connectivity. This work is still ongoing, but we predict to see more robust astrocytic architecture in males that have undergone LBN, while in LBN females, we expect to see either no change or deterioration in astrocytic morphology. Changes in astrocytic morphology can affect neurotransmitter levels, resulting in alterations in behavioral output. Thus, one mechanism by which LBN affects impulsivity in males may be via changes in cortical astrocytes.

Disclosures: C. Deckers: None. **E.A. Witt:** None. **E. Harder:** None. **K.J. Reissner:** None. **D.A. Bangasser:** None.

Poster

394. Effects of Early Life Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 394.18

Topic: F.03. Stress and the Brain

Support: 5R01DK126085-02

Title: Molecular profiling of developing vagal sensory neurons following early life stress

Authors: *A. K. KAMITAKAHARA[1], M. MCCOY[2], S. BENNISON[2], L. M. RINAMAN[3], P. R. LEVITT[1];
[1]Children's Hosp. Los Angeles, Univ. of Southern California, Los Angeles, CA; [2]Children's Hosp. Los Angeles, Los Angeles, CA; [3]Dept. of Psychology, Florida State Univ., Tallahassee, FL

Abstract: Early life stress (ELS) has profound consequences on lifespan health including increased risk for obesity and diabetes, dysregulated hypothalamic-pituitary-adrenal function, systemic inflammation, and gastrointestinal dysmotility. The deleterious multi-system effects of ELS stem, in part, from blunted sympathetic nervous system responsiveness and reduced parasympathetic vagal tone. Vagal sensory afferents in the nodose ganglion serve as a major communication route from the viscera to the brain that modulates both sympathetic and

parasympathetic responses. To identify molecular alterations responsible for maladaptative vagal sensory-motor function, transcriptomics analyses were performed in tissue from developing nodose ganglia following ELS. A well-established "limited resources" mouse model of postnatal ELS was used that generates lifelong cognitive, metabolic, inflammatory, and visceral dysfunction. Following the ELS period, on postnatal day 9, tissue was collected from the vagal nodose-jugular complex and 10x genomic single-cell sequencing was performed. Initial processing of control care-as-usual (CAU) samples revealed the presence of several unique subtypes of vagal sensory neurons consistent with data that has thus far only been characterized in adults. Several subclasses of satellite glia were also present, along with discretely clustered populations of endothelial cells and microglia. The neurons within the fused jugular-nodose ganglion express either *Prdm12* or *Phox2b* in adults. These cellular populations were readily identified, along with an as yet undescribed population of neurons that expressed neither marker. This novel neuronal population was differentiated by expression of acetylcholinesterase (*Ache*) known for its canonical role in the degradation of the neurotransmitter acetylcholine, as well as numerous non-canonical developmental functions in neurite outgrowth, cell adhesion, and synaptogenesis. Single-cell sequencing completed for the first time in the developing nodose ganglion demonstrates readily identifiable sensory neuronal subtypes in CAU pups. Ongoing work will compare these molecular profiles to those following ELS to identify putative mechanisms underlying vagal functional and connectional deficits.

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Poster

394. Effects of Early Life Stress

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Program #/Poster #: 394.19

Topic: F.03. Stress and the Brain

Support: NIH DA052909
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NIH MH078105
Emory National Primate Research Center (ENPRC) Base Grant OD P51OD011132

Title: Exosomal RNA signatures of Early Life Stress in Non-Human Primates

Authors: *L. KOROBKOVA[1,2,3], H. WALUM[4], E. L. MORIN[4], E. R. SIEBERT[4], M. M. SANCHEZ[5,4], B. G. DIAS[1,6,3];
[1]Developmental Neurosci. and Neurogenetics Program, The Saban Res. Inst., Los Angeles, CA; [2]Neurosci. Grad. Program, USC, Los Angeles, CA; [3]Div. of Endocrinol., Children's Hosp. LA, Los Angeles, CA; [4]Div. of Cognitive Neuroscience, Emory Natl. Primate Res. Ctr., Emory

Univ., Atlanta, GA; [5]Dept. of Psychiatry, Emory Univ. Sch. of Med., Atlanta, GA; [6]Dept. of Pediatrics, Keck Sch. of Med. of USC, Los Angeles, CA

Abstract: Exposure to early life stress (ELS), including childhood maltreatment, is one of most significant risk factors for the emergence of psychopathology. Despite this relationship being well established, individual variability exists and it is challenging to distinguish those impacted by the perniciousness of ELS from those who escape relatively unscathed. Identifying biological signatures of ELS is one way to achieve this objective. In this study, we combined a translationally relevant non-human primate model of ELS that leverages naturally occurring adverse caregiving of infant macaques with the profiling of RNA in circulating plasma exosomes to identify exosomal RNA signatures of exposure to ELS. We isolated exosomes from plasma collected from adolescent rhesus macaques that experienced Care as Usual (CAU) vs Infant Maltreatment (MALT). Next, we performed total RNA sequencing, aligned these reads to the MacaM genome, after finally, analyzing differentially expressed genes (DEG) and molecular pathways in CAU vs MALT samples. Additionally, we assigned microbial taxonomic labels to reads that did not map to the MacaM genome and assessed the composition of microbiota in circulating exosomes collected from CAU and MALT animals. DEG analysis revealed genes that were both, highly expressed and highly suppressed, in MALT compared to CAU. Gene enrichment analysis revealed that genes related to translation, ATP synthesis, mitochondrial function and immune response were downregulated in MALT samples, while genes involved in ion transport, metabolism and cell differentiation were upregulated in these samples compared to CAU. Finally, we found that MALT altered the diversity of microbiotic signatures found in exosomes. Specifically, we identified the presence of 35 unique bacterial species in MALT samples. Our study demonstrated that analysis of exosomal RNA can be a useful strategy to assess biological signatures of ELS and suggests that immune function and the microbiome may be robust signatures of infant maltreatment.

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Poster

394. Effects of Early Life Stress

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Program #/Poster #: 394.20

Topic: F.03. Stress and the Brain

Support: CIHR Project grant to N.M.
FRQ-S scholarship to M.W.

Title: A new method to isolate microvessels from human postmortem neocortex for RNA sequencing

Authors: *M. WAKID, D. A. ALMEIDA, R. A. RAHIMIAN, Z. AOUABED, J.-F. A. THEROUX, M. DAVOLI, V. YERKO, G. A. TURECKI, N. A. MECHAWAR;
Douglas Mental Hlth. Univ. Inst., Montreal, QC, Canada

Abstract: Introduction: Childhood abuse (CA), is experienced globally by approximately 1 billion youth aged between 2-17 years (Hillis et al., 2016, *Pediatrics*, 137:1). Consistent evidence of neuroinflammation and activated microglia (Lehmann et al., 2018, *Scientific Reports*, 8:5) but also microbleeds (Lehmann et al., 2018, *Scientific Reports*, 8:5), abnormal blood vessel morphology and downregulated tight junction protein claudin5 (Cldn5) are observed in the brains of mice exposed to chronic stress (Menard et al., 2017, *Nat Neurosci*, 20:3; Dudek et al., 2020, *Proc Natl Acad Sci USA*, 117:2). However, the impact CA has on cerebrovascular integrity has yet to be sufficiently investigated in humans.
Hypothesis & Objectives: We hypothesize that a history of early-life stress exacerbates the alterations in cerebrovasculature suggested by previous studies of chronic stress experienced in adulthood. As a first step to test this hypothesis and circumvent the challenges surrounding single-nuclei sequencing, we developed an alternative method to isolate intact microvessels from snap-frozen human cerebral cortex in order to explore cerebrovascular differences in the ventromedial prefrontal cortex (vmPFC) from depressed suicides with a history of CA and matched controls (CTRL).
Methods: Well-characterized frozen postmortem brain samples from adult male and female depressed suicides with a history of severe CA and matched sudden-death controls (n=26/group) were obtained from the Douglas-Bell Canada Brain Bank. We developed a protocol to enrich and isolate microvessels using mechanical homogenization and centrifugation-separation that is gentle enough to maintain the structural integrity and multicellular composition of intact microvessels for downstream investigation.
Results: Through immunohistochemical characterization, we show that isolated microvessel fragments are comprised of endothelial cells, astrocytic end-feet, pericytes as well as tight junction proteins that seal endothelial cells. Total RNA extracted from microvessels is compatible with library preparation and RNA-sequencing on the NovaSeq6000 system (Genome Quebec).
Significance: To our knowledge, this is the first time that microvessels have been effectively isolated from human brain tissue and analyzed as an intact structure to explore the molecular characteristics of the cerebrovasculature with high-throughput approaches such as RNAseq. The advantages of this simple protocol are manifold: it does not require a single-nuclei sorting setup to isolate fluorescence-labeled cells, nor does it require enzymatic dissociation or fresh brain tissue.

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Poster

395. Integrative Physiology and Behavior: Sleep Systems II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 395.01

Topic: F.07. Biological Rhythms and Sleep

Title: Temporal evolution of brain connectivity upon awakening from slow wave sleep varies by cognitive task

Authors: *L. O. JIMENEZ[1,2], K. BANSAL[3,2,1], C. L. HILDITCH[4], N. L. SHATTUCK[5], J. O. GARCIA[1,2], E. E. FLYNN-EVANS[6];
[1]DEVCOM Army Res. Lab., Aberdeen, MD; [2]Cognitive Sci., Univ. of California, Irvine, Irvine, CA; [3]Biomed. Engin., Columbia Univ., New York, NY; [4]Dept. of Psychology, San José State Univ., San Jose, CA; [5]Operations Res. Department,, Naval Postgraduate Sch., Monterey, CA; [6]Human Systems Integration Div., NASA Ames Res. Ctr., Moffett Field, CA

Abstract: Sleep inertia refers to the state of transition between sleep and wake characterized by impaired alertness, confusion, and reduced cognitive and behavioral performance. While the neurobehavioral symptoms of sleep inertia are well-described, less is known about the temporal evolution of brain connectivity that characterize sleep inertia and the cognitive specificity of these effects. Previously, using electroencephalography (EEG), we have shown during a psychomotor vigilance task (PVT), that upon awakening, global power within lower frequency bands returns to baseline levels before higher frequencies. This observation was also accompanied by changes in network metrics in the lower frequency bands, specifically, change in average *clustering coefficient*, which measures how likely two neighbors of a node are connected to one another, and average *path length*, which measures the average shortest path between every node pair. Here we extend these findings to a restful awake segment (Karolinska Drowsiness Test; KDT), a Go/No-Go task (GNG), and an arithmetic task (MATH), to understand the specificity and task interactions of these effects. After mild restriction the night before (5h time-in-bed), participants were brought to the laboratory and participated in a baseline assessment of task performance and neural metrics before sleep. Participants were then allowed to sleep and were woken up in slow wave sleep (SWS) by an experimenter who gave them an intervention (blue-enriched light, dim red light) and then led them through the four tasks (PVT, MATH, GNG, KDT) consecutively four times (T1-4). Similar to our previous findings, for the KDT, GNG, and MATH tasks, high frequency differences in global power between baseline and other test bouts were largely retained. Interestingly, clustering and path length differences between baseline and the test bouts (T1-4) were largely absent in the low frequencies; however, within the beta band whilst participants were engaged in the MATH task, we observed a significant change from baseline in T2-4 for both clustering and path length. This effect was largely attenuated with an intervention of blue-enriched light; however, the blue-enriched light intervention also significantly increased clustering coefficient in the delta band above baseline levels. Considering the stability of the power effects across tasks and the specificity of the network metrics effects to tasks, these two approaches suggest that two different neural schemes underlying sleep inertia, one that gradually recovers and impacts all that we do (i.e., power) and one that is sensitive to task and intervention, reconfiguring the brain as new task demands emerge.

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Poster

395. Integrative Physiology and Behavior: Sleep Systems II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 395.02

Topic: F.07. Biological Rhythms and Sleep

Support: Grant-in-Aid for Transformative Research Areas (A)
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Grant-in-Aid for Scientific Research on Innovative Areas (Research in a proposed research area)
Grant-in-Aid for Scientific Research (C)

Title: Oscillatory population-level activity of dorsal raphe serotonergic neurons is inscribed in sleep structure

Authors: *T. KATO[1], Y. MITSUKURA[3], M. MIMURA[1], N. TAKATA[2], K. F. TANAKA[2]; [1]Dept. of Neuropsychiatry, [2]Div. of Brain Sciences, Inst. for Advanced Med. Res., Keio Univ. Sch. of Med., Tokyo, Japan; [3]Dept. of Syst. Design Engin., Fac. of Sci. and Technol. of Keio Univ., Kanagawa, Japan

Abstract: Dorsal raphe (DR) 5-HT neurons regulate sleep-wake transitions. Previous studies demonstrated that single-unit activity of DR 5-HT neurons is high during wakefulness, decreases during non-rapid eye movement (NREM) sleep, and ceases during rapid eye movement (REM) sleep. However, characteristics of the population-level activity of DR 5-HT neurons, which influence the entire brain, are largely unknown. Here, we measured population activities of 5-HT neurons in the male and female mouse DR across the sleep-wake cycle by ratiometric fiber photometry. We found a slow oscillatory activity of compound intracellular Ca^{2+} signals during NREM sleep. The trough of the concave 5-HT activity increased across sleep progression, but 5-HT activity always returned to that seen during the wake period. When the trough reached a minimum and remained there, REM sleep was initiated. We also found a unique coupling of the oscillatory 5-HT activity and wide-band EEG power fluctuation. Furthermore, optogenetic activation of 5-HT neurons during NREM sleep triggered a high EMG power and induced wakefulness, demonstrating a causal role of 5-HT neuron activation. Optogenetic inhibition induced REM sleep or sustained NREM, with an EEG power increase and EEG fluctuation, and pharmacological silencing of 5-HT activity using a selective serotonin reuptake inhibitor led to sustained NREM, with an EEG power decrease and EEG fluctuation. These inhibitory manipulations supported the association between oscillatory 5-HT activity and EEG fluctuation. We propose that NREM sleep is not a monotonous state, but rather it contains dynamic changes that coincide with the oscillatory population-level activity of DR 5-HT neurons.

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Poster

395. Integrative Physiology and Behavior: Sleep Systems II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 395.03

Topic: F.07. Biological Rhythms and Sleep

Support: National Institute on Alcohol Abuse and Alcoholism (ZIAAA000550, PI: Nora D. Volkow)

Title: Brain correlates of rest-activity rhythm profiles in healthy adults

Authors: *R. ZHANG[1], D. TOMASI[1], E. SHOKRI-KOJORI[1], N. D. VOLKOW[2];
[1]NIH/NIAAA, Bethesda, MD; [2]NIDA/NIH, NIH, Natl. Inst. On Drug Abuse, Bethesda, MD

Abstract: Certain patterns of rest-activity rhythms (RAR) such as delayed phase, low physical activity, insufficient and inconsistent sleep are associated with increased risk for drug use and mood problems. However, the underlying brain mechanisms remain largely unknown.In the current study, we assessed RAR of n=93 healthy adults (age range: 19-73; 45% females) using one-week actigraphy combined with self-reported sleep and chronotype. Resting-state fMRI was performed within 21 days prior to each participant's actigraphy data. Instead of examining brain correlates of individual RAR parameters, we first identified four independent RAR factors: phase, daytime activity duration, sleep regularity and duration, physical activity using principal component analysis. Using whole-brain ROI-ROI approach, we found that delayed phase was associated with greater resting state functional connectivity (RSFC) in the salience network. Based on the four RAR factors, we further identified two phenotypes: high-risk and low-risk RAR profiles using hierarchical clustering. 30% of participants were classified into the high-risk RAR phenotype. Using machine learning approach, resting state functional connectivity during rest predicted phenotypes with an accuracy of 77.8% in an independent hold-out sample. High-risk RAR phenotype showed greater RSFC between salience network and posterior default mode network and lower RSFC between visual attention network and cerebellum than low-risk RAR phenotype.

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Poster

395. Integrative Physiology and Behavior: Sleep Systems II

Location: SDCC Halls B-H

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Program #/Poster #: 395.04

Topic: F.07. Biological Rhythms and Sleep

Support: Jazz Pharmaceuticals Grant IST 19-11124

Title: Pattern and degree of sleep disruption: a model for hypersomnia identification

Authors: A. CAIRNS, ***C. GRAY**, D. LEWIN;
BioSerenity, Atlanta, GA

Abstract: Introduction: Central disorders of hypersomnia (CDH), including Narcolepsy type 1 (NT1) and type 2 (NT2) as well as Idiopathic Hypersomnia (IH) are differentiated with the daytime multiple sleep latency test (MSLT). Those with NT1 often have MSLT outcomes that reflect a trait-like, repeatable finding. In contrast, differential diagnosis of NT2 and IH via the MSLT is notably challenging as data often yields equivocal and/or inconsistent outcomes. This study aimed to explore a novel method of CDH classification based on nocturnal features of EEG disruption.
Methods: Polysomnograms (PSG) preceding an MSLT for patients being evaluated for hypersomnia were extracted from a large real-world database. Studies were excluded if the patient reported shift/night work, if another sleep pathology was identified on the PSG, or if EEG tracings were of inadequate quality. PSGs were visually scored and power spectral analyzed. Data elements representing sleep/REM disruption were imputed into an unsupervised cluster analytic technique. Optimal cluster solution was identified using Bayesian information criterion.
Results: Three distinct clusters were identified, each with homogeneous patterns of sleep disruption. Cluster 1 (14% of sample) was characterized by the highest degree of sleep and REM lability (REM transition index (RTI) = 4.5/hr, $p<.001$; wake-REM sequence = 4.9, $p<.001$)). Arousals were frequent (arousal index (AI) = 15.1/hr)), but non-sustained. Cluster 2 (31% of sample) was characterized by frequent and sustained arousals (AI = 15.5/hr; WASO = 66.4 min, respectively), frequent sleep/wake transitions (20.9/hr), but low REM lability (RTI = 2.0/hr; wake-REM sequence = 0.94). Cluster 3 (55% of sample) was characterized by the lowest degree of sleep and REM fragmentation (AI = 10.2/hr, sleep/wake transitions = 13.0/hr, WASO = 25.9 min, RTI = 1.8/hr; all p's$<.001$). Cluster distribution was similar across MSLT outcomes of IH and NT2 ($p=.429$).
Discussion: We identified clusters of nocturnal EEG features in patients being evaluated for hypersomnia that, at face value, overlap with the conceptual framework of differential diagnosis of NT2 from IH. However, these feature groups were essentially random based on the MSLT, supporting the growing body of literature that the MSLT is insufficient for CDH identification.

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Poster

395. Integrative Physiology and Behavior: Sleep Systems II

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Program #/Poster #: 395.05

Topic: F.07. Biological Rhythms and Sleep

Support: NIH T32 NS115753
UCLA Integrative Biology & Physiology Eureka Scholarship
NIH R01 MH60670
NIH U01 NS108930

Title: Different simultaneous sleep states between the hippocampus and neocortex in humans

Authors: *R. GUTHRIE[1], D. CILIBERTI[2], E. A. MANKIN[4], G. R. POE[3];
[1]Univ. of California Los Angeles, Los Angeles, CA; [2]Dept. of Neurosurg., [3]Dept. of Integrative Biol. and Physiol., UCLA, Los Angeles, CA; [4]UCLA, Dept. of Neurosurg., Los Angeles, CA

Abstract: Although sleep is generally thought of as a homogenous brain state, previous work in rodents has reported that different areas of the brain could simultaneously be in different sleep states. **In this study, we explored whether, in humans, the hippocampus and neocortex can simultaneously be in different states during sleep.**For this purpose, we analyzed whole night scalp electroencephalography (EEG) and intracranial recordings (iEEG) from eight participants undergoing implantation of depth electrodes as part of their clinical evaluation of pharmacoresistant epilepsy. Using standard scoring criteria, the iEEG from the posterior hippocampus and the midline scalp electrode signals were sleep scored manually and independently from each other.Our analyses provided strong evidence of the presence of asynchronous states between the hippocampus and the neocortex during sleep. First, we found that the hippocampus entered the deeper stages of sleep prior to the neocortex and that the amount of time spent in each state varied by region. Secondly, about one third of the night was spent in asynchronous states between regions (mean +/- SEM: 33.92% +/- 2.9%; range: 25%-49.6%). Remarkably, we also found that, although some state transitions were synchronous, non-simultaneous transitions were more common. Furthermore, bouts of different sleep states occurring simultaneously at both sites could last up to 35 mins. In order to test whether scoring in each site matched standard criteria, we evaluated the power spectral density (PSD) profiles from each recording within each state as scored. PSD for epochs of same simultaneous and different simultaneous states showed that the cortical PSD profile in different simultaneous states closely matched the designated state's PSD when that state was simultaneously present in both structures. **These results indicate that whole subcortical sleep bouts can be missed when sleep scoring is based on scalp electrodes alone.** The phenomenon of subcortical structures simultaneously existing in sleep states different from the neocortex could mean that past studies of sleep function and homeostatic control may have missed covert sleep state occurrences. Future studies seeking to explore sleep function should be designed with the knowledge that it is common for sleep states to fluctuate independently between brain structures throughout the night. Furthermore, novel spectral analysis aimed at revealing subcortical states non-invasively should be pursued.

Disclosures: R. Guthrie: None. **D. Ciliberti:** None. **E.A. Mankin:** None. **G.R. Poe:** None.

Poster

395. Integrative Physiology and Behavior: Sleep Systems II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 395.06

Topic: F.07. Biological Rhythms and Sleep

Support: T34GM141639

Title: Decoding arousal state information from spike train recordings in freely behaving mice

Authors: *J. JOSEPH, A. SCHNEIDER, K. B. HENGEN;
Washington Univ. In St. Louis, St. Louis, MO

Abstract: Arousal states such as sleep and wake are typically described by broad signals such as local field potentials. However, the extent to which arousal states affect the computations of single neurons are not well described. Previous studies hypothesize an arousal state imposes some general effect on the activity of some group of neurons (e.g., wake increases firing rate of pyramidal neurons), while one or more other groups of neurons are not so affected. We hypothesize that arousal state might exert an effect on every individual neuron's activity, however this effect may be unique to the neuron and potentially highly complex. To evaluate this possibility, we collected multi-day, multisite extracellular recordings of ensembles of single units in different subcortical and cortical regions of freely behaving mice (n = 8). We employed several machine-learning models with the capacity to identify increasingly complex effects in the spike trains of each unit which predict arousal state. First, to test whether standard metrics are sufficient to predict arousal state, we trained a logistic regression on standard distributional spiking statistics such as firing rate (FR) mean and interspike interval (ISI) coefficient of variation. Next, to test whether single unit spike patterns are more predictive of arousal state, we used a multilayer perceptron (MLP) trained on histograms of ISI distributions and FR distributions. Preliminary results suggest that as one recognizes unique and increasingly complex effects an increasing number of neurons' activity is revealed to be systematically modulated by arousal state. Additionally, our preliminary results may suggest that spiking patterns on shorter time scales are predictive of arousal state in ways distinct from distributional patterns over longer timescales. Ongoing work employs a deep learning architecture that takes as input firing patterns during short intervals and pools them across many timescales into new features using an attentional mechanism. These results will help us understand the extent and timescale of the diverse effects of arousal state on brain activity in diverse neural circuitry.

Disclosures: J. Joseph: None. **A. Schneider:** None. **K.B. Hengen:** None.

Poster

395. Integrative Physiology and Behavior: Sleep Systems II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 395.07

Topic: F.07. Biological Rhythms and Sleep

Title: A manifold of heterogeneous vigilance states across cortical areas

Authors: *J. H. WANG[1], R. KWAPICH[2], S. CHAUVETTE[3], I. V. TIMOFEEV[4], T. A. ENGEL[2];
[1]Cold Spring Harbor Lab. Sch. of Biol. Sci., Cold Spring Harbor Lab. Sch. of Biol. Sci., Cold Spring Harbor, NY; [2]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [3]CRIUSMQ, Quebec, QC, Canada; [4]CRIUSMQ, Univ. Laval, Quebec, QC, Canada

Abstract: Brain states are conventionally divided into wake, slow wave sleep (SWS) and rapid eye movement (REM) sleep based on distinct patterns of neural activity and muscle tone. However, recently available large-scale recordings indicate that this conventional division of brain states is insufficient to account for rich heterogeneity of neural dynamics on the global scale. During sleep, neural activity in some brain regions can exhibit awake signatures and vice versa. While brain states provide the backdrop for any activity underlying behavioral functions, the spatiotemporal structure of multi-regional brain states remains unexplored. We simultaneously recorded electromyogram (EMG) and local field potentials (LFP) at 14 sites across the mouse cortex during the natural variation of sleep and wake cycles continuously over multiple days. To characterize the heterogeneity of brain states in these multi-regional recordings, we developed an approach to uncover a low- dimensional manifold on which these states evolve. We use unsupervised dimensionality reduction based on a variational autoencoder (VAE) that predicts the next point in time. We trained the model on activity from an individual channel to uncover a local characterization of brain states. For single channels, the inferred manifold revealed three major clusters corresponding with human-expert labels of the basic wake, SWS, and REM states. Classical frequency bands, such as alpha, beta, and gamma, contributed nonlinearly to the inferred manifold. Applying the model to other electrodes, we found profound differences in the expression of states across cortical areas, particularly, the lack of REM-like activity in the lateral somatosensory cortex. We found that heterogeneity of states largely appears during transition periods between primary states, suggesting a more continuous global manifold. Our work provides a framework for quantifying heterogeneous brains states and shows that the regional co-existence of wake and sleep states is a common feature of global brain activity.

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Poster

395. Integrative Physiology and Behavior: Sleep Systems II

Location: SDCC Halls B-H

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Program #/Poster #: 395.08

Topic: F.07. Biological Rhythms and Sleep

Support: One Mind Rising Star Award
NARSAD Young Investigator Grant
1907 Trailblazer Award

Title: Total sleep deprivation alters spectral BOLD dynamics during an affective matching task

Authors: *S. WILLIAMS[1], Z. YANG[4], Z. DIAMANDIS[2], N. TACUGUE[1], Z. VALDIVIEZO[1], T. LY[1], M. AON[1], I. VINAL[1], E. SCHIMMELPFENNIG[1], N. M. LEONARD[1], J. YEE[3], D. ZIMMERMAN[1], L. D. LEWIS[1];
[2]Biomed. Engin., [1]Boston Univ., Boston, MA; [3]Boston Univ., Allston, MA; [4]Boston Univ. Grad. Program For Neurosci., Boston, MA

Abstract: Sleep deprivation profoundly alters affective processing, disrupting both sustained affective states ('mood'), and the ability to regulate affective reactions. Dysfunctional affective processing is associated with changes in blood oxygenation-level (BOLD) signals in limbic areas such as the amygdala and anterior cingulate cortex. These altered limbic area BOLD signals have traditionally been studied in the context of the magnitude of an average evoked response or the strength of functional connectivity between regions. However, how the intrinsic dynamics within these regions is affected by mood state is not well understood. We examined the frequency content of BOLD signals to investigate how spontaneous dynamics within limbic structures is affected by mood changes after sleep deprivation. The frequency content of BOLD signals carries information related to arousal state, with increases in low-frequency power indicating sleep or sleep-like states. We hypothesized that the spectra of BOLD signals during sleep-deprived wakefulness might resemble the spectra of sleep for brain areas involved in affective processing. To test whether total sleep deprivation affects spectral BOLD dynamics in limbic brain areas, we collected fast functional magnetic resonance imaging (fMRI) data with a short repetition time (TR=378 milliseconds) while subjects performed an emotional face-matching task. Subjects performed the task across two sessions, a well-rested session and a 26-hour total sleep deprivation session. All subjects were sleep deprived under laboratory conditions with whole-night real-time eye monitoring to ensure wakefulness. Our rapid acquisition of fMRI data allowed us measure spectral dynamics up to 1.3 Hz. The fast sampling rate also allowed us to resolve respiratory (0.2 - 0.4 Hz) and cardiac rhythms (0.8 - 1.2 Hz) which typically become aliased with more traditional sampling rates and which can confound interpretations of the neural contributions to the observed spectra. Our data show that multiple cortical and subcortical regions exhibit increased power at low frequencies (< 0.1 Hz) after total sleep deprivation, including arousal-related subcortical regions and cortical emotional processing areas. These results suggest that disruptions in affective processing observed after sleep deprivation may in part be due to sleep-like rhythms that interfere with the typical dynamics that support healthy affective processing under well-rested conditions.

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Poster

395. Integrative Physiology and Behavior: Sleep Systems II

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Program #/Poster #: 395.09

Topic: F.07. Biological Rhythms and Sleep

Support: NIH grant R01NS078168
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Title: Arousal state dependence of the cortical blood volume - pupil diameter relationship

Authors: *P. J. DREW[1], K. W. GHERES[2], K. L. TURNER[3];
[1]Pennsylvania State Univ., University Park, PA; [2]Huck Inst. of the Life Sci., Pennsylvania State Univ. Univ. Park, University Park, PA; [3]Dept. of Biomed. Engin., Penn State Univ., University Park, PA

Abstract: Arousal state profoundly affects neural activity and vascular dynamics in the cortex, with sleep causing large changes in local field potentials (LFP) and increases in blood flow. Here we investigate how arousal state dictates the relationship between non-invasive measures of arousal (pupil diameter and blink rate), with neural activity and blood volume in the somatosensory cortex. In both male and female head-fixed mice, pupil diameter was consistently smaller during REM and NREM sleep than in the awake state. Pupil diameter changes were coherent with both changes in blood volume and gamma-band power in the somatosensory cortex, but the strength and sign of this relationship varied with behavioral state. We observed a strong negative correlation between pupil diameter and both blood volume and gamma-band power during periods of rest and sleep, though the correlations between pupil diameter and blood volume became positive during periods of alertness and active whisking, consistent with fidgeting and other body motions driving vasodilation in the somatosensory cortex. Blinking was associated with increases in arousal, and large decreases in blood volume when the mouse blinked while asleep. Coherence of bilateral measures of gamma-band power and blood volume dropped following a blink, indicating a 'reset' of vascular and neural activity. Using only pupil diameter, we could determine if the mouse is awake or asleep with high accuracy with a temporal resolution of seconds. Our results demonstrate a very strong reciprocal relationship between pupil diameter and hemodynamics signals in unanesthetized mice, reflecting the pronounced effects of arousal on cerebrovascular dynamics.

Disclosures: P.J. Drew: None. **K.W. Gheres:** None. **K.L. Turner:** None.

Poster

395. Integrative Physiology and Behavior: Sleep Systems II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 395.10

Topic: F.07. Biological Rhythms and Sleep

Support: CFI JELF 37931

Title: Sleep Wake Dependent Organization of Claustrum Neurons Innervating the Retrosplenial Cortex

Authors: *B. A. MARRIOTT[1], A. DO[2], V. CATTAUD[2], J. C. JACKSON[2];
[1]Neurosci. and Mental Hlth. Inst., [2]Physiol., Univ. of Alberta, Edmonton, AB, Canada

Abstract: The claustrum is a small subcortical nucleus that has been recently implicated in sleep, but there has been conflicting data about when and how the claustrum is active across waking and sleep states in the mouse. The claustrum's thin, sheet-like shape and depth in the brain have stymied conventional techniques to observe claustrum activity *in-vivo*. To overcome these technical limitations, we have combined virally mediated, pathway specific expression of jGCaMP8m with *in-vivo* thin-skull two-photon imaging of claustrum axons during waking, slow wave sleep, and rapid eye movement sleep. With this methodology, we present a characterization of cell specific retrosplenial-cortex-projecting claustrum neuron activity through the sleep-wake cycle coupled with pupil recordings. Preliminary data suggests the CLA-RSP pathway is most active during slow wave sleep and quiet awake states. Additionally, a subset of axons exhibit activity correlated with periods of low slow wave power during microarousals. This data shows that this claustrocortical pathway is comprised of cells that exhibit a heterogenous relationship with sleep and wake states. Therefore, different subpopulations may have a differential role in shaping activity in the cortex in a brain state dependent manner.

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Poster

395. Integrative Physiology and Behavior: Sleep Systems II

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Program #/Poster #: 395.11

Topic: F.07. Biological Rhythms and Sleep

Support: NIH F31 NS118963-01A1

Title: Neuronal and homeostatic regulation of sleep by the preoptic area and tuberomammillary nucleus

Authors: *J. MAURER, S. CHUNG;
Neurosci., Univ. of Pennsylvania, Philadelphia, PA

Abstract: While sleep is evolutionarily conserved across all animals studied, the precise function of sleep remains unknown. It is vital that organisms receive an adequate amount of sleep, as sleep deprivation has profound physiological effects. The preoptic area (POA) of the hypothalamus contains sleep-active GABAergic neurons and activation of their axons

innervating the wake-active tuberomammillary nucleus histamine (TMN^{HIS}) neurons are critical for sleep regulation. However, it is not yet understood exactly how the activity of POA GABAergic axonal projections to the TMN (POA^{GABA}→TMN) changes in response to increased sleep need and whether they are necessary to integrate homeostatic pressure. Using fiber photometry in mice, we have found that TMN^{HIS} neurons are most active at wake onset, but as mice transition from wake to NREM sleep the activity gradually decreases and continues to decrease until they reach their lowest activity during REM sleep. Conversely, fiber photometry also revealed that POA^{GABA}→TMN neurons are sleep active, with most mice displaying the highest population activity during REM sleep. Following sleep deprivation, the sleep-active POA^{GABA}→TMN neurons display elevated activity during sleep rebound across all sleep states, suggesting an important role of these neurons in regulating sleep homeostasis. Using optogenetics, we found that inhibition of TMN^{HIS} neurons during sleep rebound produces a deeper quality of sleep, suggesting the inhibition of these neurons is a critical component of regulating sleep in response to sleep need. Future experiments aim to address whether inhibition of POA^{GABA}→TMN neurons is necessary for sleep rebound and how individual POA^{GABA}→TMN neurons respond to sleep loss. Together, these studies will identify novel circuit mechanisms by which the POA and TMN coordinate their activity during sleep/wake and periods of homeostatic sleep pressure.

Disclosures: **J. Maurer:** None. **S. Chung:** None.

Poster

395. Integrative Physiology and Behavior: Sleep Systems II

Location: SDCC Halls B-H

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Topic: F.07. Biological Rhythms and Sleep

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Title: GABAergic neurons in ventral midbrain/pons are involved in mania-like behaviors with altered sleep homeostasis and sleep architecture

Authors: *T. HONDA[1,2], Y. TAKATA[2], Y. CHERASSE[2], S. MIZUNO[3], F. SUGIYAMA[3], S. TAKAHASHI[2,3], H. FUNATO[2,4], M. YANAGISAWA[2,5], M. LAZARUS[2], Y. OISHI[2];
[1]Picower Inst. for Learning and Memory, Dept. of Brain and Cognitive Sci., Massachusetts Inst.

of Technol. (MIT), Cambridge, MA; [2]Intl. Inst. for Integrative Sleep Med. (WPI-IIIS), Univ. of Tsukuba, Tsukuba, Japan; [3]Lab. Animal Resource Ctr. and Trans-border Med. Res. Ctr., Univ. of Tsukuba, Tsukuba, Japan; [4]Toho Univ. Sch. of Med., Tokyo, Japan; [5]Dept. of Mol. Genet., Univ. of Texas Southwestern (UTSW) Med. Ctr., Dallas, TX

Abstract: In sleep biology, the neural mechanism of sleep homeostasis, which is known as the existence of rebound sleep after sleep deprivation, remains as a core unrevealed question. In sleep medicine, sleep problems are highly associated with neuropsychiatric disorders such as bipolar disorder, characterized by periods of depression and mania. Patients with mania persistently exhibit hyperactivity, elevated mood, and decreased need for sleep. However, brain areas and neuronal populations involved in mania remain elusive. In this study, we selectively ablated GABAergic neurons in ventral medial midbrain/pons (VMP) by Cre-dependent viral expression of diphtheria toxin subunit A (AAV-FLEX-DTA) in VMP of VGAT-Cre mice (VGAT-Cre$^{DTA/VMP}$). For controls, we prepared the mice expressing humanized *Renilla reniformis*-derived GFP (AAV-FLEX-hrGFP) in VMP GABAergic neurons (VGAT-Cre$^{hrGFP/VMP}$). For these mice, we performed the comprehensive behavioral test battery and electroencephalography (EEG)/electromyography (EMG)-based sleep/wake analysis. To investigate the involvement of the dopamine system, we newly generated dopamine D_2 receptors knockout mice in VGAT-Cre background by CRISPR/Cas9 system (VGAT-Cre;$D_2R^{-/-}$). We found that ablation of VMP GABAergic neurons induced mania-like behaviors in mice, including hyperactivity, anti-depressive behaviors, reduced anxiety, increased risk-taking behaviors, distractibility, and an extremely shortened sleep time. Strikingly, these mice also exhibited no rebound sleep after sleep deprivation, suggesting abnormal sleep homeostatic regulation. Furthermore, dopamine D_2 receptor deficiency largely abolished the sleep reduction induced by ablating the VMP GABAergic neurons without affecting the hyperactivity and anti-depressive behaviors. In summary, our data demonstrate that VMP GABAergic neurons are involved in the expression of mania-like behaviors, which can be segregated to the short-sleep and other phenotypes on the basis of the dopamine D_2 receptors. In this conference, we also discuss our recent pharmacological studies using VGAT-Cre$^{DTA/VMP}$ mice. Overall, our study may provide novel insights into the pathophysiology of bipolar disorder and the mechanism of sleep homeostasis.

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Poster

395. Integrative Physiology and Behavior: Sleep Systems II

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Program #/Poster #: 395.13

Title: WITHDRAWN

Poster

395. Integrative Physiology and Behavior: Sleep Systems II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 395.14

Topic: F.07. Biological Rhythms and Sleep

Support: NIH Grant R01AG054081

Title: Automatic segmentation of sleep spindles: a variational switching state-space approach

Authors: *M. HE[1], P. DAS[2], G. C. HOTAN[4], P. L. PURDON[3];
[1]MIT, MIT, Cambridge, MA; [2]MGH Charlstown Navy Yard, Massachusetts Gen. Hosp., Charlestown, MA; [3]Massachusetts Gen. Hosp., Somerville, MA; [4]Inst. of High Performance Computing, Singapore, Singapore

Abstract: Sleep spindles are bursts of oscillatory activity in the 12-16 Hz frequency range, lasting ~0.5-3 s, and occurring during non-rapid eye movement (NREM) sleep. On electroencephalograms (EEG), spindles appear as discrete events with distinctive waxing and waning waveforms. Spindles are traditionally marked via visual inspection; however, recent findings on their integral roles in normal sleep physiology, memory consolidation, and neurodegenerative diseases have spurred a growing interest in automatic detection of spindles. Existing detection algorithms typically check if the momentary power within a predefined frequency range exceeds a heuristically set threshold. Such ad-hoc procedures are biased to select only the strongest spindles and fail to characterize temporal dynamics of spindle waveforms adequately. In this work, we introduce a generative model for sleep spindles based on parametric switching state-space models to explicitly capture the oscillatory and transient nature of the underlying process. To this end, we model sleep EEG during NREM sleep as a superposition of two state-space oscillation models, representing slow oscillations and spindles respectively. We then employ a hidden Markov process that determines whether a spindle is present at a given point in time, thus modeling the transientness of spindles. We use an instance of generalized Expectation-Maximization algorithm under a structured variational approximation to learn the model parameters and infer the posterior distributions of hidden oscillation states and discrete switching state probabilities in an unsupervised manner. The discrete switching state probabilities provide a probabilistic partition of sleep EEG into spindle segments with full statistical characterization. Additionally, the hidden oscillation states extract the temporal evolution of spindles and slow waves in terms of instantaneous amplitude and phase. We analyzed NREM sleep EEG recorded from a healthy young adult: our method correctly identified spindles even when the spindle activity was weak. Compared to existing thresholding methods, our data-driven algorithm is robust to outliers and noise of similar strength as spindles, because the state-space models can adjust to the temporal variations of spindles and slow waves over time. In summary, our work provides a novel framework to reliably detect the presence of spindles and allows inferences on dynamic features of the underlying sleep oscillations. These advances pave ways for future clinical and research studies of sleep spindles, such as in assessing sleep quality or in developing biomarkers of psychiatric and neurodegenerative diseases.

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Poster

395. Integrative Physiology and Behavior: Sleep Systems II

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Program #/Poster #: 395.15

Topic: F.07. Biological Rhythms and Sleep

Support: NIH - NINDS Grant 1UH3NS113769-01A1

Title: Sleep-stage specific spectral dynamics of subthalamic nucleus local field potentials identify distinct clusters of Parkinson's disease patients

Authors: *E. M. RADCLIFFE #[1,2], K. SALTOUN #[5], E. CHRISTENSEN[3], A. ABOSCH[6], J. ZYLBERBERG[7], J. A. THOMPSON[2,4,1];
[1]Dept. of Bioengineering, Univ. of Colorado Denver | Anschutz Med. Campus, Aurora, CO; [2]Dept. of Neurosurg., [3]Dept. of Physiol. and Biophysics, [4]Dept. of Neurol., Univ. of Colorado Sch. of Med., Aurora, CO; [5]Neurosci., McGill Univ., Montreal, QC, Canada; [6]Dept. of Neurosurg., Univ. of Nebraska Med. Ctr., Omaha, NE; [7]Dept. or Physics and Astronomy, York Univ., Toronto, ON, Canada

Abstract: Sleep is essential for regulating physiological and cognitive function. Dysregulated sleep can severely degrade quality of life, is highly comorbid with Parkinson's disease (PD), and may be an early indicator of PD. Subthalamic nucleus (STN) deep brain stimulation (DBS) effectively treats PD motor symptoms and may attenuate sleep dysfunction. To account for fluctuations in symptoms and clinical states, closed loop adaptive DBS (aDBS) aims to optimize therapeutic benefit for individuals with neurological conditions by adjusting neurostimulation parameters in response to concurrently recorded, patient-specific brain activity. A necessary criterion in the development of aDBS systems is the ability to accurately correlate brain-derived signals to specific physiological and pathological states. Prior work by co-authors evaluated local field potentials (LFPs) recorded from STN-DBS electrodes in PD patients (n=10) and discovered diverse sleep-state specific power spectral distributions across subjects with consistent power spectral patterns correlated with distinct sleep states. A feedforward neural network model developed to classify sleep stage based on STN LFP power obtained good accuracy across all subjects. However, this model performed poorly for specific subsets of subjects and sleep states. One hypothesis is that this patient- and sleep-state specific reduction in model accuracy is due to the extreme heterogeneity in sleep structure between subjects. We assessed whether subsets of PD patients exhibit shared spectral characteristics in their sleep-state specific STN LFP power spectral distributions to identify patterns that might further inform predictive sleep state classification algorithms. Consistent with previous studies, we computed each patient's mean power per canonical frequency band per 30-second LFP epoch from each bipolar contact configuration across a full night of sleep (mean 7.5 hours). Using a series of tailored dimensionality reduction and machine learning-driven clustering methods, we then investigated

sleep-state specific variance within the mean LFP power spectral data across patients. Our results reveal heterogeneous relationships between STN LFP power spectrum dynamics and sleep state montages and further delineate distinct patient profile groupings within the PD patient dataset. Characterization of sleep-state specific power spectral montages may inform the development of more precise sleep state classification models that can be translated into biomarker-driven aDBS control systems to treat sleep dysfunction for diverse PD patient profiles.

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Poster

395. Integrative Physiology and Behavior: Sleep Systems II

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Program #/Poster #: 395.16

Topic: F.07. Biological Rhythms and Sleep

Support: NIH R01NS118442

Title: Sleep restores an optimal computational regime in the plastic cortical network

Authors: *Y. XU[1], K. B. HENGEN[2];
[1]Biol., Washington Univ. in St. Louis, Saint Louis, MO; [2]Biol., Washington Univ. In St. Louis, Saint Louis, MO

Abstract: Sleep is believed to play a vital role in promoting stable brain function, but how sleep contributes to robust and efficient neural computation at the network level remains unknown. We recently demonstrated that cortical dynamics are homeostatically organized around criticality, a computational regime that optimizes information processing, such as information capacity and dynamic range. Our modeling also revealed that Hebbian plasticity disrupts the critical regime, which raises the question of how diverse circuits maintain this optimal regime in the face of daily experience. To address the hypothesis that the restoration of criticality is a central feature of why brains sleep, we implanted micro-electrode arrays into the primary visual cortex of freely-behaving rats and tracked the activity of ensembles of single units for >10 d. We examined critical dynamics as a function of brain state, behavior, and environment. Our data revealed that in the context of unperturbed free behavior, time spent awake has a positive correlation with the network's distance to criticality, and time spent asleep serves to counteract this effect. Sleep deprivation causally disrupted critical dynamics. These results demonstrated that sleep functions to homeostatically restore the critical regime, which is progressively undermined during waking experience. Powerfully, the extent to which neural dynamics deviate from criticality predicts both the amount of future sleep as well as prior sleep/wake history, further suggesting that maintenance of criticality is a core purpose of sleep. Our results establish a theory-driven model describing how sleep and wake modify the computational regime of the neural networks and offer a novel, systems-level explanation of the cognitive benefit of sleep.

Disclosures: **Y. Xu:** None. **K.B. Hengen:** None.

Poster

395. Integrative Physiology and Behavior: Sleep Systems II

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Topic: F.07. Biological Rhythms and Sleep

Support: NIH MH117155

Title: Spindle and slow-oscillation properties in a large-scale thalamocortical network model of the human brain.

Authors: Y. SOKOLOV[1], B. Q. ROSEN[2], E. DELANOIS[3], O. C. GONZALEZ[5], *G. KRISHNAN[1], E. HALGREN[4], M. V. BAZHENOV[6];
[1]Dept. of Med., [2]Neurosci. Grad. Program, [3]Dept. of Computer Sci. and Engin., [4]Neurosci. Grad. Program, Departments of Radiology and Neurosci., UC San Diego, San Diego, CA; [5]Dept. of Psychiatry and Behavioral Sci., Stanford Univ., Stanford University, CA; [6]Dept. of Medicine, Neurosci. Grad. Program, Univ. of California San Diego, La Jolla, CA

Abstract: Spindles and slow-oscillations are characteristic of stage N2 and N3 of non-rapid-eye-movement (NREM) sleep, respectively. However, how cortical connectivity may affect these activity states during NREM is not yet fully understood. To address this issue, we developed a large-scale thalamocortical network model with a realistic cortical connectivity that is able to transit from N2 to N3 of NREM. The base model has ~9500 cortical 'modules' equally positioned across the surface of one hemisphere. Each module has pyramidal and inhibitory map neurons arrayed in 6 layers. Connections within the module follow those found in multipatch recordings. The thalamus is represented by ~640 Hodgkin-Huxley modules, each with a matrix and core element comprised of a thalamo-cortical and reticular neuron. The network has a cortical connectivity based on DTI tractography between Human Connectome Project parcels, originating and terminating in layers according to the relative hierarchical positions of the parcels. To investigate the role of long-range projections on activity patterns, we compared this base model to uniform and localized cortical connectivity. We found that a small density of distance-dependent long-range random cortical connections is enough to synchronize the cortical up-states of slow-oscillations. We also showed that the pruning of feed-forward or feed-back connections reduces cortical spindle oscillations. We analyzed the duration and frequency of cortical spindles in different regions of the cortex when the laminar organization due to hierarchical organization was varied, and demonstrated that increased laminar organization increases the duration and frequency of cortical spindles compared to removing the hierarchical organization. These findings suggest predominantly local connectivity is sufficient for generating local slow waves while long-range connections are necessary for broader synchrony. It further suggests that while spindles are generated by thalamic oscillators, sleep spindling is modulated by the large-scale cortical hierarchy.

Disclosures: **Y. Sokolov:** None. **B.Q. Rosen:** None. **E. Delanois:** None. **O.C. Gonzalez:** None. **G. Krishnan:** None. **E. Halgren:** None. **M.V. Bazhenov:** None.

Poster

395. Integrative Physiology and Behavior: Sleep Systems II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 395.18

Topic: F.07. Biological Rhythms and Sleep

Support: NHLBI R01HL149133

Title: Neural population dynamics in midbrain and pons during sleep

Authors: *D. LOZANO, J. STUCYNSKI, F. WEBER;
Univ. of Pennsylvania, Philadelphia, PA

Abstract: Rapid eye movement (REM) sleep is characterized by a desynchronized electroencephalogram (EEG), muscle atonia, and vivid dreaming. The core circuits generating REM sleep are distributed throughout the brainstem and are composed of REM sleep-promoting (REM-on) and REM sleep-suppressing (REM-off) neurons. While much progress has been made in identifying specific neuronal populations that promote or suppress REM sleep, the neural dynamics that arise from the interactions between these REM regulatory populations and the dynamical mechanisms which gate transitions into REM sleep are largely unknown. We have previously demonstrated using optogenetic manipulations in mice that GABAergic neurons in the dorsomedial medulla (dmM) promote the induction of REM sleep via their projections to the dorsal and median raphe nuclei. To identify the neural dynamics within these downstream areas underlying NREM-to-REM sleep transitions, we employed Neuropixels probes to record the population activity in the dorsal and medial raphe, as well as neighboring midbrain and pontine brain areas, during spontaneous sleep in mice. We applied principal component analysis (PCA) to describe the population dynamics across the recorded brain regions. We found that the population activity during NREM and REM sleep is captured within low-dimensional subspaces. Furthermore we found that wakefulness, NREM, and REM sleep correspond to distinct areas along the trajectories traversed by the population activity within the PCA space. The REM sleep-promoting effect of dmM stimulation largely resulted from an activation of REM-on and inactivation of REM-off neurons, effectively forcing the population activity towards REM sleep. Overall, these results demonstrate that the population activity during sleep in midbrain and pons areas are low-dimensional and provide a geometric description of how inputs from the REM-promoting dmM neurons affect the population dynamics within these areas to induce NREM-to-REM sleep transitions.

Disclosures: **D. Lozano:** None. **J. Stucynski:** None. **F. Weber:** None.

Poster

395. Integrative Physiology and Behavior: Sleep Systems II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 395.19

Topic: F.07. Biological Rhythms and Sleep

Support: NIH R35 GM127102
NIH F31 GM140592

Title: Drosophila phototransduction occurs in arousal/circadian neurons via color-specific external and internal photoreceptors

Authors: *D. AU, J. LIU, S. PARK, A. FODEN, T. HOLMES;
Univ. of California, Irvine, Irvine, CA

Abstract: Cryptochrome (CRY) is classically associated with its role in regulating the circadian molecular clock via light-induced degradation of the Timeless (TIM) clock protein in flies. Recent discoveries in our lab have identified additional processes occurring as a result of CRY phototransduction, such as light-evoked excitation of the ventral lateral subset in the circadian/arousal neural circuit. CRY is primarily a short-wavelength photoreceptor, having peak absorption around blue (450 nm) and UV (365 nm), however, light-evoked excitation using red light (635 nm) also elicits an acute response in the ventral lateral neurons. There has been longstanding anatomical evidence that rhodopsin-expressing photoreceptors from the eyes, including red light sensitive rhodopsin 6, project to a central hub in the circadian/arousal circuit; the accessory medulla, and subsequently to ventral lateral neurons. We explored the photoelectrical input contributions of these external photoreceptor systems, as well as internally expressed rhodopsin 7 (Rh7) and CRY to determine their functional convergence on the large ventral lateral neurons (l-LNvs) in flies. Patch-clamp electrophysiology was performed on l-LNvs using UV (365 nm), violet (405 nm), blue (450 nm), and red (635 nm) LED light stimulation. Fly groups that were tested consisted of externally blind gl60j, cry^{01}, $rh7^{1}$, and double mutants gl60j-cry^{01}, and $rh7^{1}$-cry^{01}. Our electrophysiology experiments show CRY and Rh7 contribute the greatest light-excitatory effect with blue and UV light. Rh7 is the predominant sensor for violet light-induced electrical excitability. Gl60j flies show the greatest attenuation of red light-induced electrical excitability in l-LNvs. Further, these neurons are critical for light-arousal and phototaxis responses. We employed a light-pulse arousal assay to measure the awakening arousal response of flies during subjective nighttime. Our results show that at lower intensities of blue light (10 µW/cm²) gl60j and $rh7^{1}$ mutant flies have the greatest loss of light-pulse arousal, whereas at high intensities (400 µW/cm²) cry^{01} and $rh7^{1}$ mutant flies exhibit the greatest phenotype. These results extend to violet light as well, with gl60j mutant flies exhibiting the greatest loss of light arousal for lower intensities, and $rh7^{1}$ having the greatest loss at higher intensities. Interestingly, gl60j mutant flies exhibit the greatest loss of arousal for both higher and lower intensities for red light pulses. These results suggest different photoreceptor systems converge input to the ventral lateral neurons and provide UV to red sensation in flies for non-image forming processes.

Disclosures: **D. Au:** None. **J. Liu:** None. **S. Park:** None. **A. Foden:** None. **T. Holmes:** None.

Poster

395. Integrative Physiology and Behavior: Sleep Systems II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 395.20

Topic: F.07. Biological Rhythms and Sleep

Support: NIH R01 GM109086
NIH R01 AG063849

Title: Intracranial electrophysiological signatures of delirium in neurosurgical patients

Authors: ***B. D. HAYUM**[1], B. M. KRAUSE[2], R. D. SANDERS[3], K. V. NOURSKI[4], M. I. BANKS[5];
[1]Banks Lab., Madison, WI; [2]Anesthesiol., Univ. of Wisconsin - Madison, Madison, WI; [3]Anaesthetics, Univ. of Sydney, Sydney, Australia; [4]The Univ. of Iowa, Iowa City, IA; [5]Dept. of Anesthesiol., Univ. of Wisconsin, Madison, WI

Abstract: Introduction: Post-operative delirium (POD) and post-ictal delirium in epilepsy (PID) are acute disorders of consciousness characterized by confusion, fluctuating arousal, impaired executive function, and perceptual disturbances. The mechanisms underlying these disorders and the degree to which these mechanisms overlap between POD and PID are unknown. Epilepsy patients undergoing intracranial electroencephalographic (iEEG) monitoring prior to surgery for resection of seizure foci can experience both POD and PID, and thus are suitable subjects for mechanistic investigations. **Methods:** iEEG recordings were obtained from adult neurosurgical patients (n = 19) implanted with electrodes in temporal, parietal, and frontal cortex to identify epileptic foci. POD was assessed daily or twice daily using the 3-Minute Diagnostic interview for Confusion Assessment Method (3D-CAM) or Confusion Assessment Method for the Intensive Care Unit (CAM-ICU) beginning 24 hours after surgery. PID was assessed immediately following seizures. Resting state data (~10 mins) were collected [1] following POD assessments at two time points: Early (40–70 hrs after surgery) and Late (140–320 hrs after surgery), [2] following PID assessments, and [3] at a third time point >4 hrs after a seizure to serve as control. Electrodes were rejected if they corresponded to seizure foci, were excessively noisy, or located in white matter or outside the brain. Power within canonical frequency bands was averaged across electrodes in each region of interest, and compared between different time points and according to delirium status. **Results:** Four of 12 patients exhibited POD (all <70 hrs post-op). Early delta (1-4 Hz) power was elevated compared to Late in 7/8 POD- patients and in 3/4 POD+ patients, with similar elevation in POD+ and POD- patients. Post-ictal delta power was elevated compared to control in 5/7 PID+ patients and 1/10 PID- patients. In all 4 patients for whom both PID+ and PID- data were collected, post-ictal elevation of delta power was greater for PID+ vs. PID-. Changes in delta power were not region-specific. Results for theta (4-8 Hz) power largely aligned with those for delta, except that theta

was elevated in only 2/7 PID+ patients. No consistent changes were observed in higher frequency bands.**Conclusions:** Delta and theta power were elevated post-operatively regardless of delirium status, suggesting non-specific effects related to recovery from the neurosurgical procedure. Post-ictal elevation of delta power was inconsistently observed and for theta power was rarely observed. Results suggest that elevation of delta power is a signature of PID.

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Poster

395. Integrative Physiology and Behavior: Sleep Systems II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 395.21

Topic: F.07. Biological Rhythms and Sleep

Support: NIH Grant R01-GM109086
NIH Grant R01-DC04290
NIH Grant S10OD025025
NIH Grant UL1-RR024979

Title: Altered functional geometry of human cortical networks during states of reduced consciousness

Authors: B. M. KRAUSE[1], D. I. CAMPBELL[2], C. K. KOVACH[3], R. N. MUELLER[3], H. KAWASAKI[4], K. V. NOURSKI[3], ***M. I. BANKS**[5];
[1]Anesthesiol., Univ. of Wisconsin - Madison, Madison, WI; [2]Univ. of Wisconsin -- Madison, Madison, WI; [3]The Univ. of Iowa, Iowa City, IA; [4]Dept Neurosurg., Univ. Iowa Hosp Clin., Iowa City, IA; [5]Univ. of Wisconsin, Madison, WI

Abstract: Introduction: Changes in the brain underlying transitions into and out of unconsciousness are postulated to be conserved no matter whether they occur during anesthesia, sleep, or other circumstances. However, identifying these common neural signatures has proven elusive. We applied diffusion map embedding (DME) to human electrophysiology data to elucidate those signatures. DME maps data into a space in which proximity represents similarity in connectivity to the sampled network. We used DME to explore changes in the functional geometry of human cortical networks during anesthesia and sleep found striking commonalities. **Methods:** Resting state intracranial electroencephalographic (iEEG) recordings were obtained during induction of propofol anesthesia or during sleep from adult neurosurgical patients implanted with electrodes (propofol: n = 15; sleep: n = 14) in temporal, parietal, and frontal cortex to identify epileptic foci. Awake states were compared with two stages of anesthesia (sedated/responsive and unresponsive) and four stages of sleep (N1, N2, N3, and REM). .

Channel-by-channel connectivity matrices were computed as pairwise gamma (30-70Hz) orthogonalized power envelope correlations. Connectivity matrices were thresholded and normalized by degree to yield the symmetric diffusion matrix $\mathbf{P_{symm}}$, which was analyzed using DME. We assigned nodes in embedding space to clusters, corresponding to distinct functional derived from a complementary dataset (Banks et al., bioRxiv 2022.02.06.479292). Distance within cluster (i.e. cluster quality, Calinski-Harabasz index) is a measure of local differentiation; inter-cluster proximity is a measure of functional integration. **Results:** Transitions into unresponsiveness during propofol anesthesia and into N2 and N3 during sleep were associated with increased cluster quality (likelihood ratio test for omitting state: propofol $\chi^2(2) = 37.7$, $p < 0.0001$; sleep $\chi^2(4) = 68.1$, $p < 0.0001$), and increased inter-cluster distance (likelihood ratio test for omitting state: propofol $\chi^2(2) = 26.7$, $p < 0.0001$; sleep $\chi^2(4) = 34.9$, $p < 0.0001$). Observed changes were not region-specific and were robust to choice of threshold (10%, ~33%, retain all positive correlations).**Conclusions:** Stages of reduced consciousness are associated with decreases in differentiation and functional integration throughout the brain, identifying a global network reorganization common to changes in consciousness during sleep and anesthesia. This framework for understanding loss and recovery of consciousness lays the foundation for evaluation of cortical state transitions in clinical settings.

Disclosures: **B.M. Krause:** None. **D.I. Campbell:** None. **C.K. Kovach:** None. **R.N. Mueller:** None. **H. Kawasaki:** None. **K.V. Nourski:** None. **M.I. Banks:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); VCENNA, Inc.. F. Consulting Fees (e.g., advisory boards); VCENNA, Inc..

Poster

395. Integrative Physiology and Behavior: Sleep Systems II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 395.22

Topic: F.08. Food and Water Intake and Energy Balance

Support: NIH DK092246
NIH AT011653
NIH DK020541

Title: Role of parasympathetic cholinergic innervation on hepatic glucose and lipid metabolism

Authors: *Y.-H. JO, J. HWANG;
Albert Einstein college of medicine, Albert Einstein college of medicine, bronx, NY

Abstract: The role of the autonomic nervous system - and the parasympathetic nervous system in particular - on hepatic glucose and lipid metabolism has been the focus of substantial debate and controversy. The anatomy and function of the parasympathetic cholinergic innervation of the liver still remains poorly characterized. Hence, we aim to define the role of this cholinergic innervation of the liver in controlling glucose and lipid metabolism.The dorsal motor nucleus of

the vagus (DMV) contains parasympathetic cholinergic neurons. Although there is a contradictory finding of the lack of cholinergic innervation to the mouse liver, prior studies with the retrograde neuronal tracers such as cholera toxin B, pseudorabies virus, and AAV encoding a Cre-inducible reporter protein strongly support that the mouse liver receives DMV cholinergic innervation. Furthermore, we recently show that hepatocytes receive direct DMV cholinergic input and express muscarinic acetylcholine receptors, suggesting that the hepatic cholinergic system is critical for proper liver function.In this study, we further investigate the role of parasympathetic cholinergic neurons in controlling energy balance, glucose homeostasis, and insulin sensitivity in C57BL/6J mice fed a high-fat diet for 10 weeks. We selectively ablate liver-projecting cholinergic neurons by retrogradely expressing diphtheria toxin subunit A (dTA) in a Cre recombinase-dependent manner. There is no difference in body weight between the two groups (males, control, n=9 mice, dTA, n=10 mice; females, control, n=6 mice, dTA, n=7 mice). Basal (non-fasting), but not fasting, glucose levels are significantly lower in males receiving retrograde AAV-mCherry-FLEX-dtA injection than in controls. While glucose tolerance tests show no significant difference in blood glucose levels in response to a bolus of glucose (i.p. 2 g/kg) between the groups, blood glucose levels during the insulin tolerance tests (i.p. 1 U/kg) are significantly lower in mice injected with retroAAV-FLEX-dtA than in controls. Interestingly, mice without liver-projecting cholinergic neurons exhibit reduced fat accumulation and triglycerides (TG) in the liver than controls. Hence, our study suggest that liver-projecting cholinergic neurons play a role for hepatic glucose and lipid metabolism.

Disclosures: **Y. Jo:** None. **J. Hwang:** None.

Poster

396. Cortical and Basal Ganglia Circuits in Aversive Processing

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 396.01

Topic: G.04. Emotion

Support: R01 DA052108
T32 DA007244

Title: Coordinated neural activity in the PrL and the NAc core does not track shifts in hedonic processing during conditioned taste aversion and its extinction

Authors: *P. L. RODRIGUEZ-ECHEMENDIA, R. M. CARELLI;
Univ. of North Carolina At Chapel Hill, Chapel Hill, NC

Abstract: Dysfunctional hedonic processing is associated with numerous psychiatric illnesses including depression and substance use disorders. Conditioned taste aversion (CTA) is a preclinical behavioral model used to investigate shifts in hedonic processing; here taste reactivity (TR) is used to track the affective properties of appetitive and aversive tastants before and after the induction of a CTA. Since various studies have linked the infralimbic cortex (IL) and its

projections to the nucleus accumbens (NAc) shell in hedonic processing, electrophysiological (local field potential, LFP) recording methods have been instrumental in examining its role in CTA and its extinction. We previously revealed that oscillatory signaling dynamics in the IL and NAc shell track shifts in affect in the naïve state, during CTA, and its extinction. Because the prelimbic cortex (PrL) and NAc core circuit has been linked to extinction-related behaviors, the goal of the present study was to determine if this circuit also plays a role in CTA and its extinction. Adult, male Sprague Dawley rats (n=6) received 30 intraoral (IO) 0.15% saccharin infusions (3.5s/inf, VT30s schedule) immediately followed by an IP injection of LiCl (127 mg/kg) on the naïve day. After recovery, rats were tested for CTA with IO saccharin infusions followed by four additional extinction days. Next, rats were given 30 IO bitter quinine infusions to examine neural responses to an innate aversive tastant. TR and LFP in the PrL-NAc core were recorded during a baseline 15-min period before each session and during all IO infusions. Behavioral results indicate that CTA elicited a shift from positive to negative TR and that positive TR was recovered within four days of extinction. However, preliminary analysis of LFP data suggests that the PrL-NAc core circuit does not track any aspect of CTA or its extinction. Ongoing studies are increasing the number of male rats and investigating female rats in the same study design to determine if sex differences are evident. Collectively, these data will provide an understanding of how real-time temporal dynamics of coordinated neural activity unfold in the PrL and NAc core during positive circumstances, when hedonic states shift to negative conditions, and when they are restored in extinction.

Disclosures: P.L. Rodriguez-Echemendia: None. **R.M. Carelli:** None.

Poster

396. Cortical and Basal Ganglia Circuits in Aversive Processing

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 396.02

Topic: G.04. Emotion

Support: NRF-2021R1I1A1A01049318
NRF-2017R1A2B4007288

Title: Dynamic changes in balance of basal ganglia circuitry control stress-related behaviors in mice

Authors: *Y. LEE[1,2], N.-E. HAN[2], S. SOHN[2], S.-Y. CHOI[1], B.-J. YOON[2];
[1]Seoul Natl. Univesity, Seoul Natl. Univ. Sch. of Dent., Seoul, Korea, Republic of; [2]Life Sci., Korea Univ., Seoul, Korea, Republic of

Abstract: The basal ganglia network has been implicated in the control of adaptive behavior, possibly by integrating motor learning and motivational processes. While the reward-related aspects of motivational control through which action selection and execution occur have been studied extensively, whether negative emotions can also influence adaptive behavior by

modulating the basal ganglia network has not been examined. Here, we show that the direct and indirect pathways of the basal ganglia within the dorsomedial region control defensive coping behaviors after predator stress exposure. We specifically activated each basal ganglia pathway using chemogenetics in the dorsomedial striatum of mice and found that the direct and indirect pathways promote active and passive coping behaviors, respectively. We also examined a transgenic mouse line (G2CT) in which synaptic transmissions onto the medium spiny neurons (MSNs) are depressed and found that the level of collaterals from direct pathway MSNs in the external segment of the globus pallidus (GPe) ('bridging collaterals') was decreased in these mice, and this was accompanied by passive coping behavior. Furthermore, additional manipulations that could further decrease or restore the level of the bridging collaterals increased passive or active coping behavior in the G2CT mice, respectively. We demonstrate that the bridging collaterals preferentially target the arkypallidal neurons in the GPe. Collectively, our data indicate that the dorsomedial region of the basal ganglia network integrates negative emotions and controls appropriate coping responses in which the bridging collateral connections to the arkypallidal neurons in the GPe play a critical regulatory role.

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Poster

396. Cortical and Basal Ganglia Circuits in Aversive Processing

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 396.03

Topic: G.04. Emotion

Title: Anatomical and functional characterization of amygdala-striatal circuits

Authors: *A. BRAINE[1,2], A. TOKARSKA[3], L. BONAMY[1,2], C. HERRY[4], G. SILBERBERG[3], J. M. BAUFRETON[1,2], F. GEORGES[1,2];
[1]Inst. of Neurodegenerative Dis., Univ. of Bordeaux, Bordeaux, France; [2]CNRS, UMR 5293, Bordeaux, France; [3]Karolinska Institutet, Karolinska Institutet, Stockholm, Sweden; [4]INSERM, Neurocentre Magendie, U1215, Bordeaux, France

Abstract: In humans and animals, changes in emotional states are known to modify posture, fine motor control, and/or coordination, inducing either beneficial or detrimental effects on motor performance. This suggests an overlap between neural circuits underlying emotions (limbic system) and motor control (basal ganglia). We thus performed an extensive review of the anatomical limbic-to-basal ganglia direct connections and chose to focus on the basolateral amygdala (BLA) to dorsal striatum (DS) projections in mice. The DS is the gate of entry of the basal ganglia and is involved in action selection and movement. The BLA is a key limbic structure involved in emotional processing such as valence-related signals, fear- and anxiety-related behavior. We hypothesize that BLA-DS projecting neurons can modulate motor behavior during emotional states. Using anatomical tracing tools, we show that the BLA topographically project to the DS, with the densest projections to the dorsomedial striatum (DMS) and further

characterized the *in vivo* and *ex-vivo* electrophysiological properties of DMS-projecting BLA neurons. These excitatory projections preferentially target medium-spiny neurons and parvalbumin interneurons. To define this neuronal sub-population, we mapped the inputs and outputs of the DMS-BLA neurons and determined the sources of their neuromodulators. Calcium imaging *in vivo* in anesthetized mice further confirms the functional connectivity of the main inputs to BLA-DMS neurons and shows that they respond to different sensory challenges (footshocks and airpuffs). Our study shows that the BLA-DMS pathway connects limbic structures to the basal ganglia and can also relay sensory, cognitive and interoceptive information to the motor system.

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Poster

396. Cortical and Basal Ganglia Circuits in Aversive Processing

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 396.04

Topic: G.06. Anxiety Disorders

Support: NIH Grant 068283

Title: Estrous cycle modulation of fear extinction and relapse: Role of a substantia nigra-to-dorsolateral striatum pathway

Authors: *A. A. HOHORST[1], M. K. TANNER[2], E. C. LOETZ[3], B. N. GREENWOOD[4]; [1]Integrative Biol., [3]Psychology, [2]Univ. of Colorado Denver, Denver, CO; [4]Psychology, Univ. of Colorado Denver, DENVER, CO

Abstract: The impaired inhibition of learned fear is a feature of stress-related psychiatric disorders such as depression, generalized anxiety disorder and post-traumatic stress disorder (PTSD). Extinction-based exposure therapy is an effective treatment strategy for these disorders but has limited long-term efficacy due to the vulnerability of fear memories to relapse. Additionally, women are up to 60% more likely to experience anxiety disorders and up to twice as likely to experience PTSD compared to men, however, these sex differences are not fully considered in the field of neuroscience. Prior data suggests that learning extinction during estrous phases characterized by high levels of ovarian hormones (proestrus and estrus, Pro/Est) enhances extinction memory and reduces relapse in females. However, how relapse levels in females exposed to extinction during Pro/Est compares to males, and mechanisms underlying gonadal hormone-modulation of extinction and relapse, are unknown. Increasing dopamine (DA) signaling in the striatum can enhance extinction memory, and females have higher stimulus-evoked striatal DA release than males and during Pro/Est. DA neurons originating in the substantia nigra (SN) and terminating in the dorsolateral striatum (DLS) contribute to stimulus-response ("habit") learning, which can be resistant to the memory-disrupting phenomena thought

to contribute to relapse. This study aims to characterize the effects of various estrous phases and sex during extinction on later extinction memory and relapse, and to determine the role of the DA^{SN-DLS} circuit in mediating the effects of Pro/Est. Females exposed to fear extinction training during Pro/Est were protected from fear relapse compared to both males and females exposed to fear extinction during the other estrous phases. Chemogenetic inhibition of the DA^{SN-DLS} circuit during extinction had no effect on extinction acquisition, but restored relapse in females that learned extinction during Pro/Est. These data suggest ovarian hormones interact with the DA^{SN-DLS} circuit to render fear extinction memory resistant to relapse.

Disclosures: A.A. Hohorst: None. **M.K. Tanner:** None. **E.C. Loetz:** None. **B.N. Greenwood:** None.

Poster

396. Cortical and Basal Ganglia Circuits in Aversive Processing

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 396.05

Topic: G.06. Anxiety Disorders

Support: NIH Grant 068283

Title: Stimulation of the substantia nigra to dorsal lateral striatum circuit during fear extinction reduces fear relapse

Authors: *R. HAN[1], J. WISEMAN[2], E. C. LOETZ[3], E. B. OLESON[3], B. N. GREENWOOD[4]; [1]Univ. of Colorado Denver, Denver, CO; [2]Univ. of Colorado Denver, Aurora, CO; [3]Psychology, Univ. of Colorado Denver, Denver, CO; [4]Psychology, Univ. of Colorado Denver, DENVER, CO

Abstract: Exposure therapy targeting fear extinction is often used to treat anxiety and post-traumatic stress disorders, but the long-term efficacy of exposure therapy is limited due to relapse phenomena such as fear renewal. Fear renewal occurs when fear responses return in contexts different from where extinction was learned. Substantia nigra (SN) dopamine (DA) activation during fear extinction promotes fear extinction and protects against fear renewal, but the specific postsynaptic targets where SN DA is acting to reduce renewal are unclear. Fear extinction supported by SN DA activation is associated with neural activity in the dorsal lateral striatum (DLS), a region implicated in habit learning processes which are resistant to contextual modulation. Therefore, the goal of the current experiment was to determine whether activation of the SN-DLS circuit during fear extinction can reduce renewal. Adult Long-Evans male rats received either control virus or AAV-Chr2-hSyn-mCherry bilaterally into the SN and bilateral optic ferrules in the DLS to optogenetically stimulate SN terminals in the DLS during fear extinction. Expression of neural activation marker cFos was used to verify the effectiveness of optogenetic stimulation. Activation of SN terminals in the DLS during fear extinction reduced fear renewal in a novel context without improving the fear extinction recall in the extinction

context. The data suggest that the SN-DLS circuit is a novel target for freeing fear extinction memory from contextual modulation.

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Poster

396. Cortical and Basal Ganglia Circuits in Aversive Processing

Location: SDCC Halls B-H

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Program #/Poster #: 396.06

Topic: G.06. Anxiety Disorders

Support: NIH Grant 068283

Title: Neural mechanism underlying estrous cycle modulation of fear extinction and relapse

Authors: *M. PRICE, A. HOHORST, M. TANNER, E. LOETZ, B. GREENWOOD;
Univ. of Colorado Denver, Denver, CO

Abstract: Fear extinction-based exposure therapy is a common behavioral therapy for anxiety-and trauma-related psychiatric disorders. However, fear memories tend to return even after successful extinction, leading to poor long-term efficacy of exposure therapy. This impaired inhibition of learned fear is a feature of stress-related psychiatric disorders such as generalized anxiety disorder and post-traumatic stress disorder. Animal models of fear and anxiety form the basis for research into treatments, but have been developed almost exclusively using males, despite the higher prevalence of anxiety-and trauma-related disorders in women compared to men. The estrous cycle can modulate extinction learning in female rats; whereby extinction training during estrous phases characterized by high levels of ovarian hormones (proestrus and estrus, Pro/Est) enhances extinction memory and reduces relapse compared to both females that learn extinction during conditions of low ovarian hormones (metestrus and diestrus, Met/Di) and males. Though brain regions activated during extinction learning have been well elucidated in males, it remains unknown how estrous cycle impacts neural activity during extinction. The goal of the current study was to determine whether brain regions implicated in fear extinction and relapse are differentially activated during fear extinction depending on sex and estrous cycle phase during which extinction is learned. Adult, male and female, Long-Evans rats were exposed to auditory fear conditioning and extinction in a novel context. Rats were euthanized 90 min after the end of extinction and cFos immunohistochemistry was performed in various brain regions of interest. Data collection and analyses are ongoing. Results could reveal circuits through which estrous phase interacts with fear extinction to render fear extinction memories resistant to relapse.

Disclosures: M. Price: None. **A. Hohorst:** None. **M. Tanner:** None. **E. Loetz:** None. **B. Greenwood:** None.

Poster

396. Cortical and Basal Ganglia Circuits in Aversive Processing

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 396.07

Topic: G.06. Anxiety Disorders

Support: R01MH120514
R01MH120637
R01MH051399
NARSAD Brain and Behavior Research Foundation

Title: Parallel dopamine circuit dynamics in chronic social stress-induced behavioral outcomes

Authors: *M. CAROLE[1], S. E. MONTGOMERY[4], L. LI[7], R. DURAND-DE CUTTOLI[2], E. TEICHMAN[5], B. JUAREZ[8], N. TZAVARAS[6], M. FLANIGAN[9], J. J. WALSH[10], S. J. RUSSO[5], E. NESTLER[3], E. S. CALIPARI[11], A. K. FRIEDMAN[12], M.-H. HAN[13];
[1]Icahn Sch. of Med., [2]Icahn Sch. of Med., New York, NY; [3]Icahn Sch. of Med., NEW YORK, NY; [4]Icahn Sch. of Med. at Mount Sinai, [5]Icahn Sch. of Med. at Mount Sinai, New York, NY; [6]Icahn Sch. of Med. at Mount Sinai, NEW YORK, NY; [7]Home, Icahn Sch. of Med. At Mount Sinai Grad. Training Program In Neurosci., New York, NY; [8]Home, Univ. of Washington, Seattle, WA; [9]Univ. of North Carolina Bowles Ctr. for Alcohol Studies, Mt. Sinai Sch. of Med., Chapel Hill, NC; [10]Univ. of North Carolina at Chapel Hill, Chapel Hill, NC; [11]Vanderbilt Univ., Vanderbilt Univ. Sch. of Med., Nashville, TN; [12]Hunter College, City Univ. of New York, Hunter College, City Univ. of New York, New York, NY; [13]Shenzhen Inst. of Advanced Technol., Shenzhen, China

Abstract: Comorbidity is more the norm than the exception. Anxiety, anhedonia, and depression, the highest burden of disability amongst psychiatric disorders, are highly comorbid (over 60%). This co-occurrence is associated with greater chronicity, disability, and 4 times higher suicide attempt rates in comorbid patients. However, the shared or segregated mechanisms remain largely unknown. Clinical and preclinical studies implicate the dopaminergic system in the emergence of anxiety disorders and depression. The ventral tegmental area (VTA) dopamine neurons encode rewarding, salient and aversive stimuli, and project to emotion-related brain regions, including the nucleus accumbens (NAc), prefrontal cortex (PFC), and amygdala to support appropriate adaptive behaviors and maintain healthy brain functions. Using a chronic social stress paradigm (CSS) that induces features of anxiety- and depressive-like behaviors, we aim to dissect the dopaminergic subcircuit mechanisms that underlie anxiety, anhedonia, and depressive-like behaviors. Following CSS, we assess social behaviors, reward processing, and approach/avoidance behaviors. We then combine neural circuit-probing techniques with electrophysiological and fiber photometry approaches to determine the characteristics and dynamics of VTA projections to BLA or NAc in the expression of stress-induced behavioral outcomes. Additionally, we perform *in vivo* photo-tagging electrophysiology and use optogenetics to causally link VTA subcircuit activity and behavioral

outcomes. We establish that CSS dramatically decreases VTA-BLA dopamine neuron activity which in turn induces anxiety-like behaviors, while changes in VTA-NAc and VTA-PFC dopamine activity result in depressive-like behaviors. We then identify that VTA-BLA circuit activity is associated with the expression of anxiety- but not depressive-like behavioral outcomes. Finally, we bidirectionally causally link the VTA-BLA subcircuit dysfunction with anxiety- but not depressive-like behaviors. Overall, these data establish a selective functional role for VTA-BLA dopamine neurons in bi-directionally controlling anxiety-related behaviors not only in anxiety-alone, but also in anxious-depressive comorbid subjects.

Disclosures: M. Carole: None. **S.E. Montgomery:** None. **L. Li:** None. **R. Durand-De Cuttoli:** None. **E. Teichman:** None. **B. Juarez:** None. **N. Tzavaras:** None. **M. Flanigan:** None. **J.J. Walsh:** None. **S.J. Russo:** None. **E. Nestler:** None. **E.S. Calipari:** None. **A.K. Friedman:** None. **M. Han:** None.

Poster

396. Cortical and Basal Ganglia Circuits in Aversive Processing

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 396.08

Topic: G.04. Emotion

Support: NIH grant R01MH121735
NIH grant P51OD011107

Title: Functional connectivity between the anterior cingulate cortex and bed nucleus of the stria terminalis and inhibited temperament in rhesus macaques

Authors: *C. M. DRZEWIECKI[1], L. J. CAMPOS[1], D. HOLLEY[1], J. P. CAPITANIO[2], A. S. FOX[3];
[1]Univ. of California at Davis, Davis, CA; [2]California Natl. Primate Res. Ctr., Univ. of California Davis, Davis, CA; [3]Psychology, Univ. of California - Davis, Davis, CA

Abstract: Children with an inhibited temperament (IT) are at an increased risk to develop anxiety disorders as they mature into adults. Studies in humans and rhesus monkeys show IT is heritable, moderately stable over time and context, and under the control of an evolutionarily conserved network of brain regions. A key component in this network is the extended amygdala, which includes the bed nucleus of the stria terminalis (BST). The BST orchestrates threat responses and other survival relevant behaviors. The extended amygdala also receives reciprocal anatomical connections from the prefrontal cortex (PFC), particularly the subgenual anterior cingulate cortex (sgACC; area 25). Recent work implicates the sgACC in fear expression and threat prediction, and prefrontal-amygdala connectivity has been well characterized in anxiety, but less is known about how the PFC interacts with the BST. Importantly, IT-related metabolism in both the BST and sgACC is heritable, highlighting a possible prefrontal-limbic circuit involved in the intergenerational transfer of IT.

Here we aimed to further explore the role of the sgACC on IT in nonhuman primates. Rhesus monkeys (macaca mulatta) at the California National Primate Research Center were previously assessed for IT as part of the BioBehavioral Assessment during infancy (3-4 months). Among those animals, 20 adolescent females were selected and phenotyped again for anxious temperament using the well-validated no eye contact (NEC) condition of the human intruder paradigm. Following this test, “resting-state" functional MRI scans were collected under light anesthesia with a Siemens Skyra 3T MRI Scanner and a dedicated rhesus 8-channel surface coil. rsfMRI data were processed using the Configurable Pipeline for the Analysis of Connectomes (C-PAC), and all data were thresholded at $p<0.05$, uncorrected. Preliminary results demonstrate significant, functional connectivity between the sgACC and BST during adolescence. Interestingly, functional connectivity was not observed between the BST and other ACC subregions, including the more anterior areas 24 and 32. Additional analyses identified several brain regions where adolescent sgACC-seeded functional connectivity was positively correlated with individual differences in infant IT, including the BST, hippocampus, periaqueductal gray, and anterior insula. These findings recapitulate many key nodes of the distributed brain networks where metabolism is associated with IT and highlight a key role for the sgACC in early-life IT. Together, these findings provide evidence for sgACC-BST connections that may contribute to anxiety-related phenotype across development.

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Poster

396. Cortical and Basal Ganglia Circuits in Aversive Processing

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Topic: G.04. Emotion

Support: NARSAD 27202
SF NRT UtB DGE-1633516
1R01MH129732-01 Cruz-Martin
1R01EB029171-01 Mertz

Title: Dynamic subpopulations of VIP interneurons in the anterior cingulate cortex activate to emotional and social stimuli

Authors: *C. JOHNSON[1,10], L. N. KRETSGE[2,3], W. W. YEN[1], B. SRIRAM[11], A. O'CONNOR[4], R. LIU[5], J. C. JIMENEZ[12], R. A. PHADKE[6], K. K. WINGFIELD[3,7], C. YEUNG[1], T. J. JINADASA[1], T. P. H. NGUYEN[4], E. SEON CHO[1], E. FUCHS[1], B. ESCUDE VELASCO[1], E. D. SPEVACK[1], F. S. HAUSMANN[1], L. A. FORNIER[1], A. BRACK[6], S. MELZER[13], A. CRUZ-MARTÍN[1,3,6,8,9];
[1]Neurobio. Section in the Dept. of Biol., [2]The Grad. Program for Neurosci., [3]Neurophotonics Ctr., [4]Dept. of Biomed. Engin., [5]MS in Statistical Practice Program, [6]Mol. Biology, Cell Biol.

and Biochem. Program, [7]Dept. of Pharmacol. and Exptl. Therapeut., [8]Ctr. for Systems Neurosci., [9]The Ctr. for Network Systems Biol., Boston Univ., Boston, MA; [10]Neurosci. Grad. Program, Brandeis Univ., Waltham, MA; [11]Praxis Precision Medicines, Inc, Boston, MA; [12]Dept. of Neurol., Univ. of California, San Francisco, San Francisco, MA; [13]Howard Hughes Med. Inst. - Harvard Med. Sch., Howard Hughes Med. Inst., Boston, MA

Abstract: The anterior cingulate cortex (ACC) has long been thought to play an important role in the integration of cognition with emotional and social stimuli. Functional studies in both humans and rodents have supported this idea however, the mechanisms that underlie the processing of these signals remain unknown. Vasoactive intestinal peptide (VIP) expressing inhibitory interneurons offer a candidate of interest in these circuits as they drive excitatory activity through disinhibition of pyramidal cells. Here we recorded the activity of VIP interneurons in the ACC (VIPACC) using in vivo Ca2+ imaging in freely moving mice during social and anxiogenic behaviors using miniature fluorescent microscopes. We found that subpopulations of VIPACC responded to particular stimuli within trials, however these representations drifted over the course of hours and days. Additionally, during social tasks we observed that these cells responded exclusively to direct social interactions and not to non-social exploratory behaviors. These findings implicate VIPACC in real time processing of social and anxiety inducing stimuli, while not encoding long-term representations of stimuli at an individual cell level.

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Poster

396. Cortical and Basal Ganglia Circuits in Aversive Processing

Location: SDCC Halls B-H

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Program #/Poster #: 396.10

Topic: G.07. Post-Traumatic Stress Disorder

Support: NIH-NIGMS #2R25GM082406
NCMHD [U54MD007579]
NIH-NIGMS R15 MH116345

Title: Infralimbic cortex-hypothalamic circuit in fear and anxiety-related behaviors

Authors: *Y. RIVERA-ESCOBALES[1], L. DIEZ-ASAD[2], M. COLÓN-ROMERO[2], J. T. PORTER[3];
[1]PONCE HEALTH SCIENCES UNIVERSITY, Ponce, Puerto Rico; [2]Ponce Hlth. Sci. Univ., Ponce, Puerto Rico; [3]Ponce Hlth. Sci. Univ., Ponce, PR

Abstract: The infralimbic cortex is associated with fear and anxiety-related behaviors in rodents. The infralimbic cortex innervates several brain regions associated with learning, memory, and autonomic responses including the posterior hypothalamic nucleus. The posterior hypothalamic nucleus is activated in response to stress and inhibiting this region decreases anxiety-like behavior. Despite this evidence, whether the infralimbic cortex-posterior hypothalamic nucleus pathway has a role in fear and anxiety-related behaviors remains unexplored. In the present study, we sought to investigate the role of the infralimbic cortex-posterior hypothalamic nucleus pathway in fear and anxiety-related behaviors. We hypothesized that activation of the infralimbic cortex-posterior hypothalamic nucleus pathway would reduce fear and anxiety-related behaviors. To test this hypothesis male and female adult rats received a bilateral injection of an AAV expressing a Cre-dependent DREADD into the infralimbic cortex and a retrograde Cre-expressing AAV into the posterior hypothalamic nucleus for clozapine-N-oxide (CNO)-induced neuronal activation. Seven weeks after the surgery, rats were trained in auditory fear conditioning and extinction and also examined for anxiety-like behaviors using the open field and zero maze tests. Animals received an intraperitoneal injection of CNO or vehicle one hour before extinction training and assessment of anxiety-related behaviors. Activation of the infralimbic cortex-posterior hypothalamic nucleus pathway in females did not affect fear or anxiety-related behaviors. We are currently performing behavior experiments on male rats to examine whether there are sex differences. In addition, we are analyzing brain tissue to confirm the viral infusions were done in the correct places and performing immunofluorescence analysis of c-Fos expression in the posterior hypothalamic nucleus to verify if there was neuronal activation due to CNO.

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Poster

396. Cortical and Basal Ganglia Circuits in Aversive Processing

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 396.11

Topic: G.01. Fear and Aversive Learning and Memory

Support: Human frontier science program postdoctoral fellowship
EMBO long-term fellowship

Title: Encoding of general danger and specific threat representations in prefrontal circuits

Authors: *M. MARTIN FERNANDEZ, A. MENEGOLLA, G. LOPEZ-FERNANDEZ, H.-R. KIM, D. JERCOG, D. GIRARD, C. DEJEAN, C. HERRY;
INSERM U1215, Bordeaux, France

Abstract: The behavioral adaptation to potential threats requires both a global representation of danger to prepare the organism to react in a timely manner but also the identification of specific

threatening situations to select the appropriate behavioral responses. The prefrontal cortex is known to control threat-related behaviors, yet it is unknown whether it encodes global defensive states and/or the identity of specific threatening encounters. We used a combination of electrophysiological recordings, neuronal decoding approaches, and optogenetic manipulations in a novel behavioral paradigm allowing the simultaneous evaluation of distinct defensive behaviors in response to different threatening situations. Our results indicate that the dorsomedial prefrontal cortex (dmPFC) encodes a global representation of danger in high dimensional spaces of the overall population activity while simultaneously encoding a specific neuronal representation of each threatening situation. Importantly, we demonstrated the persistence of global danger neuronal representation in error trials that instead lacked threat-identity representation. Consistently, the optogenetic inhibition of prefrontal neurons impaired the overall behavioral performance and the discrimination of different threatening situations without any bias towards active or passive behaviors. Together these data indicate that the prefrontal cortex encodes both a global representation of danger and a specific representation of threat identity to control the selection of defensive behaviors.

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Poster

397. Fear and Aversive Learning and Memory: Cellular and Molecular

Location: SDCC Halls B-H

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Topic: G.01. Fear and Aversive Learning and Memory

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Toyoaki Scholarship Foundation
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Japanese Government (MEXT) Scholarship
Mills College Barrett Undergraduate Research Program

Title: Mapping of a C. elegans electricity response mutant

Authors: *J. J. YOUNG[1], L. TEE[2], J. ROSECRANS[1], Q. MUHAMMAD[1], E. RIVERA[1], H. AL-JUBARI[1], V. LAM[1], E. KEOMANY[1,2], K. KIMURA[2];
[1]Mills Col., Mills Col., Oakland, CA; [2]Nagoya City Univ., Nagoya, Japan

Abstract: We recently discovered that C. elegans runs in random directions when 30V alternating current is applied to an agar plate on which they have been placed. This running response exhibits features that suggest a fear-like emotional state, including that the running response persists after the electricity is removed. Here we report on a forward genetic screen we

carried out to identify components that play roles in this electricity response, and on efforts to map the causative locus for one of our mutants, named J102. After mutagenizing worms, we subjected them to repeated rounds of electricity application, and collected worms that did not respond. This involved placing worms on an agar plate with a small OP50 patch, then applying 30V alternating current to the plate and collecting worms that remained in the food patch after about one minute. These non-responders were allowed to reproduce, and additional assays were performed on the resulting lines to further weed out lines without robust phenotypes. Next we tested 9 lines for dominance relationship and number of causative mutations, after which we proceeded with 5 of these lines. We backcrossed each of those 5 lines at least 2x each, and did complementation tests, resulting in 3 complementation groups. We then sent samples of genomic DNA for all five of these samples for whole genome sequencing (WGS). We are currently following up on the ”J102” mutant. WGS results show the highest density of mutations on regions of chromosomes V and X. We are currently pursuing a mapping-by-sequencing approach utilizing the Hawaiian strain for outcrossing. We are obtaining outcrossed lines that are homozygous for the mutation and will pool the DNA from these lines and send that DNA for whole genome sequencing. In this way we hope to get close to identifying the causal mutation.

Disclosures: **J.J. Young:** None. **L. Tee:** None. **J. Rosecrans:** None. **Q. Muhammad:** None. **E. Rivera:** None. **H. Al-Jubari:** None. **V. Lam:** None. **E. Keomany:** None. **K. Kimura:** None.

Poster

397. Fear and Aversive Learning and Memory: Cellular and Molecular

Location: SDCC Halls B-H

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Program #/Poster #: 397.02

Topic: G.01. Fear and Aversive Learning and Memory

Support: UNAM PAPIIT IN205622
CONACYT CVU-998761

Title: Exposure to an environmental enrichment attenuates the strength of conditioned taste aversion through the restoration of bdnf levels in the insular cortex

Authors: *B. GUTIERREZ[1], M. L. ESCOBAR[1], A. RIVERA-OLVERA[2];
[1]Facultad de Psicologia, [2]Facultad de Psicologia, Ciudad de México, Mexico

Abstract: It has been reported that animals housed in an enriched environment (EE) show alower psychological and physiological impact from stressors when compared withanimals housed in standard conditions, in addition to modifying the salience of strongaversive stimuli. Changes in brain-derived neurotrophic factor (BDNF) expression inthe brain, have been proposed to be the predominant mechanism by which an EEprovides beneficial effects on health and cognition. BDNF is considered an essentialprotein for the regulation of mechanisms related to long-term memory in the adultbrain. Our previous studies have demonstrated that in the insular cortex (IC), a brainregion of the temporal lobe implicated in the acquisition,

consolidation, and retentionof conditioned taste aversion (CTA) task, BDNF can reverse the CTA memory deficitcaused by a protein synthesis inhibitor. Likewise, our research group have alsoshown that BDNF is required for the maintenance of CTA long-term memory. Herewe evaluate the effects of the exposure to an enriched environment on the CTAmemory strength, using a weak and strong version of this paradigm. The exposureto an EE for 21 days was able to attenuate the strong-CTA response through therestoration of BDNF levels in the IC of adult rats. These results provide evidencethat environmental enrichment is capable of reducing the strength of an aversivememory trace, restoring the BDNF levels in a neocortical region of the adult brain.Keywords: Environmental enrichment, BDNF, Insular cortex, CTA.

Disclosures: B. Gutierrez: None. **M.L. Escobar:** None. **A. Rivera-Olvera:** None.

Poster

397. Fear and Aversive Learning and Memory: Cellular and Molecular

Location: SDCC Halls B-H

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Program #/Poster #: 397.03

Topic: G.01. Fear and Aversive Learning and Memory

Support: Sloan Fellowship

Title: Stress induced emotional dysregulation in a mouse model of Tuberous Sclerosis Complex

Authors: *P. SHRESTHA;
Stony Brook Univ., Stony Brook, NY

Abstract: Tuberous Sclerosis Complex (TSC) is a neurodevelopmental disorder caused by mutations in Tsc1 or Tsc2, the genes that encode the protein components of TSC complex. TSC is one of the leading monogenic causes of autism spectrum disorder, and is associated with several neuropsychiatric disorders, collectively referred to as TSC associated neuropsychiatric disorders (TAND). TSC protein complex acts as an intracellular signal integrator and puts a brake on anabolic signaling mediated by mammalian target of Rapamycin complex (mTORC1), this occurs through its inhibition of small GTP-ase Rheb, which is a direct activator of mTORC1. Aside from the canonical TSC-Rheb-mTORC1 pathway, TSC complex has recently been identified as a regulator of integrated stress response via TSC-Rheb mediated activation of eIF2alpha kinase PERK. Here, we show that heterozygous deletion of Tsc2 in Oxytocin receptor expressing cells (OxtRCs) precipitates behavioral susceptibility to social isolation stress. The manifestation of this behavioral susceptibility to stress is sex-specific. Male heterozygous null mice, upon prolonged social isolation, exhibit anxiety in tests that involve exploration of the environmental context or novel objects, specifically the open field test and marble burying test. On the other hand, social isolation reveals social anxiety in the female heterozygous null mice. We did not detect any impairment in associative learning and memory in the mutant mice, or motor stereotypy, indicating that OxtRC-specific haploinsufficiency of Tsc2 leads to aggravation of distinct indices of anxiety while sparing other behavior modules. Systemic administration of

PERK inhibitor, GSK2606414, rescued all indices of anxiety-like behavior in male and female mutant mice whereas systemic Rapamycin, an mTORC1 inhibitor, only rescued anxiety in the marble-burying test for male mutants. Further, cell type-specific knockdown of Rheb in prefrontal OxtRCs rescued arena-related exploratory anxiety in male mice as well as social anxiety in female mutant mice. Our data indicate that aberrant behavior responses to stress are primarily mediated by TSC-Rheb-PERK signaling axis in Oxytocin responsive cells in the medial prefrontal cortex, and provide insight into the molecular underpinnings for emotional dysregulation evident in TSC.

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Poster

397. Fear and Aversive Learning and Memory: Cellular and Molecular

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Program #/Poster #: 397.04

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH Grant DA049044

Title: Organic Cation Transporter 3 on Serotonin Neurons Sex-Dependently Modulates Fear and Anxiety Behaviors

Authors: *N. CLAUSS[1], L. HONAN[1], A. W. A. OWENS[2], R. E. HORTON[1], G. M. TONEY[1], L. C. DAWS[2];
[1]Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX; [2]Univ. of Texas Hlth. Sci. Ctr. At San Antonio, San Antonio, TX

Abstract: Dysregulation in serotonergic neurotransmission has long been implicated in neuropsychiatric illness, including stress related disorders. Thus, understanding the role serotonergic regulation plays in circuitry relevant to emotion is fundamental to develop therapeutics to treat stress-related disorders. A large body of literature exists investigating the role of high-affinity, low-capacity ‘uptake-1’ transport mechanisms (serotonin, dopamine and norepinephrine transporters) in monoaminergic modulation of emotion-regulating circuitry. To date, pharmacological targeting of these ‘uptake-1’ transporters has demonstrated variable clinical efficacy, suggesting a role for other mechanisms in manifestation of disorders associated with monoaminergic dysregulation. Evidence from our laboratory and others suggests that the corticosterone-sensitive, organic cation transporter 3 (OCT3), a low-affinity, high-capacity ‘uptake-2’ transporter, has a significant impact on monoaminergic homeostasis and may play a major role in stress-related disorders. Here we used ePet-cre crossed with our Oct3 floxed mice to knockout OCT3 from serotonin neurons during embryogenesis. We characterized serotonin clearance in basolateral amygdala (BLA) of adult male and female offspring using *in vivo* high-speed chronoamperometry. BLA is densely innervated by serotonin neurons (mainly from the dorsal raphe) and essential to processing and consolidation of fear memory. Consistent with

OCT3 being an important regulator of serotonin homeostasis, we found that serotonin clearance in BLA was impaired in mice with loss of OCT3 on serotonin neurons, and this impairment was exacerbated with increasing extracellular serotonin, regardless of sex. Furthermore, we found that male, but not female, mice lacking OCT3 on serotonin neurons trended to show greater fear memory (more time spent freezing in cued and context conditions following fear memory acquisition). Consistent with this finding, male, but not female, mice lacking OCT3 on serotonin neurons showed greater anxiety-like behavior in the elevated plus maze (less time in open arms and more time in closed arms) relative to their control counterparts. Ongoing studies are using a tamoxifen-inducible knock out approach (tamoxifen treatment of Oct3 floxed crossed with Nestin-cre/ERT2 mice) to assess the impacts of conditional loss of OCT3 from neurons and glia in adult mice. Preliminary findings suggest that OCT3 plays an important sex-dependent role in formation of fear memory and anxiety-related behaviors.

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Poster

397. Fear and Aversive Learning and Memory: Cellular and Molecular

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Program #/Poster #: 397.05

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIAAA Intramural Research Program

Title: Functional Roles of Astrocyte Calcium Elevations in the Basolateral Amygdala

Authors: *O. BUKALO, S. ZIMMERMAN, V. OFFENBERG, A. MENDEZ, C. WEINHOLTZ, T. CAMPBELL, M. YDE, A. HOLMES;
Natl. Inst. on Alcohol Abuse and Alcoholism, NIH, Rockville, MD

Abstract: The ability to retrieve associations between environmental stimuli and previously encountered threat represents a fundamental form of memory crucial to survival. Recent studies suggest astrocytes support fear memory by modulating memory-encoding neural circuits and neuronal engrams in cortical and limbic regions. However, the precise mechanisms by which this occurs remain unknown. Here, we monitored and manipulated astrocyte activity in vivo with fiber photometry in the basolateral amygdala (BLA), a brain region critical to the formation, retrieval, and extinction of fear memories. First, our data demonstrate that population BLA astrocyte Ca^{2+} activity signals the retrieval of a cued threat memory then tracks the extinction-induced shift from a high to low fear state and subsequent return of high fear during context-driven renewal. Next, we sought to assess whether Ca^{2+} activity in BLA astrocytes causally contributed to formation/retrieval of fear memory. To do so, we selectively expressed in BLA astrocytes a viral construct containing the plasma membrane Ca^{2+} extruder (hPMCA2w/b) known to attenuate Ca^{2+}-dependent signaling in astrocytes. We found normal levels of freezing

on conditioning in hPMCA2w/b-expressing animals, relative to controls, but lower levels during extinction training, and extinction retrieval. To further dissociate the significance of astrocytic Ca^{2+} signaling in fear memory, we employed a chemogenetic manipulation by expressing viral constructs for hM3D(Gq)- or hM4D(Gi)-coupled DREADD in BLA astrocytes. Systemic injection of the inert ligand clozapine n-oxide (CNO), prior to extinction training, have an opposite effect on freezing behavior. We found that freezing levels were markedly lower in hM3D-, but higher in hM4D-expressing animals as compared to mice expressing control viral construct, during early extinction trials, consistent with an impairment or improvement in fear memory retrieval. Using in vivo fiber photometry Ca^{2+} imaging during CNO application, we observed a different dynamic of Ca^{2+} signal in astrocytes in hM3D- and hM4D-expressing animals. In hM3D mice CNO administration produced a sizable short-lasting increase in Ca^{2+} activity, followed by attenuation of astrocytic Ca^{2+} activity sustained at least till the end of extinction session. In contrast, in hM4D-expressing mice, we observed an increase in number of astrocytic Ca^{2+} events after CNO administration, which persists till the end of extinction session. Altogether, our data suggests that Ca^{2+} responses in astrocytes are not only tightly correlated with fear state, but also that BLA astrocyte Ca^{2+} activity is necessary for fear memory retrieval.

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Poster

397. Fear and Aversive Learning and Memory: Cellular and Molecular

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 397.06

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH (R01 NS106915
VA (IO1 BX003893

Title: Fear conditioning reduces HCN currents in cerebellar stellate cells via endocannabinoid signaling

Authors: *G. KOGIAS[1], K. L. CARZOLI[1], S. J. LIU[2];
[1]LSU Hlth. Sci. Ctr. Cell Biol. & Anat., NEW ORLEANS, LA; [2]LSU Hlth. Sci. Ctr. Cell Biol. & Anat., New Orleans, LA

Abstract: Cerebellar activity is critical for the formation of associative fear memory. Our recent study showed that fear conditioning reduces endocannabinoid (eCB) signaling in cerebellar lobules V/VI and this is required for memory consolidation. Endocannabinoids are known to suppress neurotransmitter release, and have been shown to enhance hyperpolarization-activated cyclic nucleotide-gated (HCN) channels in hippocampal CA1 neurons. Given that genetic deletion and pharmacological inhibition of HCN enhances intrinsic membrane properties and alters spatial and motor learning and memory, a learning-induced change in hyperpolarization-

activated currents (I_h) can alter neural circuit activity and modulate animal behavior. Stellate cells in the cerebellar cortex express HCN channels and control the activity of Purkinje cells *via* tonic and feedforward inhibition. Here we tested whether associative fear conditioning altered I_h via reducing endocannabinoid signaling, using a combined behavioral and electrophysiological whole cell voltage clamp recording approach. We found that fear conditioning selectively induced a lasting decrease in I_h amplitude and a depolarizing shift of I_h activation in cerebellar stellate cells in vermal lobules V/VI, a region involved in the consolidation of associative fear memory. In naïve mice, application of a cannabinoid receptor 1 (CB1R) receptor neutral antagonist NESS0327, reduced the amplitude of I_h and produced a hyperpolarizing shift in I_h activation, suggesting that tonic eCB levels enhance HCN channels. After fear conditioning CB1R agonist application reversed the learning-induced change in I_h amplitude and activation while NESS0327 failed to alter I_h properties. Inhibition of a 2-AG degradation enzyme that elevates endogenous 2-AG levels, also increased I_h amplitude and induced a depolarizing shift of I_h activation to the same level as naïve mice. Therefore, a learning-induced decrease in eCB signalling drives the decrease in I_h amplitude and the hyperpolarizing shift in I_h activation. We next characterized the signaling pathway that is activated by CB1Rs and leads to changes in I_h in postsynaptic stellate cells by including a Gβγ, JNK, NOS or guanylyl cyclase inhibitor in the recording electrode. Each of these inhibitors completely abolished the WIN55,212-2-induced increase in I_h amplitude and the depolarizing shift of I_h activation in conditioned mice. In contrast, adenylyl cyclase inhibitor failed to prevent CB1R agonist-induced changes in HCN currents. Thus, a CB1R-Gβγ-JNK-dependent regulation of cGMP in stellate cells leads to the learning-induced decrease in I_h.

Disclosures: **G. Kogias:** None. **K.L. Carzoli:** None. **S.J. Liu:** None.

Poster

397. Fear and Aversive Learning and Memory: Cellular and Molecular

Location: SDCC Halls B-H

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Program #/Poster #: 397.07

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH Grant MH113325
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Title: Conditioned flight behavior is increased by ASIC1A disruption

Authors: *R. J. TAUGHER-HEBL, M. M. CONLON, R. FAN, J. A. WEMMIE;
Psychiatry, The Univ. of Iowa, Iowa City, IA

Abstract: Surviving existential threats often requires defensive responses that are optimally timed and appropriately matched to the threat. Inadequate, exaggerated, or ill-timed responses can further increase danger. To study mechanisms by which organisms tailor defensive responses

to impending threats, a recent paper modified classical fear conditioning paradigms to simultaneously probe both conditioned freezing and conditioned flight behaviors. This paradigm uses a compound stimulus consisting of a pure tone followed by white noise terminating with a foot shock. Mice conditioned by this paradigm switch their defensive responses from freezing to flight upon presentation of the white noise which indicates that a potential shock is increasingly imminent. In previous studies we found that acid-sensing ion channel-1A (ASIC1A) in the basolateral amygdala is critical for synaptic plasticity and conditioned freezing behavior. Here we sought to test whether ASIC1A might play a similar role in conditioned flight behaviors. Wild-type and *Asic1a*$^{-/-}$ mice were conditioned to 10 pairings of the compound stimulus (tone + white noise) that co-terminated with a 1 s, 0.9 mA foot shock. Conditioned freezing and flight responses to the tone and white noise were tested without shocks 24 hr later in the conditioning (threat) context or in a neutral (safe) context. As anticipated, in the threat context, wild-type mice exhibited robust conditioned flight responses to white noise. Surprisingly, in these same conditions *Asic1a*$^{-/-}$ mice exhibited normal conditioned flight responses, despite markedly impaired conditioned freezing. Moreover, compared to wild type mice, *Asic1a*$^{-/-}$ mice exhibited exaggerated flight responses to pure tones in the threat context, and exaggerated flight responses to the white noise in the safe context. These findings suggest not all conditioned defense responses are reduced by ASIC1A disruption, rather loss of ASIC1A may impair the appropriate matching of defensive responses to the threat. Interestingly, this imbalance between adaptive and maladaptive defensive responses in *Asic1a*$^{-/-}$ mice is reminiscent of altered defensive responses in patients suffering from PTSD. Dissecting circuit level abnormalities in these mice may shed important light on ways to normalize maladaptive responses to diverse and perceived threats.

Disclosures: **R.J. Taugher-Hebl:** None. **M.M. Conlon:** None. **R. Fan:** None. **J.A. Wemmie:** None.

Poster

397. Fear and Aversive Learning and Memory: Cellular and Molecular

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Program #/Poster #: 397.08

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH R01 MH119283
NIH R01MH104373

Title: Gq neuromodulation of cholecystokinin-expressing interneurons in the basolateral amygdala suppresses fear learning via patterned inhibitory input to principal neurons

Authors: X. FU[1], ***B. SWEETEN**[1], J. L. MAGUIRE[2], J. G. TASKER[1];
[1]Cell and Mol. Biol., Tulane Univ., New Orleans, LA; [2]Neurosci., Tufts Univ. Sch. of Med., Boston, MA

Abstract: Neuromodulatory systems regulate changes between emotional behavioral states, but the mechanisms underlying the neuromodulatory control of basolateral amygdala (BLA) circuits remain largely unknown. Norepinephrine (NE) release in the BLA during emotional arousal plays an essential role in fear processing, however cell type-specific noradrenergic modulation in the BLA has not been fully characterized. Our previous data showed that NE activation of Gq-coupled α1A adrenoreceptors stimulates two types of dissociable perisomatic inhibitory synaptic inputs to BLA principal neurons, repetitive IPSC bursts by parvalbumin (PV)-expressing interneurons and single, low-frequency trains of IPSCs by putative cholesystokinin-expressing (CCK) basket cells (Fu et al., 2022). Here, we tested the role of BLA CCK interneurons in the Gq modulation of BLA circuits and fear learning by combining brain slice electrophysiology, intersectional viral targeting, and genetic manipulations. We confirmed that NE α1A adrenoreceptor and Gq-DREADD activation of CCK interneurons generate synchronized, low-frequency trains of rhythmic CB1-sensitive IPSCs in BLA principal neurons. Additionally, α1A adrenoreceptors or Gq-DREADD expressed specifically in CCK interneurons in a global adra1A knockout mouse decreased fear memory acquisition and recall. To more specifically target CCK basket cells, we developed an intersectional viral strategy combining an AAV expressing a Gq-DREADD controlled by a GABA neuron-specific promoter (hDLX) with an AAV expressing Cre under the control of the CB1 receptor promoter. Overall, these data reveal an inhibitory role of noradrenergic Gq activation of BLA CCK interneurons in fear learning via patterned, synchronized inhibitory inputs to the principal neurons. In combination with the parvalbumin-expressing basket cells, the Gq modulation of CCK neurons in the BLA may fine-tune associative fear learning by balancing the respective contributions of different patterns of perisomatic inhibition.

Disclosures: X. Fu: None. **B. Sweeten:** None. **J.L. Maguire:** None. **J.G. Tasker:** None.

Poster

397. Fear and Aversive Learning and Memory: Cellular and Molecular

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 397.09

Topic: G.01. Fear and Aversive Learning and Memory

Title: Heterogeneity of excitatory neurons of the basolateral amygdala: from transcriptome to calcium imaging and behavior

Authors: *H. LIM, C. PETERS, R. KLEIN;
Dept. of Molecules – Signaling – Develop., Max Planck Inst. for Biol. Intelligence (in foundation), Planegg, Germany

Abstract: The basolateral amygdala (BLA) integrates emotional, social and metabolic information to participate in a variety of defensive and appetitive behaviors. The circuit mechanisms by which the BLA mediates these different functions are poorly understood. One important aspect is the heterogeneity of excitatory neurons of the BLA. On the one hand, BLA

neurons have shown a high degree of heterogeneity that varies in transcriptome, physiology and circuit properties. Genetically distinct BLA populations were found to respond to negative and positive valence stimuli, respectively, and to control the respective behaviors. On the other hand, BLA neurons can be recruited from the same population into active ensembles during different kinds of explorative behaviors, suggesting e.g. that the BLA encodes social exploration behavior in a valence-independent manner. In our study, we have combined single cell transcriptomics to identify heterogeneities among excitatory BLA neurons, with multi fluorescent in situ hybridization to map cell types to sub-regions of the BLA. We then selected a small number of Cre transgenic lines representing distinct BLA cell types and performed deep brain calcium imaging in response to appetitive and aversive stimuli. Finally, we used optogenetics to manipulate these BLA cell types to modulate valence-specific behavior. Our preliminary results suggest that excitatory neurons of the BLA consist of heterogeneous subpopulations that are organized in space, and are involved in valence-specific behaviors. Two populations of the anterior BA are involved in aversive behaviors, whereas one population of the posterior BA is involved in appetitive behaviors.

Disclosures: H. Lim: None. **C. Peters:** None. **R. Klein:** None.

Poster

397. Fear and Aversive Learning and Memory: Cellular and Molecular

Location: SDCC Halls B-H

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Program #/Poster #: 397.10

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH DA047981
5T32MH119049-03

Title: Investigating Gilz, an X-linked Gene, in the Development and Persistence of Maladaptive Memory and Stress-induced Reinstatement

Authors: *C. CHINN[1], J. ROUNDS[1], J. L. KWAPIS[2], M. A. WOOD[3];
[1]UC Irvine, Irvine, CA; [2]Dept. of Biology, Ctr. for Mol. Investigation of Neurolog. Disord, Penn State Univ., University Park, PA; [3]Neurobiol & Behavior, Univ. of California Irvine, Irvine, CA

Abstract: The effects of stress responses on memory vary widely, from modulating fight or flight behavior to influencing the salience of a traumatic experience. The latter is often associated with post-traumatic stress disorder (PTSD), a pervasive stress and trauma related disorder with long-term effects on mood and memory with a higher prevalence in females. PTSD is commonly comorbid with substance use disorder (SUD), making it extremely difficult to treat both diseases in the same individual. Regarding the establishment of PTSD, many studies focus on memory consolidation and stress response pathways, yet recent work highlights the importance of epigenetic mechanisms, as they can establish persistent changes in cell function and long-lasting changes in behavior. Our lab has shown that a focal deletion of histone

deacetylase 3 (HDAC3) in the dorsal hippocampus increased expression of Gilz (glucocorticoid-induced leucine zipper) after a learning event. This suggests HDAC3, a powerful epigenetic negative regulator of gene expression and memory formation, may be critical for modulating Gilz transcription in long-term memory processes. Further, siRNA knockdown of Gilz in the nucleus accumbens impaired long-term potentiation. Thus, Gilz, a glucocorticoid induced downstream target gene and an X-chromosome linked gene, may provide novel insight into sex-differences observed in PTSD-related long-term memory processes. Using stress-enhanced fear learning and cocaine place preference, this work will demonstrate the effects of Gilz, a potential key HDAC3 target gene, in sex-specific maladaptive stress response and memory processes that affect stress-induced relapse of drug-seeking behavior.

Disclosures: C. Chinn: None. **J. Rounds:** None. **J.L. Kwapis:** None. **M.A. Wood:** None.

Poster

397. Fear and Aversive Learning and Memory: Cellular and Molecular

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Program #/Poster #: 397.11

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIAAA F30 AA028178
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NIAAA T32 AA007577

Title: Ppar alpha mediates a fear conditioning-induced increase in magl expression and promotes memory consolidation

Authors: *M. A. FAROOQ[1], M. REYNOLDS[2], S. J. LIU[1];
[1]Cell Biol. and Anat., LSU Health, New Orleans, New Orleans, LA; [2]Tulane Univ., New Orleans, LA

Abstract: Intrusive memories and hyperarousal are some of the symptoms of post-traumatic stress disorder (PTSD). Fear conditioning is a model for PTSD in which an animal learns to associate a tone with a foot shock. The cerebellum, a brain region traditionally known for motor functions is required for fear memory consolidation. A recent publication from our lab showed that fear conditioning accelerates endocannabinoid degradation and lowers tonic endocannabinoid signaling in the cerebellum. This is required for memory consolidation. MAGL is an enzyme that degrades 2-arachidonoylglycerol (2-AG), which is a major endocannabinoid in the cerebellum. In this study, we investigate the mechanisms underlying the increase in MAGL expression and subsequent activity. We hypothesize that the fear conditioning-induced increase in MAGL expression is mediated by a transcription factor called peroxisome proliferator-activated receptor alpha (PPARα). PPARα binds to the promoter region of the MAGL gene and enhances its transcription. First, we demonstrated that fear conditioning increased MAGL

enzymatic activity using a fluorescence-based enzyme activity assay in lobules V/VI of the cerebellar vermis, a region critical for fear memory consolidation. We next tested if PPARα antagonism could prevent the fear conditioning-induced increase in MAGL expression and activity and consequent reduction in endocannabinoid signaling. Mice received an injection of saline or GW6471 (2mg/kg, i.p), a PPARα antagonist, 30 minutes prior to fear conditioning or an unpaired control protocol. We found that fear conditioning no longer induced an increase in MAGL expression assessed via immunohistochemistry and enzymatic activity in lobules V/VI. Using in vitro electrophysiology, we found that GW6471 pretreatment prevented the learning-induced decrease in endocannabinoid signaling. We further determined the impact of PPARα antagonist on fear memory consolidation and found that GW6471 injection impaired cued memory retention. These findings show that fear conditioning increases MAGL expression and activity via a PPARα-dependent pathway that reduces endocannabinoid tone, promoting memory consolidation.

Disclosures: M.A. Farooq: None. **M. Reynolds:** None. **S.J. Liu:** None.

Poster

397. Fear and Aversive Learning and Memory: Cellular and Molecular

Location: SDCC Halls B-H

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Program #/Poster #: 397.12

Topic: G.01. Fear and Aversive Learning and Memory

Support: NHMRC

Title: Experience-dependent accumulation of G-quadruplex DNA serves as a transcriptional control device to regulate the consolidation and stability of fear-related memories

Authors: *P. MARSHALL[1], J. DAVIES[1], **Y. LEE**[1], Q. ZHAO[1], W.-S. LIAU[1], A. PERIYAKARUPPIAH[1], E. ZAJACZKOWSKI[1], L. LEIGHTON[1], S. MADUGALLE[1], L. KACZMARCZYK[2], W. JACKSON[2], R. SPITALE[3], A. CHEN[4], T. BREDY[1];
[1]Univ. of Queensland, Brisbane, Australia; [2]Dept. of Biomed. and Clin. Sci. (BKV), Linköping, Sweden; [3]Univ. of California Irvine, Irvine, CA; [4]Weizmann Inst. of Sci., Rehovot, Israel

Abstract: DNA can adopt more than 20 different conformational states; however, beyond the right-handed double helix, little is known about the dynamic nature of DNA and whether altered DNA structure states contribute to experience-dependent transcriptional activity in the adult brain. A genome-wide analysis of G-quadruplex DNA (G4-DNA) in neurons activated by behavioural experience revealed a transient, cell-specific, increase in the accumulation of G4-DNA following fear extinction learning. Critically, knockdown of the G4-specific helicase DHX36 led to a global increase in G4-DNA, which was associated with Pol II stalling, an overall reduction in transcriptional activity, and impaired memory. Targeted resolution of G4-DNA using site-directed dCas9-DHX36 at the Gephyrin locus caused a reduction in G4-DNA-associated RNA expression and impaired the formation fear extinction memory. In contrast, the

same manipulation within the cell-adhesion like-1 locus resulted in an increase in G4-DNA-associated RNA expression and destabilization of the original fear memory trace. These findings reveal a causal relationship between dynamic G4-DNA structure and experience-dependent gene expression, with variable gene-specific effects on the consolidation and stability of fear-related memories.

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Poster

397. Fear and Aversive Learning and Memory: Cellular and Molecular

Location: SDCC Halls B-H

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Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH R01MH109588

Title: Fear extinction is regulated by the activity of long noncoding RNAs at the synapse

Authors: *T. BREDY, W.-S. LIAU;
Queensland Brain Inst., St Lucia, Australia

Abstract: Long noncoding RNAs (lncRNAs) represent a multidimensional class of regulatory molecules involved in many aspects of brain function. Emerging evidence indicates that lncRNAs are expressed at the synapse; however, a direct role for their activity in this subcellular compartment in memory formation has yet to be demonstrated. Using lncRNA capture-seq on synaptosomes, we identified a significant number of lncRNAs that accumulate at synapses within the infralimbic prefrontal cortex of adult male C57/Bl6 mice. Among these is a splice variant related to the stress-associated lncRNA, Gas5. RNA immunoprecipitation followed by mass spectrometry and single molecule imaging revealed that this Gas5 isoform, in association with the RNA binding proteins G3bp2 and Caprin1, regulates the activity-dependent trafficking and clustering of RNA granules in dendrites. In addition, we found that cell-type-specific, state-dependent, and synapse-specific knockdown of the Gas5 variant led to impaired fear extinction memory. These findings identify a new mechanism of fear extinction that involves the dynamic interaction between local lncRNA activity and the coordination of activity-dependent trafficking and nanoscale clustering of RNA granules in the synaptic compartment.

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Poster

398. Cellular and Molecular Mechanisms of Reward Circuits

Location: SDCC Halls B-H

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Program #/Poster #: 398.01

Title: WITHDRAWN

Poster

398. Cellular and Molecular Mechanisms of Reward Circuits

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 398.02

Topic: G.02. Reward and Appetitive Learning and Memory

Support: RO1-DK111475

Title: Melanin concentrating hormone neurons interact with estrous cycle stage to modulate behavioral state transitions in a time-dependent feeding task

Authors: *L. RAYCRAFT[1], K. SAPKOWSKI[1], D. ZHAO[2], D. KASHY[2], A. W. JOHNSON[1]; [1]Psychology, [2]Michigan State Univ., East Lansing, MI

Abstract: Many psychological factors influence eating behavior, including both time perception and motivation. While the timing of food intake is often framed from a circadian perspective, multiple timescales likely contribute to the regulation and dysregulation of feeding behavior. Interval timing is a unique form of timing that enables individuals to perceive time in the seconds to minutes range and is critical for learning and decision making. In the present study, we examined whether the regulatory feeding peptide Melanin Concentrating Hormone (MCH) could influence timing-dependent motivational responding in Sprague Dawley rats in a peak interval (PI) timing task. Interestingly, chemogenetic excitation of MCH neurons in the lateral hypothalamic area (LHA) prolonged high rate responding after the criterion duration in female, but not male, rats. This change occurred without affecting time perception and depended on estrous cycle stage. Specifically, LHA-MCH excitation prolonged high rate responding in female rats tested during metestrus/ diestrus (M/D) but not proestrus/ estrus (P/E). Given that the ventral striatum has been implicated in motivational responding in the PI task, we next examined whether the motivational effects of LHA-MCH excitation depended on projections to this region in cycling female rats. Using a dual-virus approach, we selectively excited LHA-MCH neurons that project to the nucleus accumbens (NAc) during the PI task. In this case, female rats tested during M/D reduced responding after the criterion duration, indicating a decrease in motivation. In sum, while a broad population of LHA-MCH neurons increased motivation to respond for food during M/D, a subset of LHA-MCH neurons that project to the NAc instead reduced motivation to respond for food during M/D. In both cases, LHA-MCH excitation selectively affected the behavioral transition from high- to low-rate responding after the time criterion, indicating that these neurons may be important for gating motivated responding in a manner that

depends on both their downstream targets and circulating gonadal hormones. Altogether, these findings suggest that LHA-MCH neurons form a diverse population of motivationally relevant neurons that interact with circulating gonadal hormones to modulate behavioral state transitions.

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Poster

398. Cellular and Molecular Mechanisms of Reward Circuits

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Title: Changes in NR2B-NMDA receptor levels differ across Layer 1 excitatory synapses of pyramidal neurons versus GABA-INs of the prefrontal cortex in ketamine's amelioration of anorexia-like behaviors of adolescent female mice

Authors: *J. LI[1], C. AOKI[1,2], R. TEMIZER[1], Y. W. CHEN[1];
[1]New York Univ., New York Univ., New York City, NY; [2]Neurosci. Inst., NYU Langone Med. Ctr., New York, NY

Abstract: Objective: A previous behavioral study showed that ketamine at a dose of 30mg/kg ameliorated anorexia-like symptoms acutely (during the 1st exposure to the environment inducing activity-based anorexia, ABA1) and during re-exposure, (ABA2, >10 days later), while 3mg/kg only ameliorated the symptoms acutely (Chen et al., 2018, DOI: 10.1002/eat.22937). We sought to understand whether cellular mechanisms involving excitatory synapses in the prefrontal cortex (PFC) might relate to the behavioral changes observed. We analyzed the levels of N-methyl-D-aspartate (NMDA) receptors that contain the NR2B subunit at excitatory synapses of postsynaptic dendritic spines of pyramidal neurons and at dendritic shafts of GABAergic interneurons (GABA-INs) in Layer 1a of the PFC. Methods: 16 female C57BI/6 J mice underwent the ABA model, which included acclimation to wheels and two cycles of food restriction periods (ABA1 and ABA2), each followed by a recovery phase. The anorexia-like behaviors were voluntary food restriction, increased wheel-running, decreased body weight, and heightened anxiety. Two groups of mice were compared in terms of levels of subcellular locations of NR2B subunits, each with a single intraperitoneal dose of 3mg/kg or 30mg/kg

ketamine. Results: The 30mg/kg group had significantly higher levels of NR2B at the synaptic cleft and in the postsynaptic cytoplasm of dendritic spines of pyramidal neurons, compared to the 3mg/kg group, with no difference for the GABA-INs' excitatory synapses. Wheel running during ABA2 correlated negatively with NR2B at spines of pyramidal neurons and positively with NR2B at excitatory synapses on GABA-INs. Both correlations fit with the idea we proposed earlier, namely that dampening the PFC-to-dorsal striatum pathway ameliorates ABA by suppressing the food restriction-evoked hyperactivity (Santiago et al., 2021, DOI: 10.1093/cercor/bhaa394). Levels of NR2B at excitatory synapses of GABA-INs correlated positively with food consumption of the 30mg/kg group during recovery from ABA1 and ABA2, indicating that boosting GABA-IN inhibition in the PFC promoted recovery of ABA animals by enhancing food consumption. Accordingly, body weight during recovery also correlated positively with NR2B levels at excitatory synapses on GABA-INs. The less efficacious 3mg/kg treatment yielded no correlations or opposite correlations for the corresponding variables. Conclusion: Thus, cell type-specific changes in NR2B-containing NMDA receptor levels provide cellular and molecular explanations for the action of the efficacious dose of ketamine.

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Poster

398. Cellular and Molecular Mechanisms of Reward Circuits

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Topic: G.02. Reward and Appetitive Learning and Memory

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SERB, GOI; CRG/2020/004971
SERB, GOI; CRG/2021/008295

Title: Gabaergic neurotransmission in lateral hypothalamus plays essential role in reward-seeking behaviour via epigenetic regulation of orexin gene expression

Authors: *N. PAWAR, S. BHUJBAL, A. SAKHARKAR;
Dept. of Biotech., Savitribai Phule Pune Univ., Pune, India

Abstract: Reward is the fundamental brain function that drives survival of species. Nose-poke operant conditioning is widely used to decipher the neural substrates of natural reward in rodents. The reward produced naturally is free from secondary effects of drugs of abuse. Lateral hypothalamus (LH) serves as motivational epicentre of the brain to seek reward. A range of classical studies uncovered cellular phenotypes in LH that encode and orchestrate behaviours encompassing the reward. However, the underlying molecular substrates that drive the interplay between neuropeptidergic subpopulations of neurons in LH engaged in motivation for reward are largely unknown. Chromatin remodelling plays an essential role in experience-dependent gene expression required for phenotypic outcomes. Herein, we have investigated the role for

GABAergic neurotransmission in coordinating the function of cocaine- and amphetamine regulated transcript peptide (CARTp)- and orexin-expressing cells via chromatin remodelling in the LH using nose-poke operant conditioning. Adult male Wistar rats (n=8) were trained for nose-poke activity to seek the sweet pellets followed by test session. The nose-poke conditioning increased protein and mRNA levels of CART and orexin in LH. Chromatin immunoprecipitation showed higher binding of G9a, a histone methyltransferase, and higher histone methylation (H3-K4me2) on both CART and orexin gene promoters in conditioned rats. CART siRNA (0.5 µg/µl) infusion in LH reduced the nose-poke activity, CART and orexin levels, and G9a binding and H3-K4me2 levels at orexin promoter. These results indicated the role for CARTp in the regulation of orexin function in LH. Interestingly, the CART and orexin are expressed in distinct subpopulation of cells, wherein the CART-positive neurons are GABAergic. Therefore, we further postulated the role for CART in GABAergic function, which in turn may regulate orexin cells. Co-infusion of muscimol, a GABA receptor agonist (50 ng/µl) rescued the effects of CART siRNA on nose-poke behaviour and epigenetic inhibition of orexin function. Moreover, infusion of bicuculline (200 ng/µl), a GABA receptor antagonist, in LH significantly lowered nose poke activity, orexin levels, and G9a binding and H3-K4me2 levels on orexin promoters. Hence, it may be suggested that the CARTp potentiate GABA function in LH and thereby regulate orexin expression via histone methylation. The study for the first time reports the novel role for GABAergic neurotransmission in epigenetic regulation of neuropeptidergic (orexin) function during reward and reinforcement.

Disclosures: **N. Pawar:** None. **S. Bhujbal:** None. **A. Sakharkar:** None.

Poster

398. Cellular and Molecular Mechanisms of Reward Circuits

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Title: Sucrose self-administration regulates circular RNA expression in the rat orbitofrontal cortex.

Authors: *K. R. DABROWSKI, G. FLORIS, A. GILLESPIE, S. E. SILLIVAN;
Ctr. for Substance Abuse Res., Lewis Katz Sch. of Medicine, Temple Univ., Philadelphia, PA

Abstract: The orbitofrontal cortex (OFC) is a vital component of the brain reward circuitry that is important for reward seeking behavior. Abnormalities in the functioning of the reward system have been associated with a range of psychiatric disorders, including major depressive disorder, substance use disorder, and schizophrenia. However, the molecular mechanisms underlying the function of the OFC in rewarding behavior are poorly understood. Moreover, delineating the

molecular pathways that sustain OFC-mediated reward will critically inform us of the neurobiology of motivation. In this study, we evaluated the role of a novel RNA species, circular RNAs (circRNAs), in reward seeking behavior in the context of a natural appetitive reward-sucrose. Using a microarray analysis, we identified 92 circRNAs differentially regulated in the OFC of male Sprague-Dawley rats that underwent sucrose self-administration (SSA) and validated 4 reward-associated circRNAs with qPCR in both male and female rats (n=12-15). Among these changes, we observed a significant downregulation of a circular RNA originating from neurexin 3 (Nrxn3), a gene involved in synaptogenesis, learning, and memory. mRNA levels of Nrxn3 were unchanged in the OFC by sucrose, suggesting that SSA differentially regulates the formation of circNrxn3 without impacting host gene expression. We evaluated the functional role of OFC circNrxn3 in reward seeking behavior by performing in vivo siRNA-mediated knock-down of circNrxn3 in the OFC of male Sprague-Dawley rats (n=9) prior to SSA. Knock-down of circNrxn3 in the OFC led to increased reward seeking behavior during sucrose self-administration on both fixed ratio 1 (FR1) and progressive ratio (PR) schedule in comparison with a scrambled siRNA control. Thus, we conclude that SSA regulates the expression of OFC circular RNA splice variants to support reward seeking behavior.

Disclosures: **K.R. Dabrowski:** None. **G. Floris:** None. **A. Gillespie:** None. **S.E. Sillivan:** None.

Poster

398. Cellular and Molecular Mechanisms of Reward Circuits

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 398.06

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant DA014241-18

Title: Activity of a direct VTA to VP GABA pathway encodes unconditioned reward value

Authors: *K. KIM[1,2], W. ZHOU[2], M. R. PICCIOTTO[2];
[1]Interdepartmental Neurosci. Program, [2]Dept. of Psychiatry, Yale Univ., New Haven, CT

Abstract: The ventral tegmental area (VTA) plays a central role in reward-motivated behaviors. Within the VTA, dopamine neurons have been thought to be the main mediators of reward learning by encoding reward prediction error and motivation to obtain rewards. While less is known about the function of VTA GABA neurons, increasing evidence suggests that these neurons also play a critical role in reward. In the current study, we show that a primary target of VTA GABA projection neurons is the ventral pallidum (VP). We used fiber photometry to record calcium fluorescence as a readout of neuronal activity in these GABAergic VTA to VP projections during various reward-seeking tasks. The genetically-encoded calcium indicator GCaMP7s was injected into the VTA of GAD65-Cre mice, and an optic fiber was implanted above the VP to record selectively from VTA terminal fibers. Recordings were performed while

the mice were trained in a cue-reward task for 5 sessions. In this cue-reward task, a tone was presented at a random intertrial interval of 30 seconds on average. The mice earned a reward (Ensure) if a nose poke was made within 5 seconds of the tone. VTA to VP GABA neurons were consistently active in response to retrieving the unconditioned reward. This reward signal was present from the first training session and was invariant across further training. We next varied the size of the reward in an FR1 task, where each nose poke resulted in a reward of a random, variable amount. The activity of VTA to VP GABA projections increased with greater reward sizes. We further tested if activity in these neurons would change when the palatability of the reward was varied, and found that the activity increased with palatability. Taken together, these experiments revealed that VTA GABA neurons projecting to the VP respond consistently to a primary, unconditioned reward and that the magnitude of this response is positively correlated with size and palatability of the reward. Furthermore, distinct from dopamine prediction error-like signals, the response of this pathway to primary reward does not change as animals learn that a cue predicts reward availability.

Disclosures: K. Kim: None. **W. Zhou:** None. **M.R. Picciotto:** None.

Poster

398. Cellular and Molecular Mechanisms of Reward Circuits

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 398.07

Topic: G.02. Reward and Appetitive Learning and Memory

Support: BBRF NARSAD grant 27735

Title: Tachykinin1-expressing neurons in the habenula encode reward outcomes

Authors: K. SUZUKI, B. HOLCOMB, *E. L. SYLWESTRAK;
Inst. of Neurosci., Univ. of Oregon, Eugene, OR

Abstract: Learning which actions lead to reward is a fundamental task of the nervous system, represented in many structures across the mammalian brain. One node in this interconnected reward network is the habenula complex, an evolutionarily conserved epithalamic structure and neuroanatomical hub that links the limbic forebrain to midbrain neuromodulatory systems, including dopaminergic and serotonergic pathways. The lateral habenula (LHb) is known for its role in processing aversive stimuli and reward prediction errors. The function of the medial habenula (MHb) is less understood. Several studies have linked the MHb to stress, anxiety, fear, addiction, novelty preference, and reward, but how the habenula segregates these behaviors—or the extent of crosstalk between information streams—is not clear. One way the nervous system organizes information about different behavioral processes is by utilizing subsets of cells for behaviors, with cell types characterized by distinct molecular signature, connectivity, morphology, or physiology. In this way, genetically- or anatomically- defined subnetworks can carry stimulus-specific information. The habenula shows rich transcriptional diversity, including

many neuromodulatory- and neuropeptide-related genes, which we propose provides a substrate for cell type-to-function mapping. Using fiber photometry recording in mice performing a reward-guided task, we find that genetically- and anatomically-defined habenular cell types encode distinct aspects of reward, including reward-predictive cues, reward history, and reward outcome. Tachykinin1-expressing neurons (Tac1) consist of two functionally and anatomically defined groups: medial habenula Tac1 neurons ($Tac1^{MHb}$) respond to positive reward outcomes, whereas lateral habenula Tac1 neurons ($Tac1^{LHb}$) respond to negative reward outcomes. We find that $Tac1^{LHb}$ responses to negative outcomes are specific to expectation-driven computations; innately aversive stimuli fail to drive activity in $Tac1^{LHb}$ neurons. Neural activity at both positive and negative outcomes evolves over time as a result of the recent history of success or failure, enabling dynamic control of foraging behavior. Using axonal projection mapping and rabies transsynaptic input tracing, we identify the inputs and outputs of positive- and negative-encoding subtypes. Taken together, these data suggest that habenula neurons encode multiple aspects of reward responses and could serve as a mechanism to dynamically modulate motivated behavior and reward learning.

Disclosures: K. Suzuki: None. **B. Holcomb:** None. **E.L. Sylwestrak:** None.

Poster

398. Cellular and Molecular Mechanisms of Reward Circuits

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 398.08

Topic: G.02. Reward and Appetitive Learning and Memory

Support: R01 DA047443

Title: Ventral tegmental area glutamate cell types differentially signal reward value

Authors: *D. MCGOVERN[1], K. A. SILETTI[1], E. D. PRÉVOST[3], A. LY[3], A. PHILLIPS[2], D. H. ROOT[1];
[1]Univ. of Colorado Boulder, Univ. of Colorado Boulder, Boulder, CO; [2]Univ. of Colorado Boulder, Univ. of Colorado Boulder, Lafayette, CO; [3]Univ. of Colorado Boulder, Univ. of Colorado Boulder Dept. of Psychology and Neurosci., Boulder, CO

Abstract: The Ventral Tegmental Area (VTA) is a cellularly heterogeneous midbrain region that contributes to drug-seeking, reinforcement learning, stress, and motivated behavior. Previous literature has established that VTA dopamine neurons, defined by the expression of tyrosine hydroxylase (TH), are recruited for consummatory reward behaviors as well as reward value judgments and economic decision making in mice. However, recent data implicate glutamate neurons, defined by the expression of vesicular glutamate transporter 2 (VGluT2), in reward as well. Several subtypes of VGluT2 neurons intermingle within the VTA but their contributions toward reward are unknown.In these projects we used a combination of transgenic mice and intersectional or subtractive viral monitoring and manipulation strategies to characterize the

contribution of 1) VTA VGluT2+VGaT+ neurons 2) VTA VGluT2+VGaT- neurons 3) and VTA VGluT2+TH+ neurons to consummatory reward value and motivated behavior.Mice were injected with GCaMP6m respective to the cell-type of interest and population level changes in intracellular calcium were recorded using fiber photometry during a two-bottle choice consummatory reward task (sucrose, fat, saccharine). Escalating concentrations of sucrose (8%, 16%, 32%) were used to assess neuronal responses to changes in reward value. VTA glutamate cell-types, regardless of neurotransmitter co-expression, scaled calcium signaling relative to sucrose concentration. Behaviorally mice developed a preference for fat consumption over sucrose but interestingly the recorded calcium change for fat was significantly less in the VGluT2+VGaT+ population. The VGluT2+VGaT- population signaled more robustly for fat than sucrose, which suggests that subjective reward value may be more salient for this population of neurons. Next we adapted a behavioral economic paradigm to assess the causal role of VGluT2+VGaT+ co-expressing neurons in sucrose reward valuation. We artificially manipulated reward price by reducing the amount of sucrose delivered as the program elapsed to devalue effort for reward. Optical stimulation of VTA VGluT2+VGaT+ neurons increased responding for reward at higher price compared to controls. These results suggest VTA VGluT2+VGaT+ neurons can amplify sweet reward value. Further, this project provides novel insights into the functional contributions of genetically-distinct VTA glutamatergic cell-types in reward processing.

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Poster

398. Cellular and Molecular Mechanisms of Reward Circuits

Location: SDCC Halls B-H

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Topic: G.02. Reward and Appetitive Learning and Memory

Support: NINDS Intramural Program Grant NS003135

Title: Paradoxical hyperactivity and improved motor learning in NALCN conditional knockout mouse

Authors: *D. COBB-LEWIS[1,2], F. CLEVER[1], Z. M. KHALIQ[1];
[1]NINDS, NIH, Bethesda, MD; [2]Inst. for Neurosci., George Washington Univ., Washington, DC

Abstract: Striatal dopamine release has been implicated in reward learning, motivation, and motor function. While phasic dopamine is known to be critical for reinforcement learning, the contribution of tonically-released dopamine to behavior has not yet been fully determined. In past studies, the non-selective sodium leak channel NALCN has been shown to play an important role in driving tonic activity in numerous pacemaker neuron types including midbrain dopaminergic neurons. Here, we evaluate the role of tonic dopamine in behavior using

conditional knockout mice that lack expression of NALCN in dopamine neurons (NALCN cKO). Consistent with our previous work, we found that the vast majority of dopamine neurons in NALCN cKO mice displayed no pacemaker activity. Cells lacking expression of NALCN could be induced to fire normally following small depolarizing current injections suggesting normal cell health. Because knockout of NALCN in cKO mice occurs from birth, we tested in separate experiments induced NALCN knockout in adults with viral expression of Cre in adult conditional-potential NALCN mice (NALCN vKO). We also found that the majority of dopamine neurons in NALCN vKO mice were silent at baseline. Examining the behavior of NALCN cKO animals, we found that these mice were paradoxically hyperactive in an open field and showed improved performance on the rotarod as compared to littermate controls. NALCN cKO animals mice also exhibited an increase in approach/avoid interactions with a novel object. NALCN cKO mice showed normal anxiety-related behaviors and cocaine-induced locomotion. The paradoxical hyperdopaminergic phenotype is not due to alterations in dopamine axonal innervation of the striatum in NALCN cKO mice, as the axonal density as measured by TH staining is similar as in controls.

Disclosures: D. Cobb-Lewis: None. **F. Clever:** None. **Z.M. Khaliq:** None.

Poster

398. Cellular and Molecular Mechanisms of Reward Circuits

Location: SDCC Halls B-H

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Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant MH73136 (to TZB)
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George E. Hewitt Foundation for Medical Research Grant (to MTB)
British Society for Neuroendocrinology Grant (to MTB)

Title: A novel cell-specific pathway mediates reward deficits following early-life stress

Authors: *M. BIRNIE, A. K. SHORT, G. B. DE CAVALHO, B. G. GUNN, A. L. PHAM, C. A. ITOGA, X. XU, L. Y. CHEN, S. V. MAHLER, Y. CHEN, T. Z. BARAM;
Univ. of California-Irvine, Univ. of California-Irvine, Irvine, CA

Abstract: Background: Disrupted operation of reward circuits is thought to underlie several emotional disorders including depression and drug abuse, disorders commonly arising after early-life stress. Yet, how early-life adversities (ELA) impact the functional maturation of reward circuitries to promote disease remains unclear. The nucleus accumbens (NAc) is a major component of the reward circuit and key structure mediating pleasure, motivation, and emotional processes. Multiple inputs converge onto the NAc to modulate reward behaviors, including the basolateral amygdala (BLA). The BLA mediates associative learning for aversive and appetitive

stimuli, and stimulation of glutamatergic projections from the BLA to NAc promotes appetitive behaviors. Here, we identified a novel projection that expresses the stress neuropeptide corticotropin-releasing hormone (CRH) to connect the basolateral amygdala (BLA) and nucleus accumbens (NAc). In the NAc, CRH+ axon terminals modulate reward and motivational behaviors. Here, we identify the role of this CRH+ BLA-NAc projection during reward in naïve and ELA mice. Methods: Pairing viral-genetic approaches with CRH-IRES-Cre mice and Cre-dependent viruses, we identified CRH+ BLA projections to the NAc. To determine the function of this novel CRH+ BLA-NAc projection we used chemo-, optogenetic and electrophysiology strategies in control (CTL) and ELA mice. In these mice, excitatory or inhibitory Cre-dependent DREADDs and optogenetic viruses were injected into BLA, followed by medial NAc shell targeted microinjections of CNO or light activation. In behavior, we tested the function of this pathway using reward, and non-reward tasks. Results: Male ELA mice have reduced preference for sucrose, palatable food, and a sex-cue, compared with CTLs. Viral-genetic tracing combined with electrophysiology identified a novel GABAergic projection that co-expresses the stress neuropeptide CRH from the BLA to the medial NAc shell. In freely behaving mice, exciting this projection using chemo- and optogenetic techniques reduced preference for sucrose, palatable food, and a sex-cue, but did not alter non-reward-mediating tasks. In adult ELA mice, chemogenetic inhibition of the GABAergic CRH+ BLA-NAc projection rescued these reward behaviors. Conclusions: Here, we identify a novel GABAergic CRH+ BLA-NAc projection and establish its role in mediating the effects of stress on reward behavior. These discoveries provide potential selective targets for prevention and intervention in the disruption of such behavior that accompanies several psychopathologies.

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Poster

398. Cellular and Molecular Mechanisms of Reward Circuits

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 398.11

Topic: G.02. Reward and Appetitive Learning and Memory

Support: Vulnerable Brain Project Grant
NIH Grant 2R25NS080686
NSF Grant DBI-1950649
NIH Grant P30 EY13079

Title: Prevalence of Mu-opioid receptors on dopaminergic axons of nucleus accumbens medial shell correlates negatively with voluntary exercise of adult mice: a dual EM-ICC study

Authors: C. J. AOKI, I. PAT-OSAGIE;
Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Exercise is widely accepted to be beneficial for health, mood and cognition, but it is challenging to convert sedentary adults to becoming regular exercisers. Conversely, over-exercise can be life-threatening to individuals diagnosed with anorexia nervosa (AN), but many AN patients have difficulty following doctor's order to stop exercising. These observations indicate than an individual's voluntary exercise level is rather stable. What brain circuits may underlie the stable individual differences in the extent of voluntary exercise? Nucleus accumbens (NAcb) is a brain region richly innervated by dopaminergic axons, heavily endowed with mu-opioid receptors (MOR) and known to play a role of encoding appetitive stimuli through the rise of dopamine. We tested the hypothesis that individual differences in exercise, measured as voluntary wheel running, is influenced by the level of dopamine in the medial shell of NAcb during wheel running which, in turn, is suppressively regulated by mu-opioid receptors (MOR) on dopaminergic axons. We also asked whether exposure to stress or exercising opportunity during adolescence might influence an individual's propensity for voluntary exercise later in life. These ideas were tested by exposing singly-housed mid-adolescence mice to a wheel, food-restriction stress (FR), or a combination of a wheel access and food scarcity for 3-4 days, followed by a 2-3 month period of housing in a standard cage without a wheel and with ad libitum food. A wheel was then installed in their home cage for 10 to 26 days, with wheel running activity monitored continuously (Med Associates, ENV-044). Following euthanasia, dual electron microscopic immunocytochemistry was employed to quantify the extent of MOR expression on dopaminergic axons in the shell of NAcb using mouse anti-tyrosine hydroxylase (Sigma #MAB318) and rabbit anti-MOR (ImmunoStar #24216). For the animals that underwent FR as adolescents, voluntary wheel running reached a plateau after 8 days. The plateau value of the last 18 days before euthanasia varied 5-fold across individuals. The expression of MOR on dopaminergic axons correlated negatively with the plateau value of wheel running ($R=-0.90$, $p=0.014$, $N=6$, 4 males and 2 females). This finding supports the hypothesis that endorphins, released during exercise, activate MOR, and this reduces excitability of dopaminergic axons, thereby reducing dopamine release, which blunts the strength of appetitive stimuli, ultimately reducing voluntary wheel running. This result will be compared to the correlations observed for the animals that underwent the adolescent experience of wheel exposure and the combination of FR and wheel.

Disclosures: C.J. Aoki: None. **I. Pat-Osagie:** None.

Poster

398. Cellular and Molecular Mechanisms of Reward Circuits

Location: SDCC Halls B-H

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Program #/Poster #: 398.12

Topic: G.02. Reward and Appetitive Learning and Memory

Support: R01 DA047981
T32 DA00726

Title: Regulation of reward-related behaviors by D1 receptors in the main intercalated cell cluster of the amygdala

Authors: *M. ANDRUS[1], E. S. KIM[1], M. LATTAL[2];
[1]Oregon Hlth. & Sci. Univ., Portland, OR; [2]Behavioral Neurosci., OREGON HEALTH & SCIENCE UNIVERSITY, Portland, OR

Abstract: The main intercalated cell mass (mITC) is a cluster of GABAergic neurons situated between the basolateral and central nuclei of the amygdala. To date, the mITC has been primarily characterized in its role in regulating fear-associated learning. Very few, if any, investigations have assessed the role of the mITC in appetitive contexts. The mITC is a site of D1-dopamine receptor expression, which indicates a potential role in appetitive processes. We used optogenetic approaches in male and female D1-Cre transgenic Long Evans rats to evaluate the role of D1-receptor expressing ITC cells in appetitive learning. We conducted two studies, one transfected a Cre-dependent channelrhodopsin and another using archaerhodopsin. Injections were made into the mITC bilaterally (ML: ±4.6, DV: -8.3, AP: -1.88), and fiber optic cannulas were implanted in the same surgery. Surgeries were done with the same virus in both Cre+ and Cre- rats in each of the studies. Rats were trained to perform a Seek-Take chain lever-pressing task for food reward. This task results in a high level of stable lever pressing across sessions, with presses on a Seek lever (variable interval 10 s schedule) leading to the insertion of a Take lever. A press on the Take lever causes a food pellet to be delivered to the subject. To assess the contribution of D1-expressing mITC cells to reward seeking, in 30 min sessions, we programmed laser stimulation to occur with every Seek press during light-cued 5-minute windows, alternating with 5-minute cue-off windows with no stimulation. In the channelrhodopsin rats, Cre+, but not Cre-, subjects increased their Seek-lever responding during cue periods. This effect was seen both when food reward continued to be delivered and in reward extinction, when the Take lever yielded no food pellet delivery. Inhibition of the D1-ITCs had no effect on reward seeking, but impaired fear extinction in a follow-up experiment. There are several possible mechanisms that could explain these findings (e.g., cue-reward associations, an unconditioned rewarding property of D1-ITCs stimulation, inhibition of anxiety/fear circuitry) that can be clarified by deeper investigation into microcircuit dynamics.

Disclosures: M. Andrus: None. **E.S. Kim:** None. **M. Lattal:** None.

Poster

398. Cellular and Molecular Mechanisms of Reward Circuits

Location: SDCC Halls B-H

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Program #/Poster #: 398.13

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIEHS R15ES029673

Title: The paternal western diet results in changes in brain mitochondrial proteome and microRNAs linked to transgenerational increases in offspring feeding behavior.

Authors: *A. K. MURASHOV[1], E. S. PAK[1], J. MAR[2], K. FISHER-WELLMAN[1], K. M. BHAT[3];
[1]East Carolina Univ., Greenville, NC; [2]Univ. of South Florida, Tampa, NC; [3]Univ. of South Florida, Tampa, FL

Abstract: A large part of the childhood obesity epidemic can be attributed to the lifestyles and eating habits of parents. The clustering of obesity in families suggests that culture, genetics, and epigenetics all work together to influence children's eating habits. We tested whether epigenetic factors, such as ancestral diet, can influence offspring feeding behavior in the current study using a fruit fly model.
For the study, we focused on preconception paternal effects in order to narrow down the range of possible mechanisms to those transmitted by gametes. This study shows that ancestral Western diet (WD) increases the offspring's feeding behavior, with concomitant alterations in locomotor activity, triglycerides, and mitochondrial density. According to our findings, these changes were linked to proteome remodeling and miRNA alterations. The study demonstrated that ancestral nutrition may play a role in programming obesity-risk behaviors and might provide insight into mechanisms of familial susceptibility to obesity and the obesity epidemic in general.

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Poster

398. Cellular and Molecular Mechanisms of Reward Circuits

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 398.14

Topic: B.09. Glial Mechanisms

Title: Single-cell Multiomic Profiling of Orbitofrontal cortex in Extended Cocaine Self-Administration Withdrawal

Authors: *S. FULTON[1], J. J. TUSCHER[2], R. A. PHILLIPS, III[4], A. LEPACK[5], J. J. DAY[3], I. MAZE[1];
[1]Icahn Sch. of Med. At Mount Sinai Grad. Training Program In Neurosci., New York, NY; [3]Neurobio., [2]Univ. of Alabama At Birmingham, Birmingham, AL; [4]Neurobio., Univ. of Alabama at Birmingham, Birmingham, AL; [5]Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: The pathophysiology of Cocaine use disorder (CUD) involves persistent neuroadaptations in Orbitofrontal Cortex (OFC), a brain region critically involved in guiding reward-based decision making and motivated behavior. Functional studies in both humans and animal models have revealed that aberrant hyperactivation in the OFC modulates compulsive drug-taking behaviors and cocaine relapse vulnerability in CUD, impairing the reward circuitry's

capacity to self-regulate. This increased OFC excitability is of high clinical relevance for the development of treatments that might address the high rates of relapse in CUD patients. However, to date, there have been no studies examining the precise cell-type specific mechanisms involved in OFC dysfunction during chronic cocaine use**.**
Although CUD has been predominantly studied in the context of neuronal function, emerging evidence indicates that dysregulation of glia may be equally important. In particular, withdrawal from chronic cocaine use represents a state of sustained physiological stress that induces chronic glial inflammatory signaling, which in turn drives aberrant activity in the surrounding neurocircuitry. This increased inflammatory state involves epigenetic regulatory mechanisms that maintain these activated states even after the initial cocaine-induced signal has waned, leading to persistent deficits in the control of motivated behavior.
Although very recent work has begun to shed some light on the importance of glial inflammation in ventral striatum during drug dependence, little is known about how these mechanisms contribute to CUD in other reward-related regions. In order to understand how inflammation stress and glial cell plasticity directly affects OFC function in CUD, we used an extended access cocaine intravenous self-administration (SA) model to profile both chromatin and transcriptional alterations in the OFC with multiomic snRNA-seq and snATAC-seq. This data set allows us to identify and characterize heterogenous glial activation states, as well as functional alterations in specific neuronal populations that may emerge CUD. Transcriptomic profiling combined with network analysis of these different activation states will facilitate understanding of the cell-type regulatory switches controlling these plastic changes to evaluate therapeutic potential in substance dependence disorders. Overall, these data represent a first ever comprehensive, cell-type specific profile of chromatin accessibility and gene expression patterns in OFC following extended abstinence from cocaine SA.

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Poster

399. Fear, Anxiety, and Pain

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 399.01

Topic: G.04. Emotion

Support: MSCA-IEF-ST 743685

Title: Commanding or being a simple intermediary: how does it affect moral behavior and related brain mechanisms?

Authors: *K. IOUMPA[1], E. CASPAR[1,2], V. GAZZOLA[1], C. KEYSERS[1];
[1]Netherlands Inst. for Neurosci., Netherlands Inst. for Neurosci., Amsterdam, Netherlands;
[2]Dept. of Exptl. Psychology, Ghent Univ., Ghent, Belgium

Abstract: Fractioning operations between several individuals along a hierarchical chain is commonly found in the way organizations function. A superior communicates a plan and a subordinate executes it. The superior then bears responsibility for the decision but is distanced from the outcomes, while the subordinate experiences authorship over the action but may experience reduced responsibility for its outcomes. As the superiors are often following instructions themselves, they also end up in intermediary roles. Experimental research has shown that this fractioning allows diffusing responsibility between components of the chain, which can disinhibit the commission of actions that harm others. However, the neural mechanisms by which being in the intermediary or commanding position disinhibit harming others remains largely unknown. Here we conducted two studies, one using fMRI and one using EEG, designed to help understand how commanding or being in an intermediary position impacts the sense of agency and the processing of victim's pain. In the age of military drones, we also explored whether commanding a human or robot agent influences these processes. In one condition, participants in our paradigm could freely decide whether or not to give an order to an agent to send painful shocks to another participant in exchange for money. In another condition participants were intermediary and received orders to order shocks or not from someone higher in the hierarchy. fMRI results revealed that activation in social cognition and empathy-related brain regions (as IFG, IPL and SII) when witnessing a victim receiving a painful shock was equally low while participants were commander or intermediary transmitting an order, with both being lower than when being the agent directly delivering the shock. EEG results showed that the neural response over P3, which is sensitive to the observation of pain in others, was higher when the executing agent was a robot compared to a human. Source reconstruction of the EEG signal revealed that this effect was mediated by areas including the insula and ACC. The sense of agency did not differ between commanders and intermediaries, no matter if the executing agent was a robot or a human. Summarizing, being a commander or intermediary seemed to reduce processing the pain of the other compared to being the agent administering the pain. Commanding a human executor led to reduced responsibility and activation compared to commanding a robot executor but not the sense of agency. These results shed some more light on how hierarchical situations can facilitate the commission of actions that harm others as responsibility is reduced and split across multiple individuals.

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Poster

399. Fear, Anxiety, and Pain

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Program #/Poster #: 399.02

Topic: G.04. Emotion

Support: University of San diego Summer Undergraduate Research Experience

Title: Rapid social transfer of pain in rats

Authors: *J. A. JOHNSON[1], C. A. BOUSTANI[2], M. FRANCO[2], E. FOLEY[2], R. STRICKLAND[3], J. M. WENZEL[4], M. L. SMITH[5];
[1]Univ. of San Diego, La Mesa, CA; [2]Univ. of San Diego, San Diego, CA; [3]Univ. of San Diego, San diego, CA; [4]Psychological Sci., Univ. of Maryland Sch. of Med., San Diego, CA; [5]Psychological Sci., UCSD, San Diego, CA

Abstract: Social behavior is critical to the survival and health of social species, ranging from rodents to humans. Empathy, or the adoption of another's sensory and/or affective state, is a core social ability. Historically, empathy was considered a "high level" process dedicated to humans, but it is now appreciated that evolutionarily conserved antecedents of empathy are evident in many species, including rodents. For example, we recently demonstrated the rapid social transfer of pain in mice, where *bystander* mice adopt the sensory and emotional state of a social partner, a key component of empathy. The aim of the current studies was to replicate this phenomenon and fully characterize the social transfer of pain in the rat. During the social transfer of pain, bystander rats socially interacted with a familiar, sex-matched partner that was experiencing inflammatory pain (due to an hindpaw injection of Complete Freunds Adjuvant; CFA). Following a one-hour social interaction, bystanders demonstrated enhanced pain behavior that was similar to the CFA-injected rats. These studies demonstrate that rats display the social transfer of pain similarly to mice, and expand the ability to explore empathy in rodent models. Rodent models of empathy are integral to understanding the neurobiology of empathy and eventually addressing social deficits in those with dysregulated empathy.

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Poster

399. Fear, Anxiety, and Pain

Location: SDCC Halls B-H

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Program #/Poster #: 399.03

Topic: G.04. Emotion

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Title: Comparing neural networks responding to vocal affective signals and their musical imitations

Authors: *C. TREVOR[1], N. B. FERNANDEZ[1,2], S. FRÜHHOLZ[1,3];
[1]Univ. of Zurich, Zurich, Switzerland; [2]Univ. of Geneva, Geneva, Switzerland; [3]Univ. of Oslo, Oslo, Norway

Abstract: Some music cognition researchers theorize that music communicates emotion by mimicking affective vocal signals, such as crying, screaming, or laughing. For example,

musicologists often describe music used to underscore frightening scenes in movies as sounding "scream-like". A well-known example is the music Bernard Herrmann wrote to accompany the infamous shower murder scene in Hitchcock's film Psycho. Although 'scream-like' is a common descriptor, the question remains: are these scary film soundtrack excerpts actually perceived similarly to human screams? We investigated this question by comparing the neural networks responding to a vocal cue (a scream) and its musical imitation (scream-like music). Thirty-two healthy and non-musician participants from the University of Zürich (18 female, age: M = 27; SD = 5.46) took part in the fMRI study. Participants performed a 1-back task on auditory stimuli (total of 480 trials) presented in a pseudorandomized order. We categorized the stimuli by affect (scream-like vs non-scream-like) and sound type (vocal vs musical) in a 2 × 2 factorial design. Contrast results show that compared with music, vocal sounds provoked stronger activations in higher-order areas of the auditory cortices and in the amygdala. Compared with non-scream-like sounds, scream-like sounds elicit higher activations in lower-order areas of the auditory cortices and in areas associated with fear processing, survival circuits, and startle responses. Directly comparing screams and scream-like music demonstrates stronger activations in the primary auditory cortex and cerebellum for screams. These results suggest that the original vocal signal (the scream) is processed by lower-order areas of the brain compared with the musical version (scream-like music), suggesting that it is potentially the more powerful or potent signal of the two. These results are consistent with a previous study that showed that while screams and scream-like music share a crucial acoustic signal communicating fear, screams present a more potent version of that signal. Therefore, while music might mimic vocal cues successfully, the original cues are more effectual, perhaps due to their direct biological significance. Our results contribute to research on musical mimicry of vocal signals, on how the brain processes fearful sounds and the voice versus music, and to investigations of music and emotion more broadly.

Disclosures: C. Trevor: None. **N.B. Fernandez:** None. **S. Frühholz:** None.

Poster

399. Fear, Anxiety, and Pain

Location: SDCC Halls B-H

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Topic: G.04. Emotion

Support: NRF Grant 2022M3E5E8081183

Title: The Involvement of Parabrachial CGRP Neurons in the Fear Learning to the Emotional Pain without Nociception

Authors: *J. HAN, J.-H. HAN;
Korea Advanced Inst. of Sci. and Technol., Daejeon, Korea, Republic of

Abstract: Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Given the definition of pain, pain is related to the physical

sensation resulting from nociception. However, we experience suffering without nociception and tissue damage, and many clinical cases reported that people suffer from emotional pain even without sensory pain. Despite the need for studies on emotional pain, little is known about it. Especially in animal models, it is challenging to study the affective aspect of pain due to the multidimensionality of the pain, which contains both a sensory and an affective dimension. In this study, we tested whether the emotional pain without nociception forms fear memory in the mouse model. To test this, we used a looming visual threat, the rapidly expanding dark disc on display above the head. As previously reported, we observed that looming threats induced a freezing response in the mice. When we used the looming threat as an unconditioned stimulus (US) and paired it with a neutral tone, the mice in the paired group showed a strong freezing response to the conditioned tone compared to the unpaired mice group. Also, the mice showed elevated freezing levels when exposed to the conditioning chamber two days after pairings. This result reveals the emotional pain induced by a looming threat is enough to form both auditory and contextual fear memory. Next, we investigated the brain region which conveys the US information about emotional pain. The parabrachial nucleus (PBN) is one of the major sensory pain pathways, and it conveys nociceptive input to the amygdala. Also, calcitonin gene-related peptide (CGRP) positive neurons in the PBN has a role in aversive learning to noxious stimuli. To test whether the PBN CGRP+ neurons are involved in processing emotional pain, we specifically expressed tetanus toxin (TetTox) in the CGRP+ neurons by using AAV1-hsyn-DIO-TetTox-nls-dTomato virus and the calca-cre transgenic mice. We observed that the specific silencing of synaptic transmission of the PBN CGRP+ neurons reduced the freezing level of the mice to both the auditory and contextual cues, indicating that the PBN CGRP+ neurons have a role in forming fear memory to the emotional pain. Our results verify that emotional pain without sensory pain works as the US to form fear memory and also reveal that PBN CGRP+ neurons are involved in processing emotional pain information in fear learning. Our fear learning paradigm using looming threats will help assess the emotional pain in the animal models, leading to a better understanding of neural circuits involved in emotional pain and the affective dimension of pain processing.

Disclosures: **J. Han:** None. **J. Han:** None.

Poster

399. Fear, Anxiety, and Pain

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Program #/Poster #: 399.05

Topic: G.04. Emotion

Support: Universidad Michoacana de San Nicolás de Hidalgo (UMSNH)
Coordinación de la Investigación Cientifica (CIC)

Title: Anxiolytic effect of ethanol and Chlorazepate on behavior activity in rats

Authors: *L. MANZO[1], A. TAFOLLA[2,3], D. LOPEZ[2];
[1]Lidia Manzo, Univ. Michoacana De San Nicolas De Hidalgo, Morelia, Mexico; [2]Univ. Michoacana de San Nicolas de Hidalgo, Morelia, Mexico; [3]Postgrado, Univ. Latina de America, Morelia, Mexico

Abstract: The origin to addictive behavior is often linked to the rewarding effects of drugs of abuse. The exposure to reward uncertainty typical of training can reduce emocional self-medication (ESM) in rats with high levels of anxiety. We demonstrated, locomotion and anxiety behavior in Wistar (nonselected) male rats exposed to 32% to 4% sucrose downshift event in a consummatory successive negative contrast (cSNC) situation were given a two bottles, 2 h preference test immediately after consummatory training, and finally to the Hole-Board (45cm x 45 cm) to assess locomotor activity and anxiety behavior. One of the two bottles contained 2% of ethanol, 1.5 mg/kg of chlorazepate or water different groups (the second bottle contained water for all groups), a benzodiazepine with and addictive profile used in the treatmen in the anxiety disorders. Because ethanol and Chlorazepate has anxiolytic properties in tasks involving reward loss, oral consumption after extinction sessions was interpreted as anti-anxiety or ESM. Three additional groups received the same postsession preference tests, but were always exposed to 4% of sucrose during consummatory training. Rats showed the cSNC effect, suppressing consummatory behavior after the downshift relative to unshifted controls. This effect was accompanied by a selective increased of ethanol oral intake during the initial downshift sessions. Such increased fluid preference did not occur in animals with access to water or unshifted controls groups with postsession to the anxiolytics during the postsession test. Our results suggest that ESM may reverse symptoms of anxiety in animal model. The effect ESM increased locomotion activity and head deeping in the Hole-Board relative to water controls. These findings providing insights into a better understanding of early stages of addictive behavior.

Disclosures: L. Manzo: None. **A. Tafolla:** None. **D. Lopez:** None.

Poster

399. Fear, Anxiety, and Pain

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Topic: G.04. Emotion

Support: NSERC
CFI
Canadian Pain Society

Title: Extended amygdala kindling alters pain sensitivity and pain-related emotional behaviours

Authors: E. XIAO, G. EKINS, K. MOZESSOHN, K. MACDONALD, ***N. M. FOURNIER**; Psychology, Trent Univ., Peterborough, ON, Canada

Abstract: A surprising number of pain conditions have been reported to occur alongside epilepsy, including chronic headache disorders, migraines, neuropathic pain, and fibromyalgia. These pain conditions may occur at a much higher rate than the general population. However, few studies have directly examined the issue of pain sensitivity in epilepsy and as a result important information regarding prevalence, diagnosis and treatment remains largely unknown. Kindling is the process by which daily administration of electrical stimulations to a particular brain region results in the gradual development and intensification of motor seizures. We found that amygdala kindling produces long-lasting increases in fear and anxiety-related behaviour in rats. Interestingly, there is evidence that many of the same neural circuits impacted amygdala kindling are also involved in processing nociceptive or pain information. This has led us to hypothesize that recurrent kindled seizures might sensitize brain regions that regulate pain responses, which in turn could lead to impairments in the processing of sensory and affective features of pain. In the present study, Long-Evans rats underwent short-term (30 stim) and extended (99 stim) amygdala kindling. At various time points (pre-kindling, 24 hrs, 48 hrs, and 1 week post-kindling), we conducted sensory/reflexive nociceptive measurements, including von Frey hair stimulation, Hargreaves test for noxious stimulation. In addition, we also examined the affective component of pain by training animals in the formalin-induced conditioned place aversion task.Our preliminary results found evidence that initial nociceptive thresholds are significantly elevated for both kindled groups for the 24-hr period immediately following the last kindling stimulation. However, rats that underwent 99 kindled stimulations showed a reduction in nociceptive threshold and evidence of hyperalgesia when examined 1-week post-kindling. Importantly, while all rats showed aversion to the formalin-paired chamber when a high concentration of formalin was used. However long-term kindled rats showed higher levels of aversion to this chamber even when a suboptimal formalin concentration was given—suggesting that kindling leads affective hypersensitivity to painful stimuli. Additional experiments are being performed to examine the neurobiological mechanisms that may underlie this enhanced responsiveness to nociceptive stimulation.

Disclosures: E. Xiao: None. **G. Ekins:** None. **K. Mozessohn:** None. **K. MacDonald:** None. **N.M. Fournier:** None.

Poster

399. Fear, Anxiety, and Pain

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Topic: G.04. Emotion

Support: NIH MH115027

Title: Complementary Roles for the Medial and Orbito-Frontal Cortex in Learning of Probabilistic Punishment

Authors: *D. JACOBS[1], A. BOGACHUK[2], B. MOGHADDAM[3];
[1]Oregon Hlth. & Sci. Univ., Portland, OR; [2]Behavioral Neurosci., Oregon Hlth. & Sci. Univ. (PO# 770008576), Portland, OR; [3]Behavioral Neurosci., Oregon Hlth. & Sci. Univ. Behavioral Neurosci., Portland, OR

Abstract: Conflict between approach and avoidance is a common source of anxiety. In the real world, resolving conflict between approaching rewards and avoiding risk of harm is not only critical for survival but is compromised in mood disorders. While there have been recent advances in assessing this relationship, the neuronal basis of how this mode of learned anxiety during motivated behavior remains poorly understood. We modeled this form of anxiety during reward seeking by training male and female adult Long-Evans rats in a task where chained instrumental responses were either safe or associated with varying probabilities of punishment (Jacobs & Moghaddam, 2020). The "risky" seek link of the chain was probabilistically punished by mild foot shock, while the "safe" take link of the chain deterministically resulted in reward delivery. We used fiber photometry to record neural calcium activity in two regions of the prefrontal cortex, the dorsomedial prefrontal cortex (dmPFC) and orbitofrontal cortex (OFC), to understand which aspects of reward motivated behavior may be differentially encoded under this model of anxiety. Significant differences were determined by permutation tests or analysis of variance comparing activity to no risk periods. An increase in probabilistic punishment produced anxiety-like suppression of reward seeking behavior. OFC and dmPFC modified their response differently during safe versus risky actions: the OFC reduced its response to safe actions and punishing outcomes, whereas the dmPFC selectively modified its response to risky actions. These changes in neural calcium activity generally returned to baseline when punishment risk was removed. Overall, we find that prefrontal cortex subregions play unique and complementary roles in adapting to anxiety during reward seeking. While the dmPFC may be important in detecting punishment contingencies, the OFC may update rewarded actions and punisher encoding after risky contingencies are learned. These findings provide mechanistic insight about changes in cortical networks that underlie learned anxiety.

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Poster

399. Fear, Anxiety, and Pain

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Support: Fonds de Recherche du Québec - Société et Culture (FRQSC)
Fonds de recherche du Québec - Santé (FRQS)

Title: The Efficacy of Playing Preferred Game on Pain Inhibition

Authors: *Z. DELDAR[1], S. DESJARDINS[1], S. DUMAS[1], Z. ARVANITIS[1], E. GOODMAN-VINCENT[1], N. KHALILI-MAHANI[2], M. ROY[1];
[1]McGill, Montreal, QC, Canada; [2]Concordia Univ., Montreal, QC, Canada

Abstract: **Introduction/Aim:** Engaging in a cognitively demanding task may distract us from painful stimuli and induce analgesia. However, most laboratory tasks are unpleasant due to their repetitive and boring nature. Furthermore, according to the Affective Game Planning for Health Applications model, pain is stressful, and it is essential to consider that interventions should not become an additional stressful condition. By contrast, video games are designed to trigger strong emotions, establish high levels of enjoyment and cause distraction. As the combined effect of enjoyment and distraction on pain reduction remain largely unexplored, we aimed to assess the impact of a gamified approach on pain inhibition and quantify inter-individual differences' role in game preference.
Methods: Sixty-four healthy young volunteers played their favorite digital game, their least favorite digital game, the 2-back task (demanding cognitive task) and a left-right task (easy cognitive task) while receiving thermal pain stimulations. Participants reported their pain intensity (visual rating scale (0-100)) as well as completed the game experience questionnaire (assesses their game experience based on the seven components of Immersion, Flow, Competence, Positive Affect, Negative Affect, Tension, and Challenge) after finishing each task.
Results: A repeated-measure ANOVA revealed that playing the preferred game produced significantly greater analgesia than completing the 2-back and Left-Right tasks ($p<0.001$). Moreover, flow and positive affect mediated the association between playing the preferred game and pain intensity.
Discussion/Conclusion: These results revealed that games are more effective in producing analgesia than most laboratory tasks, and preferred games are more effective than non-preferred games. Moreover, these effects are mediated by experiencing more flow and more positive affect. In fact, people can identify their preferred games, and playing their preferred games amplifies analgesia. These findings align with the recently proposed framework (Affective Game Planning for Health Applications), which emphasizes minimizing game stress when designing games for health applications. Future studies and interventions need to consider inter-individual differences in preference on pain inhibition and the efficacy of cognitively distracting interventions on pain.

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Poster

399. Fear, Anxiety, and Pain

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Title: Anxiety-related behavior in adult mice after early-life adversity

Authors: *K. TEGANG, S. C.-H. LIM, R. HEN, W.-L. CHANG;
Columbia Univ. and New York State Psychiatric Inst., New York, NY

Abstract: Background: Early life adversity (ELA), including neglect, extreme poverty, racism, and trauma, impacts the risk of developing psychiatric illness later in life. In fact, humans exposed to ELA are at higher risk of developing anxiety disorders and other disorders that include anxiety symptoms in adulthood. Several groups have shown that ELA can be induced in rodents by exposing dams and their pups to limited bedding and nesting materials during the early post-natal period. Innate anxiety-related behavior can then be tested in the open field test (OFT), where mice tend to avoid the central zone of the arena. This has traditionally been quantified as a binary "center" vs. "periphery" score. In this study, we tested adult mice in the open field after ELA. We then examine the behavioral data with continuous measures in addition to evaluating their exploration of the center or periphery.
Methods: c57BL/6J mice and mice on a c57BL/6J background were paired, and then females (all first-time dams) were single-housed prior to litters being born. On post-natal day 4 (P4), ELA dams and pups were transferred to a home cage, with a wire mesh bottom and half as much cotton nestlet as usual, where they remained for 1 week. On P11, they were returned to standard housing conditions. Control dams and litters remained in the standard housing conditions throughout. On P21, all mice were weaned and housed in standard conditions until adulthood. The mice in this study underwent viral injection and implantation of microendoscopes for calcium imaging (data not presented). Mice were subjected to the OFT at 12-16 weeks of age and allowed to freely explore the open arena for 10 minutes. Behavioral videos were labeled using Deep Lab Cut (DLC), followed by analysis in Python.
Results: Despite implanted hardware and a moving cable, we could accurately track multiple body parts of the mice using DLC. There was no difference in percent time spent in the central zone between the ELA (17.26 %, n=10) and control mice (17.54 %, n= 12). The continuous distance from the center point of the arena was similar between groups. Both male and female mice were included in the study, but the cohort is currently underpowered to evaluate for sex differences. Distance traveled and other measures will also be presented.
Discussion: We did not observe any gross differences in anxiety-related behavior in the OFT after ELA. Other measures, such as time spent in corners, and the time course of exploratory behaviors, should also be evaluated to test for an anxiety-related phenotype. In the future, behavioral data will be compared against neural data, and mice will be added to increase the statistical power to evaluate sex differences in ELA effects.

Disclosures: K. Tegang: None. **S.C. Lim:** None. **R. Hen:** None. **W. Chang:** None.

Poster

399. Fear, Anxiety, and Pain

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Topic: G.04. Emotion

Title: mGlu7 inhibition modulates glutamatergic synaptic transmission and disrupts fear memory reconsolidation

Authors: E. H. VAN DEN BURG[1], C. LEROUX[1], A. STEFANELLI[1], D. MOTA CASSEIRO[1], B. BOURY-JAMOT[2], E. PRALONG[3], R. T. DANIEL[3], J.-L. PAPARIN[4], ***R. LUTJENS**[5], R. STOOP[6];
[1]Ctr. for Psychiatric Neurosciences, [2]Ctr. d'Etude de Comportement, [3]Section of Neurosurgery, Dept. of Clin. Neurosciences, Lausanne Univ. Hosp. Ctr. (CHUV), Lausanne, Switzerland; [4]Addex Therapeut., Geneva, Switzerland; [5]Addex Therapeut., Geneve, Switzerland; [6]Univ. of Lausanne, Prilly, Lausanne, Switzerland

Abstract: Anxiety disorders are amongst the most frequent neurological disorders, and efficient treatment is lacking. Recently, metabotropic glutamate receptors (mGlu) have been proposed as new targets for treatment. mGlu7 is an attractive candidate as it influences anxiety and is found in the lateral amygdala (LA), where fear learning occurs. Using ex-vivo electrophysiology and fear conditioning tests, we have explored the anxiolytic potential of a highly selective mGlu7 negative allosteric modulator, ADX71743, in the LA of rats and in amygdala and cortical brain tissue from human patients. We found that ADX71743 increased glutamatergic synaptic transmission under low frequency stimulation conditions, as evidenced by an enhanced frequency of spontaneous excitatory postsynaptic currents (EPSCs), and higher electrically- and optogenetically-evoked EPSC amplitudes. In contrast, ADX71743 prevented the induction of long-term potentiation (LTP) at thalamus-to-LA synapses that was induced by a high frequency stimulation protocol. This was paralleled by reduced freezing behavior of rats injected with ADX71743 in the LA before cued fear conditioning. Furthermore, ADX71743 infusion after fear memory recall disrupted fear memory reconsolidation as evidenced by suppressed freezing three days later. Tested in amygdala and cortical neurons of human brain tissue, ADX71743 increased spontaneous EPSC frequency similarly as in rats. Our data indicate that negative allosteric modulation of mGlu7 constitutes a new potential mechanism for the treatment of traumatic fear memories and phobia following fear memory recall, with promising translational value considering the comparable effects of ADX71743 on neuronal transmission in rats and humans.

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Poster

399. Fear, Anxiety, and Pain

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2E30410-20-085

Title: Subspace alignment as a computational mechanism of the integration between cue and stimulus intensity in pain perception

Authors: *J.-W. KIM[1,2,3], S. GIM[1,2], S. M. YOO[1,2], C.-W. WOO[1,2,3];
[1]Ctr. for Neurosci. Imaging Research, Inst. for Basic Sci., Suwon, Korea, Republic of; [2]Dept. of Biomed. Engin., [3]Dept. of Intelligent Precision Healthcare Convergence, Sungkyunkwan Univ., Suwon, Korea, Republic of

Abstract: Pain is a multidimensional experience that comprises external sensory inputs and internal processes such as prior expectations. Previous studies have examined which brain regions engage in or mediate the different components of pain. However, these brain mapping studies cannot provide the computational mechanisms of how the brain integrates information from multiple internal and external sources. Here, we adopted the dynamical systems perspective in the analysis of human functional Magnetic Resonance Imaging (fMRI) data to understand how different brain regions integrate information of pain-predictive cues and nociceptive inputs. We hypothesized an alignment of the subspaces encoding cue and stimulus effects as the integration of the cue and stimulus information and the extent to which the two subspaces are aligned changes along the cortical hierarchy. We implemented a cue-induced expectation pain experiment (N = 56), in which we delivered pain predictive cues and noxious stimuli to participants. When we analyzed an alignment index, a ratio of the degree to which cue and stimulus are explained by both subspaces, respectively, we found that the limbic network shows the most, and the visual network shows the least alignment. Note that each network is at the top and bottom of the cortical hierarchy, respectively. Then, we measured the amount of cue and stimulus information in each subspace using the decodability and examined the population dynamics for different experimental conditions in the visual, limbic, and somatomotor networks. We hypothesized that the limbic network would contain both cue and stimuli information in population dynamics at the low-dimensional manifold. In the visual network, the cue information was dominant which was encoded with a form of a rotational dynamics occupying distinct location of the subspace. In the somatomotor network, the stimulus intensity information was dominant. Interestingly, the intensity of the stimulus generated dynamics with different curvature - i.e., the higher stimulus intensity exhibits higher curvature. In the limbic network, population dynamics at cue and stimuli subspace encoded each information in a way similar to that of the visual and somatomotor networks, respectively. This result implies the integration of cue and stimulus information is most prominent at the top of the cortical hierarchy, which was also consistent with the degree of the alignment of subspaces. These findings provide the

computation-level understanding of how cue and stimulus information is integrated, going beyond the “box-and-arrow” explanation of cognitive and pain processes.

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Poster

399. Fear, Anxiety, and Pain

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Topic: G.04. Emotion

Support: NIMH 5F30MH124424-03

Title: A neural population code for anxiety in the ventral hippocampus

Authors: *S. LIM, R. HEN;
Columbia Univ., New York, NY

Abstract: The ventral hippocampus is a critical limbic structure involved in the expression of anxiety-like emotional states, however much is still unknown about how neurons in this region encode anxiogenic stimuli and contexts. We used *in-vivo* freely-moving calcium imaging with miniaturized microscopes (Inscopix nVista3.0) to record neural activity in the ventral hippocampus of mice (C57/BL6, n=3) exploring both low and high-anxiety environments. We found that high-lux elicited robust avoidance of the center in both square and circular open field mazes compared to low-lux conditions (6.4% ± 5.1 high-lux, 13.5% ± 4.3 low-lux percent time in center). Furthermore, by using simultaneously collected head accelerometer data in combination with an auto-regressive hidden Markov model (ARHMM) we extracted sub-second behavioral motifs which could distinguish between high and low lux conditions (62.2% ± 9.1 decoding performance). We observed an elevated calcium transient rate difference between the center and the wall zones in the high-lux conditions (p=0.001 paired t-test). However, this rate difference did not appear to be driven by a large subset of ventral hippocampal neurons that were specifically tuned to the center of the maze (p=0.09, p=0.11, p=0.01 permutation test per mouse). However, by using linear support vector classifiers (SVC) we could decode high versus low lux conditions as well as center versus wall regions but not square versus circle contexts from the ventral hippocampal neural data (48.9% ± 2.5% high vs. low lux, 72.5% ± 3.5 center vs. wall zone, 34.2% ± 10.2 square vs. circle context, decoding performance). Additionally, cross-condition decoding analyses, where a linear SVC is trained on data from one pair of sessions and tested on data from a different pair of sessions, revealed that high versus low lux conditions are abstractly represented in the ventral hippocampus (62.0% ± 2.3 cross-condition decoding performance). In contrast to previous studies which have looked at specific subpopulations of ventral hippocampal neurons, these results suggest that the ventral hippocampus encodes an anxiety-like emotional state through a population code which can be read out by downstream

brain regions rather than through highly selective neurons which encode anxiety-like states via a simple rate code.

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Poster

399. Fear, Anxiety, and Pain

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Support: NIMH Grant MH097988

Title: Pituitary adenylate cyclase activating polypeptide (PACAP) projections from the lateral parabrachial nucleus (LPBn) to the bed nucleus of the stria terminalis (BNST) are critical for stress and anxiety-like responding

Authors: *M. N. BOUCHER[1], K. S. ABEDRABBO[2], D. H. NGUYEN[1], P. A. MCNULTY[1], V. MAY[2], S. E. HAMMACK[1];
[1]Psychological Sci., [2]Neurolog. Sci., Univ. of Vermont, Burlington, VT

Abstract: Understanding the neural circuitry underlying emotional behavior is critical for the improvement of treatments for stress-related psychiatric disorders. The bed nucleus of the stria terminalis (BNST) has been long studied for its role in many stress-related pathologies such as anxiety, pain, depression, and addiction. Our prior work has demonstrated that pituitary adenylate cyclase-activating polypeptide (PACAP) receptor activation in the BNST is both necessary and sufficient for many of the behavioral consequences of chronic stress, including increased anxiety-like behavior in both rats and mice. While the BNST contains local PACAP-expressing neurons, a major source of afferent PACAP is the lateral parabrachial nucleus (LPBn). We recently reported that chemogenetic activation of LPBn PACAP afferents in the BNST increases anxiety-like behavior; here we further characterize the role of LPBn PACAP afferents to the BNST in stress- and anxiety-responding. We demonstrate that the anxiogenic effect of LPBn PACAP afferent stimulation may require PACAP receptor activation, and we also demonstrate that BNST CNO infusions in the absence of Gq-coupled designer receptors activated by designer drugs do not cause changes in anxiety-like behavior. We also demonstrate that that stressor exposure increases PACAP mRNA expression in the LPBn and may increase cfos protein immunostaining as well.

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Poster

399. Fear, Anxiety, and Pain

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NARSAD Young Investigator Grant 25287

Title: Serotonergic modulation of ventral hippocampus underlies sex-related differences in anxiety

Authors: *S. VAN DER VELDT*, F. HENDERSON*, A.-S. SIMARD, F. PERREAULT, A. GRAVEL-CHOUINARD, G. DUCHARME, B. AMILHON;
Univ. de Montréal / CHU Sainte-Justine Res. Ctr., Montréal, QC, Canada

Abstract: Anxiety disorders are among the most common psychiatric conditions worldwide, with women being almost twice more likely than men to be diagnosed with an anxiety disorder throughout their lifetime (Kessler et al., 2005; Craske et al., 2017). Serotonergic (5-HT) neurons from the median raphe (MnR) are heavily involved in the regulation of mood and anxiety (Ohmura et al., 2014; Teissier et al., 2015), yet the neural substrates underlying sex-related differences in anxiety are still largely unknown. The ventral hippocampus (vHP), a region that has been described as a major modulator of anxiety through oscillatory communication with other brain areas (Adhikari et al., 2010; Padilla-Coreano et al., 2016), receives dense 5-HT inputs from the MnR. We hypothesized that ventral hippocampal-projecting 5-HT neurons are instrumental in controlling anxiety. Using a combination of pathway-specific optogenetic activation and local field potential recordings in mice of both sexes, we observe that cell-type specific activation of MnR serotonergic neurons strongly increases the power of 3-4 Hz oscillations in the vHP, a frequency previously found to be implicated in the long-range network synchronization during emotional behaviors (Sirota & Buzsáki, 2005; Karalis et al., 2016). Using a Cre-dependent retrograde viral vector to express the calcium indicator GCaMP6s, preliminary fiber photometry results show that increased ventral hippocampal-projecting 5-HT neurons population activity coincides with exploration of aversive environments. Strikingly, excitatory opsin ChR2 mediated optogenetic activation of these projection neurons robustly increases anxiety levels in a sex-dependent manner, with female mice showing increased anxiety in a battery of previously validated anxiety tests. This work shows that 5-HT release in the ventral hippocampus directly increases anxiety and associated changes in oscillation properties. Together, these results provide a novel mechanistic insight into a previously under-investigated sexual dimorphism in the raphe-ventral hippocampus serotonergic pathway, thereby paving the way for new therapeutic avenues for the treatment of anxiety disorders in females.

Disclosures: S. van der Veldt*: None. **F. Henderson*:** None. **A. Simard:** None. **F. Perreault:** None. **A. Gravel-Chouinard:** None. **G. Ducharme:** None. **B. Amilhon:** None.

Poster

399. Fear, Anxiety, and Pain

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 399.15

Topic: G.04. Emotion

Support: NIH P20 GM103643
NIH P20 GM103423
NIH R15 HD091841

Title: Cea-crf cells mediate the effects of nicu-like medical trauma on juvenile fear conditioning and pain sensitivity in a sex-specific manner.

Authors: *M. A. BURMAN[1], S. MCCOMAS[1], E. DITOMASO[1], J. ZUKE[2];
[1]Sch. of Social and Behavioral Sci., [2]Ctr. for Excellence in the Neurosciences, Univ. of New England, Biddeford, ME

Abstract: Over the past several decades infant admission to the neonatal intensive care unit (NICU) has been on the rise, leading to a decline in infant mortality. However, NICU infants are predisposed to later-life mental health challenges, including alterations of fear, anxiety, depression, and sensory thresholds. Similar to our previous work, these studies utilize a rat model of NICU procedures, to examine the role of Corticotropin Releasing Factor (CRF)-expressing cells in the Central Nucleus of the Amygdala (CeA) in these effects. Newborn offspring from hemizygous transgenic CRF-Cre X SAS-SD crossings were taken from their mother 4 times daily and received either a brief paw needle prick (neonatal pain group) or nonpainful tactile handling (neonatal handled group) over postnatal days (PND) 1-7. Control rats were left undisturbed. On PND 8 rats (Cre+ and Cre-) received an intracranial injection of pAAV-hSyn-DIO-hM4D(Gi)-mCherry targeted at the CeA , allowing the Cre+ rats to express an inhibitory DREADD receptor. The Cre- served as the non-active controls. As the pain of AAV injection caused a neonatal-pain phenotype, additional control rats were left non-injected. On PND 24, all subjects received an injection of clozapine N-oxide (CNO), prior to fear conditioning. Conditioning consisted of 10 tone-shock pairings (10-s 4-kHz 67-dB tone with a 2-s 1.0-mA shock). Subsequent days consisted of contextual fear testing, auditory fear testing and sensory withdrawal threshold testing. Consistent with our previous work, neonatal trauma caused only a modest decrease in measures of conditioned freezing at this age. Moreover, we once again observed a fear conditioning-induced tactile hypersensitivity on the Von Frey test that appeared to be stronger in male rats. Silencing CeA CRF cells caused a further reduction in conditioned freezing that was more prevalent in females compared to males. In contrast, silencing the CRF-cells reversed the tactile hypersensitivity more strongly in males, compared to females. These data are further evidence of a strong sexual dimorphism in the role of CeA CRF-expressing cells.

Disclosures: M.A. Burman: None. **S. McComas:** None. **E. DiTomaso:** None. **J. Zuke:** None.

Poster

399. Fear, Anxiety, and Pain

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 399.16

Topic: G.04. Emotion

Title: Hyperbaric Oxygen Therapy and Anxio-depressive Pain Behaviors in a Pre-Clinical Fibromyalgia Model

Authors: *C. M. ARGENBRIGHT, P. N. FUCHS;
Psychology, The Univ. of Texas At Arlington, Arlington, TX

Abstract: Fibromyalgia (FM) is a chronic, widespread pain disorder generally of a non-inflammatory nature with many known affective and cognitive comorbidities. There is promise in the implementation of hyperbaric oxygen therapy (HBOT) for alleviating FM pain and comorbidities, despite no work investigating the efficacy of this treatment in prominent preclinical FM models. This project aimed to investigate the affective components, specifically anhedonia and anxiety, associated with an acidic saline model of FM in rats. We also sought to find evidence for the potential efficacy of HBOT in the treatment of a pre-clinical model of FM and its associated comorbidities. Forty-eight female Sprague Dawley rats were randomized to a FM pain condition or a saline control condition. Animals were then randomized to receive two 60-minute treatments of HBOT at 2.0 atmospheric absolutes (ATA, pressure equivalent to a depth of 33 feet of sea water) or a control treatment. Mechanical thresholds were taken every 24 hours to investigate the effects of HBOT after one session, two sessions, and the period following treatment. Anhedonia was investigated using an in-cage, 72-hour sucrose preference test, while measures of anxiety-like behavior were investigated using the open-field paradigm. Results revealed that the acidic saline model was efficacious in replicating pain thresholds indicative of FM-like pain ($p < 0.001$). Data did not provide support for the presence of anxio-depressive comorbidities associated with FM ($p > 0.05$). HBOT also did not effectively increase mechanical thresholds as expected ($p > 0.05$). Future studies should seek to investigate the experimental circumstances within which the acidic saline model produces negative affect, as well as identify a more efficacious dosage-response curve associated with HBOT in this preclinical model.

Disclosures: C.M. Argenbright: None. **P.N. Fuchs:** None.

Poster

399. Fear, Anxiety, and Pain

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 399.17

Topic: G.04. Emotion

Support: 5R01MH076136-14

Title: Deconstructing emotion regulation: A system-identification approach using Bayes factors

Authors: *K. BO[1], T. KRAYNAK[2], M. KWON[1], M. SUN[1], P. J. GIANAROS[3], T. D. WAGER[1];
[1]Psychological and Brain Sci., Dartmouth Col., Hanover, NH; [2]Dept. of Epidemiology, Univ. of Pittsburgh, Pittsburgh, PA; [3]Univ. Pittsburgh, Pittsburgh, PA

Abstract: Appraisals are thoughts and beliefs about events and how they relate to the self. They are fundamental to emotion generation, as they imbue stimuli and events with personal meaning. They are also fundamentally involved in emotion regulation, as events can be *reappraised* in light of goals and context information, generating new meanings and emotions. Despite the tight conceptual links between appraisal and reappraisal, studies have usually mapped their neural bases separately. The extent of functional overlap between reappraisal and spontaneous appraisal processes that occurs when generating emotions has not been systematically characterized, and it remains unclear whether some regions are selectively activated by reappraisal alone and which emotion-related brain responses are targeted by reappraisal. Here, we applied a systems identification approach to two large community samples (n=182 and n=178), who viewed and reappraised images from the International Affective Picture System (IAPS) during fMRI scanning. We used Bayes factors to quantify evidence for both activation and null effects for emotion-generation (Look Negative - Look Neutral) and reappraisal (Reappraise - Look Negative) contrasts. This allowed us to take an axiomatic approach to identifying brain regions matching four potential system components: (1) *Reappraisal only* regions responding only to reappraisal demand, not negative images; (2) *Common appraisal* regions activated by negative images and further increased during reappraisal; (3) *Non-Modifiable emotion-generation* regions activated by negative images but unaffected by reappraisal; and (4) *Modifiable emotion-generation regions* activated by negative images and reduced by reappraisal. We identified regions matching each component consistently across the two datasets. Reappraisal-only regions included anterior prefrontal cortex, temporal-parietal junction and temporal pole. Common appraisal regions included large areas within fronto-parietal cortex, insula, and lateral and medial prefrontal cortex. Among emotion generation-related regions, most subcortical regions were non-modifiable by reappraisal, including amygdala, PAG, parabrachial complex, and thalamus. Reappraisal reduced emotion-related activity mainly in visual and attention-related cortical regions. Finally, fMRI activity in reappraisal only, common appraisal, and modifiable emotion regions individually and jointly predicted individual differences in reappraisal success, indicating a critical role of these system components in successful emotion regulation.

Disclosures: K. Bo: None. **T. Kraynak:** None. **M. Kwon:** None. **M. Sun:** None. **P.J. Gianaros:** None. **T.D. Wager:** None.

Poster

399. Fear, Anxiety, and Pain

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Program #/Poster #: 399.18

Topic: G.04. Emotion

Support: NIH R01 MH 122712
NIH R01 MH122712S1

Title: Sex differences in stress coping strategies as a predictor for post- stress avoidance behavior

Authors: *K. M. PRICE, A. M. POLTER;
Dept. of Pharmacol. and Physiol., The George Washington Univ., Washington, DC

Abstract: Generalized anxiety disorder and major depression are more likely to be diagnosed in females than in males, and stressful experiences are a major precipitating factor for these disorders. While the experience of stress is ubiquitous, only some individuals develop anxiety and depression. One potential factor that could underly selective susceptibility is the adoption of maladaptive coping strategies during stress itself. In order to probe the relationship between stress coping, sex, and stress- linked behavioral outcomes, we used the subchronic variable stress (SCVS) model, in which male and female mice are exposed to footshock, restraint, and tail suspension stress over a six-day period. Following stress, female, but not male mice exhibit anhedonia and increased anxiety-like behavior. We hypothesized that these differences in behavioral outcomes may arise from sex-specific coping behavior adopted during the stress itself. In this study, we exposed male and female mice to SCVS and assessed the correlation between coping during the tail suspension phase of the stress (TS) and avoidance behavior in the elevated plus maze (EPM). We found a negative correlation between time mobile during TS and time spent in the open arms of the EPM for both sexes ($r(12) = -0.60$, $p=0.02$). This suggests that more time spent mobile, or actively coping with stress, is associated with less avoidance in the EPM. There was no correlation between time spent mobile and open arm time when data was disaggregated by sex. However, on the second day of TS, females spent significantly more time mobile than males (Mann- Whitney $U=4$, $n_1=5$, $n_2=7$, $p=0.03$). These data suggest that differences in coping styles during stress are predictive of avoidance behavior after stress. Future studies analyzing the dynamics of sex-specific differences in coping behavior during SCVS and the neural circuitry underlying these differences will be valuable in understanding sex-specific responses to stress and how this contributes to vulnerability to stress-linked disorders.

Disclosures: K.M. Price: None. **A.M. Polter:** None.

Poster

399. Fear, Anxiety, and Pain

Location: SDCC Halls B-H

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Program #/Poster #: 399.19

Topic: G.04. Emotion

Title: Examining vaporized and edible THC/CBD products for treating seizure-induced changes in emotional behaviour

Authors: *R. C. GOM[1], S. HARRIS[1], A. G. GEORGE[1], M. N. HILL[1], R. COLANGELI[1], G. TESKEY[2];
[1]Univ. of Calgary, [2]Univ. of Calgary, Calgary, AB, Canada

Abstract: Rationale: Up to half of people with epilepsy have emotional comorbidities, including depression and anxiety disorders, which can severely impair quality of life. Reduced endocannabinoid signaling following repeated seizures has been shown to drive these emotional behaviors. Pharmacologically boosting cannabinoid receptor 1 (CB1) signaling by inhibiting enzymes which break down molecules that bind to CB1 has previously been shown to reverse the behavioral impairments caused by repeated seizures. The objective of this project was to determine if vaporized and edible phytocannabinoids are effective given that this is a more ecologically valid method for the treatment of seizure-induced behavioral changes. **Methods:** Young adult Long-Evans rats were stereotaxically implanted with bipolar stimulating electrodes in the right basolateral amygdala (BLA) and an electrical kindling protocol was used to model temporal lobe epilepsy. Rats received either tetrahydrocannabinol (THC) or cannabidiol (CBD), in either vaporized or edible form prior to behavioral testing. Performance on behavioral assays including auditory fear conditioning, and the elevated plus maze was assessed to determine if the compound and method of administration was effective in restoring typical behavioral responses. **Results:** Orally administered THC increased the time spent in open arms of the elevated plus maze. There were no differences between any experimental measures when either THC or CBD were vaporized. Rats that were administered vehicle and had seizures showed impaired fear memory retention, seen as decreased freezing in response to the conditioned tone, 24 and 48 hours after auditory fear conditioning relative to no seizure controls. At both 24 and 48 hours, seizure rats that were exposed to vaporized THC exhibited an improvement in fear memory, seen as freezing behavior similar to no seizure controls, at earlier tones but no difference at later tones. **Conclusions:** These experiments illustrate that an exogenous vaporized or oral THC/CBD can modulate fear processing, memory, and anxiety-like behaviors and could be utilized in future treatments of comorbid psychiatric conditions in people with epilepsy.

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Poster

399. Fear, Anxiety, and Pain

Location: SDCC Halls B-H

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Program #/Poster #: 399.20

Topic: G.04. Emotion

Support: NSERC

Title: Accelerated forgetting of a previous context fear memory after exposure to elevated platform stress

Authors: J. THIYAGARAJAH, E. XIAO, E. BOLTON, T. D. MENDOZA, G. EKINS, J. SACZKOWSKI, *H. LEHMANN, N. M. FOURNIER;
Dept. of Psychology, Trent Univ., Peterborough, ON, Canada

Abstract: Exposure to stress can have a significant influence on neurobiological processes important for learning and memory. A large body of work has shown that repeated exposure to stress can enhance fear memory but impair in the extinction of these memories. This observation may be mediated, at least in part, from differential effects of chronic stress on mechanisms of adaptive neural plasticity involving limbic and cortical structures, such as the amygdala, hippocampus, and medial prefrontal cortex. However, for most of these studies, stress exposure typically preceded fear and extinction learning. Thus, the impact of previously acquired memories formed before the exposure to stress is not well understood. In the present study, male Long-Evans rats were trained over two days using a strong contextual fear learning procedure (5 footshocks at 1.2 mA each per day) to produce a memory that would be resistant to extinction from repeated testing. Following acquisition, a subset of rats were exposed over two consecutive days to elevated platform stress for 30 minutes. The next day rats were returned to the conditioning chamber for 5 minutes to measure for context-induced behavioral freezing, and this test was repeated 24 and 48 hrs later. Our results show that while freezing levels on the first day were extremely high and comparable for both groups, the stress-exposed rats showed a significant and rapid reduction in freezing behaviour over the second and third day of testing compared to non-stressed controls. In a separate experiment, rats were exposed to the elevated platform stress one week after context fear conditioning. The stressed exposed rats did not show this reduction in fear memory over repeated testing suggesting that timing of the stressor was critical. Studies are currently underway to explore the potential molecular and cellular processes that underlie stress facilitated loss of fear memory.

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Poster

399. Fear, Anxiety, and Pain

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Title: Dissecting the accumbal dynorphinergic outputs underlying affective pain

Authors: O. IDOWU[1], H.-J. YOON[3], R. SANDOVAL[4], C. CAHILL[5], M. PIGNATELLI[2], J. MORON-CONCEPCION[2], ***N. MASSALY**[1,5];
[1]Washington Univ., [2]Washington Univ. In St. Louis, Saint Louis, MO; [3]Vanderbildt Univ., Nashville, TN; [4]UCSD, San Diego, CA; [5]UCLA, Los Angeles, CA

Abstract: Pain represents a growing epidemic in the U.S., afflicting more than 30% of the population. Despite the availability of effective treatments for acute nociceptive pain conditions, negative affective states induced by persistent or chronic pain remain under- or untreated. The nucleus accumbens (NAc) is a critical component of the mesolimbic system and is involved in integrating both reinforcing and aversive properties of external stimuli. Activation of kappa opioid receptors (KORs) through exogenous or endogenous agonist, dynorphin, produces dysphoric effects and impairs active coping strategies in preclinical models of pain. Using chemogenetic approaches and microPET imaging, we recently demonstrated that 1) dynorphin-containing (Dyn+) neurons in the NAc are necessary to drive pain-induced negative affect and 2) inflammatory pain increases overall central KORs occupancy. However, the nature of the downstream structures through which Dyn+ neurons mediate behavioral adaptations to pain remain to be determined. Indeed, NAc Dyn+ neurons project to many structures involved in motivation including the Ventral Pallidum, the Ventral Tegmental Area (VTA) and the Lateral Hypothalamus (LH). Recent evidence has uncovered that NAc toLH Dyn+ neurons are necessary to drive stress-induced anhedonia. In this line of thoughts, we used a combination of ex vivo physiology, imaging and behavioral pharmacology and determined that pain 1) increases the excitability of NAc to LH Dyn+ projections 2) increases KOR function in the LH and 3) engages LH KOR signaling to decrease reward-driven motivation. Our results participate in further understanding the allostatic changes in Dyn+ NAc synaptic efferents in pain and their impact on negative affective states.

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Poster

399. Fear, Anxiety, and Pain

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Title: 22khz ultrasonic calling and body freezing may represent two systems of fear in rodent fear conditioning paradigms

Authors: *B. ZHENG, L. BAO, D. YU, B. YIN;
Sch. of Psychologhy, Fujian Normal Univ., Fuzhou, China

Abstract: The majority of rodent research on fear adopts the fear conditioning (FC) paradigms in which the time percentage of body freezing in a given trial is regarded the sole indicator for fear expression. Recently the model for two systems of fear proposed by J. E. LeDoux has brought us new insights on the issue. In this model, fear is regarded as a kind of subjective emotional experience that can be consciously perceived but be independent of survival-oriented physiology and behavior. Although rodents are not able to express fear via self-report, studies have shown that the 22kHz ultrasonic call emitted by rodents may be identical to human crying both functionally and with similar neural basis. Therefore, we aimed to examine whether 22kHz calling and body freezing have different meanings in FC paradigms. On Day 1, we habituated 128 5-6 months old SD rats which had been subjected to restrained stress before to the FC chambers (Coulbourn Insrtuments, Holliston, MA) and randomly assigned them to eight groups. All groups went through the following procedures: on Day 2, we had them learn the CS-US associations via three trials of delayed tone-shock pairings (CS-tone: 85dB, 9kHz, 20s; US-shock: 0.7mA, 1s, co-terminated with the tone; ITI: 180s); on Day 3, we measured their fear expression in a contextual test and a cued test (new context); on Days 4 & 5, we measured their fear expression via three rounds of cued generalization tests and one original cue retest. Among the eight groups, one group had the standard parameters as described above, two groups were manipulated on the cue length during fear learning (5s vs 80s), two groups were manipulated on the cue length during the cued test (5s vs 80s), two groups were manipulated on the shock intensity during fear learning (weak: 0.5mA, 0.5s; strong: 0.9mA, 2s); the control group received no shocks during fear learning. We found the following: 1. Both the standard experimental group and the control group displayed body freezing during fear learning and cued tests, but only the experimental group emitted 22kHz calls. 2. The frequency of 22kHz calls increased with the shock intensity and decreased as tests went on: the frequency of the weak-shock group's 22kHz calls decreased fastest, but the level of their body freezing remained identical to the standard group. 3. During fear learning and cued tests, we observed discrepancy in the dynamics of body freezing and 22kHz calling in response to the long CS between the 80s-cue-learning group and the 80s-cue-test group. Along with other details not presented here, we concluded that 22kHz calling and body freezing are dissociable and may represent two systems of fear in the rodent FC paradigms.

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Poster

399. Fear, Anxiety, and Pain

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Program #/Poster #: 399.23

Topic: G.04. Emotion

Title: Opposing effects of postnatal and juvenile fluoxetine treatment on emotional behaviour, protein translation and bioenergetics.

Authors: *U. GHAI[1], S. E. FANIBUNDA[3], P. CHACHRA[2], A. SARKAR[4], M. MAHESHWARI[5], S. VIVEK[5], V. VAIDYA[5];
[1]Dept. of Biol. Sci., [2]Tata Inst. of Fundamental Res., Mumbai, India; [3]KHS-MRC and VaidyaLab-Alliance, Mumbai, India; [4]Biomed. Sci., Florida State Univ., Tallahassee, FL; [5]Tata Inst. Of Fundamental Res., Mumbai, India

Abstract: Prior evidence indicates that treatment with selective serotonin reuptake inhibitors (SSRI)may evoke distinct changes in mood-related behavior depending on the temporal window of drug administration. We focused on two early time windows, namely: Postnatal and juvenile windows. Postnatal Fluoxetine (PNFlx) evoked long-lasting increases in anxiety- and despair-like behavior, whereas Juvenile Fluoxetine (JFlx) elicited persistent decreases in anxiety- and despair-like behavior. The starkly differing behavioral outcomes were accompanied by differential global transcriptome changes in the mPFC, with minimal overlap in gene regulation. Further, the mTOR signaling pathway was differentially regulated in PNFlx versus JFlx animals. Opposing patterns of proteins involved in mitochondrial biogenesis and function were observed, with a decline in the mPFC of PNFlx animals in contrast to the increase following JFlx treatment. We found contrasting effects in ATP levels and OxPhos efficiency in PNFlx versus JFlx treatment. We observed a significant increase in mitochondrial function in PNFlx animals treated with NAM, reversing the mitochondrial decline and the pro-depressive effects observed with PNFlx. We show that SSRI treatment in postnatal versus juvenile windows evokes opposing effects on anxiety- and despair-like behavior, along with a differential and minimal overlap of the mPFC transcriptome, differential regulation of the mTOR pathway accompanied by opposing changes in the bioenergetics. We further establish a causal link between bioenergetics and despair-like behavior by reversing the pro-depressive effects of PNFlx using Nicotinamide treatment. Our findings highlight two distinct windows of early life in which SSRI exposure evokes starkly differing effects on behavior, gene regulation, and bioenergetics.

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Poster

399. Fear, Anxiety, and Pain

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Program #/Poster #: 399.24

Topic: G.04. Emotion

Support: NWO Research Talent Grant 406.18.214
ERC Consolidator Grant 2017_772337

Title: Reduced activity in the nucleus accumbens predicts subsequent costly fearful avoidance behaviour in humans

Authors: *A. M. HULSMAN[1,2], F. H. KLAASSEN[2], L. D. DE VOOGD[2], K. ROELOFS[1,2], F. KLUMPERS[1,2];
[1]Behavioural Sci. Institute, Radboud Univ., Nijmegen, Netherlands; [2]Donders Institute, Radboud Univ., Nijmegen, Netherlands

Abstract: Excessive avoidance is a key symptom of anxiety disorders and might be a better predictor of poor disease outcome than current anxiety levels. In excessive avoidance, potential rewards are often sacrificed to avoid potential threats. Although costly avoidance plays an important role in anxiety, little is known about the underlying neural mechanisms. In addition, previous human research typically does not investigate how the brain weighs and integrates reward and threat information when making approach-avoidance decisions. Therefore, we developed a Fearful Avoidance Task (FAT) in which active approach-avoidance decisions are made under varying reward (monetary gains) and threat (electrical stimulation) prospects. In this study, 31 participants (17 females) performed the FAT in the MRI scanner. Our pre-registered analyses (Hulsman et al., 2021 https://osf.io/7sm9k) showed that the FAT successfully induced an approach-avoidance conflict: threat-induced avoidance decreased with increasing reward. We found involvement of brain regions that are important for both appetitive and defensive processing when making approach-avoidance decisions. Parametric modulation analysis showed that increased reward was associated with increased "risky" approach behaviour and increased activity in the dorsal anterior cingulate cortex, nucleus accumbens, periaqueductal gray, bed nucleus of the stria terminalis, and amygdala. Next, we investigated whether neural activity preceding approach-avoidance decisions predicted subsequent choice. We found that reduced nucleus accumbens activity significantly and uniquely predicted decisions to avoid. Interestingly, activity in regions traditionally associated with threat processing was not predictive of avoidance. These findings highlight the relevance of reward circuitry in avoidance, not only as a reinforcement following avoidance but also in the build-up to the decision to avoid.

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Poster

399. Fear, Anxiety, and Pain

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Program #/Poster #: 399.25

Topic: G.04. Emotion

Support: ERC_CoG - 2017_772337

Title: The neural computations underlying passive and active approach-avoidance decision-making under acute threat

Authors: *F. H. KLAASSEN[1], L. D. DE VOOGD[1], A. M. HULSMAN[1,2], J. X. O'REILLY[3], F. KLUMPERS[1,2], B. FIGNER[1,2], K. ROELOFS[1,2];
[1]Donders Inst. for Brain, Cognition, and Behaviour, [2]Behavioural Sci. Inst. (BSI), Radboud Univ., Nijmegen, Netherlands; [3]Dept. of Exptl. Psychology, Oxford Univ., Oxford, United Kingdom

Abstract: When deciding whether to approach or avoid an acute threat, it is crucial to optimally weigh the potential positive and negative choice outcomes. We previously showed that freezing-like anticipatory heart rate deceleration (bradycardia) may affect the integration of the potential outcomes, depending on whether an action has to be performed or not (Klaassen et al., 2021). However, it remains unclear how bradycardia modulates neural pathways underlying value-based computations.
In this fMRI study, 58 human participants made passive and active approach-avoidance decisions in prospect of receiving varying money (1-5 euros) and shock (1-5 stimulations) amounts. Simultaneously, we measured heart rate and BOLD-fMRI. We hypothesized that freezing-states are accompanied by alterations in the neural decision-making circuitry. Namely, by modulating activity in brain regions involved in threat (PAG, amygdala), value integration (dACC), and/or switching to action (pgACC; Livermore et al., 2021).
Replicating Klaassen et al. (2021) we observed increased approach vs avoid choices for higher money vs shock amounts, respectively. Additionally, stronger trial-by-trial bradycardia was associated with faster response times, as well as a stronger effect of shocks on avoidance. Neurally, we found increased BOLD activity in the amygdala, ventral striatum, and vmPFC in anticipation of approach vs. avoid decisions. Here we also observed activity related to anticipation of increased money amounts (striatum and mPFC) and shock amounts (anterior insula and dMCC). Ongoing analyses using computational modelling will test whether the interaction of bradycardia with threat, value integration, and action is associated with neural activity in our regions of interest. Together, our results will indicate how the physiological state of freezing may feed into approach-avoidance decisions.

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Poster

399. Fear, Anxiety, and Pain

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Support: IBS-R015-D1
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2E30410-20-085

Title: Know pain, know gain: Shared brain representations of sensory pleasure and pain

Authors: *S. LEE[1,3], J.-J. LEE[1,3], J. HAN[4], M. CHOI[5,3], C.-W. WOO[1,2,3];
[1]Dept. of Biomed. Engin., [2]Dept. of Intelligent Precision Healthcare Convergence, Sungkyunkwan Univ., Suwon-si, Korea, Republic of; [3]Ctr. for Neurosci. Imaging Research, Inst. for Basic Sci. (IBS), Suwon-si, Korea, Republic of; [4]Korea Brain Res. Inst., Daegu, Korea, Republic of; [5]Dept. of Biol. Sci., Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: Pain and pleasure are often considered to be the opposite ends of a continuum. They are known to influence each other even though they engage distinct peripheral and spinal-level processes. However, it remains unclear how these two opposite-valenced affective experiences are connected at brain systems level. Here, we aimed to identify the brain regions important for both pain and pleasure experiences induced by capsaicin and chocolate. First, we selected 48 *a priori* regions-of-interest (ROIs) known to be responsive to either pain or pleasure based on the previous studies. We conducted an fMRI experiment (Study 1: n = 58, 27 females, mean of age = 22.81, SD of age = 2.83) and trained a multivariate predictive model for each ROI to identify brain regions that showed significant predictions both for pain and pleasure. We found that the ventromedial and lateral prefrontal cortices, the orbitofrontal cortex, the anterior insula, and the amygdala encoded both pain and pleasure information among the 48 ROIs. Then, we hypothesized two-dimensional information that can be shared across pain and pleasure—affective intensity and affective valence and developed multivariate predictive models of the two dimensions. The predictive models of affective intensity and valence showed significant prediction performances across two datasets (Study 1 training dataset with leave-one-participant-out cross-validation, intensity prediction: r = 0.25, P << 2.22×10^{-16} and valence prediction: r = 0.11, P = 0.0014; Study 2 independent test dataset [n = 62], intensity prediction: r = 0.17, P = 2.47×10^{-10} and valence prediction: r = 0.14, P = 3.55×10^{-4}). When we examined the patterns of predictive weights, we found that the predictive models of affective valence versus intensity involved distinct sub-populations of voxels within the common brain regions. In addition, these sub-populations showed distinct patterns of large-scale network connectivity—the valence model was largely associated with the limbic and default mode networks, whereas the intensity model was largely associated with the ventral attention network. In sum, this study provides new insights into the shared brain representations of pain and pleasure and their dimensional components, promoting the systems-level understanding of human affective experiences.

Disclosures: **S. Lee:** None. **J. Lee:** None. **J. Han:** None. **M. Choi:** None. **C. Woo:** None.

Poster

399. Fear, Anxiety, and Pain

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Title: Identification of a human parabrachial-amygdala pathway with 7T fMRI

Authors: *B. KIM[1], P. A. KRAGEL[2], M. ČEKO[3], J. THERIAULT[4], D. CHEN[5], A. SATPUTE[7], L. W. WALD[8], M. A. LINDQUIST[9], L. F. BARRETT[6], T. D. WAGER[1]; [1]Psychological and Brain Sci., Dartmouth Col., Hanover, NH; [2]Psychology, Emory Univ., Atlanta, GA; [3]Inst. of Cognitive Sci., Boulder, CO; [4]Psychology, Northeastern Univ., Boston, MA; [5]Psychology, Northeastern Univ., Allston, MA; [6]Northeastern Univ., Boston, MA; [7]Pomona Col., New York, NY; [8]Harvard Med. Sch., Boston, MA; [9]Johns Hopkins Univ., Baltimore, MD

Abstract: The spinothalamic pathway is widely regarded as the canonical 'pain pathway', but in rodents, 95% of nociceptive spinal neurons project to the parabrachial nucleus (PBN). PBN Calcitonin gene-related peptide (CGRP) neurons project to the central nucleus of the amygdala (CeA), forming a critical pathway for multiple types of aversive, unconditioned threat behaviors in nonhuman animals, including chronic pain states. However, the PBN→CeA pathway has not been definitively identified or extensively studied in humans. We recently developed a technique called Multivariate Pathway Identification (MPathI), which incorporates multivariate pattern analyses and semi-supervised learning into connectivity analysis to identify functional pathways from human neuroimaging data. We previously used MPathI to characterize the superior colliculus-pulvinar-amygdala pathway, distinct thalamocortical pathways, and a novel hypothalamic-periaqueductal gray pathway not previously described in humans. In all these cases, MPathI outperformed region-of-interest (ROI) based connectivity in identifying patterns of robust, neuroanatomically specific functional connectivity related to human affect. Here, we applied MPathI to 7T fMRI data acquired during pain avoidance learning (n = 41; 4 runs each) to test whether the human PBN→CeA pathway could be identified and distinguished from a portion of the spinothalamic pathway projecting from ventral posterior lateral (VPL) thalamus to dorsal posterior insula (dpIns). We also tested whether MPathI identified these pathways with greater precision (higher correlation and lower inter-subject error) and greater neuroanatomical specificity than standard ROI analyses. We found optimal local patterns for connecting the PBN→CeA and VPL→dpIns for each individual and used leave one run out cross-validation to obtain unbiased estimates of connectivity strength for on-target (e.g., PBN→CeA) and off-target (e.g., PBN→dpIns) pathways. With MPathI, PBN→CeA connections were strong (average r = 0.16 , t = 9.71, p < .0001, *Cohen's d* = 1.52) but off-target PBN→dpIns connections were non-significant (d = 0.28). VPL→dpIns connections were strong (average r = 0.59 , t = 23.9, p < .0001, d = 3.74) but off-target VPL→CeA connections were non-significant (d = 0.24). Connectivity was significant but less neuroanatomically specific with standard ROIs (on- vs. off-target x MPathI vs ROI interaction t = 5.43 and 4.64, p < .0001 for PBN and VPL pathways, respectively). Further characterizing PBN pathways in human affect and pain could be a key to linking mechanistic insights from animal studies to human mental health, motivation, and substance use.

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Poster

399. Fear, Anxiety, and Pain

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Title: Individual Variability in Brain Representations of Pain

Authors: *L. KOHOUTOVA[1,3,2], L. Y. ATLAS[4,5,6], C. BÜCHEL[7], J. BUHLE[8], S. GEUTER[9,10], M. JEPMA[12], L. KOBAN[13], A. KRISHNAN[14], D. LEE[1,3,2], S. LEE[1,3,2], M. ROY[15], S. M. SCHAFER[11], L. SCHMIDT[13], T. D. WAGER[16], C.-W. WOO[1,3,2];
[1]Dept. of Biomed. Engin., [2]Dept. of Intelligent Precision Healthcare Convergence, Sungkyunkwan Univ., Suwon, Korea, Republic of; [3]Ctr. for Neurosci. Imaging Res., Inst. for Basic Sci., Suwon, Korea, Republic of; [4]Natl. Ctr. for Complementary and Integrative Hlth., [5]Natl. Inst. on Drug Abuse, [6]Natl. Inst. of Mental Hlth., NIH, Bethesda, MD; [7]Dept. of Systems Neurosci., Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany; [8]Dept. of Psychology, USC, Los Angeles, CA; [9]Dept. of Biostatistics, Johns Hopkins Univ., Baltimore, MD; [10]Inst. of Cognitive Sci., [11]Dept. of Psychology and Neurosci., Univ. of Colorado Boulder, Boulder, CO; [12]Dept. of Psychology, Univ. of Amsterdam, Amsterdam, Netherlands; [13]Control-Interoception-Attention Team, Paris Brain Inst. (ICM), INSERM, CNRS, Sorbonne Univ., Paris, France; [14]Dept. of Psychology, Brooklyn Col. of the City Univ. of New York, New York, NY; [15]Dept. of Psychology, McGill Univ., Montreal, QC, Canada; [16]Dept. of Psychological and Brain Sci., Dartmouth Col., Hanover, NH

Abstract: Pain is a multidimensional experience that is processed by systems distributed across the whole brain. There are brain regions that have been associated with pain processing such as the insula, midcingulate cortex, somatosensory cortices, cerebellum, prefrontal cortex, and others, but not all these regions are consistently reported in studies as pain-processing. Therefore, viewing any fixed set of brain regions as the core pain-processing system would be an over-simplification. Moreover, lesion studies have shown that even though there are lesions in brain regions known to be important for pain, such as the anterior midcingulate cortex or insula, the ability to perceive pain is often not affected. In fact, it appears that pain shows the feature of degeneracy. In other words, there may be a slightly different combination of brain regions and pathways that gives rise to the pain experience in each individual. However, although it is crucial to understand this inter-individual variability in pain processing, for example, for enhancing pain treatment, previous brain mapping studies of pain have mostly focused on group-level analyses in which important individual-specific features can be lost. Here, we aimed to step towards the personalised brain mapping of pain and identify which brain regions show stable or variable pain-predictive patterns across individuals. We trained idiographic pain-predictive models with

13 single-trial functional magnetic resonance imaging datasets (n = 404, discovery set) and quantified voxel-level importance for individualized pain prediction finding 21 important pain-predictive regions. Within the regions, we then examined the inter-individual variability of pain-predictive weights using voxel-wise variance and their patterns using the representational similarity analysis. Higher-order transmodal regions, such as ventromedial and ventrolateral prefrontal cortices, showed larger individual variability, whereas unimodal regions, such as somatomotor cortices, showed more stable pain representations across individuals. We replicated this result in an independent dataset (n = 124). We also found 10 clusters of regions that exhibited similar patterns of inter-individual variability. Overall, our study identified cerebral sources of individual differences in pain processing, providing potential targets for personalized assessment and treatment of pain.

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Poster

400. Animal Models of Anxiety: Pharmacology and Neural Circuitry

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Topic: G.06. Anxiety Disorders

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Title: Effect of maternal dietary deficiency of Omega-3 Fatty Acids followed by post weaning supplementation on adolescent anxiety, learning, and microglial morphology

Authors: *A. BOGACHUK[1], D. S. JACOBS[2], B. MOGHADDAM[3];
[1]Behavioral Neurosci., Oregon Hlth. & Sci. Univ. (PO# 770008576), Portland, OR; [2]BEHN, Oregon Hlth. & Sci. Univ., Portland, OR; [3]Behavioral Neurosci., Oregon Hlth. & Sci. Univ. Behavioral Neurosci., Portland, OR

Abstract: Dietary deficiency in omega-3 fatty acids (n-3 FAs) is a potential risk factor for impaired cognitive and affective functions and may increase the likelihood of developing psychiatric disorders. Dopamine (DA) neurons in the midbrain are reported to be sensitive to this deficiency (e.g. Ahmad 2008). Our recent work (Bondi et al, 2014) has shown that adolescents are more susceptible to this dietary deficiency, displaying a different pattern of dopamine related changes, increased anxiety, and deficits in learning and behavioral flexibility. Here, we asked if these behavioral deficits in adolescents can be reversed by n-3 FA supplementation after weaning, and if the microglial phenotype in regions containing dopamine neurons or terminals is influenced by the deficient diet and supplementation. We compared behavior and microglial morphology in brain regions of adolescent male and female rats in three groups: (1) offspring of

second-generation n-3 FA deficient mothers that stayed on deficient diet after weaning (2) offspring of second-generation n-3 FA deficient mothers that were shifted to adequate diet after weaning, and (3) offspring of second-generation n-3 FA adequate mothers that stayed on adequate diet. We find that the adolescent DEF/DEF group exhibited increased innate anxiety and subtle behavioral differences during instrumental learning as compared to ADQ/ADQ. Supplementation after weaning did not reverse the impact on learning, however, there was a moderate reduction of anxiety. Differences in microglial activation were observed in the substantia nigra, dorsal striatum, and basolateral amygdala of adequate and deficient diet rats. Supplementation produced a microglial phenotype more closely resembling that of adequate diet animals across regions. These data confirm that changes in microglial phenotype in a sustained n-3 FA dietary deficiency can be overcome by supplementation. While adolescent dietary supplementation shows potential to rescue ongoing microglial activation, it does not fully reverse the detrimental impact of the maternal diet.

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Poster

400. Animal Models of Anxiety: Pharmacology and Neural Circuitry

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Title: Effects of closed head injury on rearing and grooming behaviors in rats

Authors: T. M. JIMÉNEZ-RIVERA, N. M. JIMÉNEZ-RIVERA, H. G. HADDOCK-MARTÍNEZ, M. GONZÁLEZ-PEDRAZA, O. MARTÍNEZ-GUZMÁN, M. CÁCERES-CHACÓN, M. RIVERA-LÓPEZ, *D. SIERRA-MERCADO;
Anat. & Neurobio., Univ. Puerto Rico Sch. of Med., San Juan, PR

Abstract: The relationship between concussions and behaviors related to emotional stress remains unclear. Recent reports suggest that concussion increases fear-behaviors in paradigms of Pavlovian fear conditioning. Here, freezing behavior is used as an index of fear. Of note, other ethologically relevant behaviors such as grooming and rearing can also be utilized as indicators of emotional stress. For this reason, we hypothesized that closed head injury would alter rearing and grooming. To help us determine this, rats were anesthetized and delivered either closed head

injury via weight drop, or sham injury for controls. After recovery, rats were exposed to an open field space. Spontaneous activity was recorded to quantify rearing and grooming behaviors in the open field. Data collection was performed by observers blind to the experimental manipulations. Specifically, the number of rears, time spent rearing, grooming bouts, and % time spent grooming were quantified. Preliminary results suggest that closed head injury (*n=10*) versus sham (*n=9*) does not effect either the number of rears (p=0.16), time spent rearing (p=0.32), the number of grooming bouts (p=0.96), nor the time spent grooming (p=0.75). Next, we will assess rearing and grooming in another context. Together, these data suggest that concussive brain injury does not affect other ethologically-relevant behaviors related to fear and anxiety. Currently, activity in brain regions involved in anxiety, namely the bed nucleus of the stria terminalis, are being quantified using cFos immunohistochemistry.

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Poster

400. Animal Models of Anxiety: Pharmacology and Neural Circuitry

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Program #/Poster #: 400.03

Topic: G.06. Anxiety Disorders

Title: Neuropharmacological effects of a Stanhopea tigrin aerial parts extract evaluated with anxiety unconditioned tests

Authors: *C. ALBA-BETANCOURT[1], A. J. ALONSO-CASTRO[2], M. A. DEVEZE-ALVAREZ[2], D. GASCA-MARTÍNEZ[3], C. CARRANZA-ALVAREZ[4];
[2]Pharm., [1]Univ. De Guanajuato, Guanajuato, Mexico; [3]Univ. Nacional Autónoma de México, Querétaro, Mexico; [4]Univ. Autónoma de San Luis Potosí, San Luis Potosí, Mexico

Abstract: Introduction Anxiety is a hypervigilance disorder and one of the most common psychiatric disorders worldwide. The traditional treatment consists of the administration of benzodiazepines, selective serotonin, and norepinephrine reuptake inhibitors, which can cause adverse side effects. The search for alternative treatments, especially those of natural origin, has become an important line of research, mainly in countries with scarce resources and which have a wide range of plant varieties. Stanhopea tigrina is an orchid endemic to Mexico. Its flowers are used to treat insolation and weakness. The aerial parts are the photosynthesis area as well as water storage area, being a fundamental part of these plants, but their neuropharmacological effects haven‘t been described yet. In this work we evaluate the possible anxiolytic properties of the Stanhopea tigrine aerial parts extract as an approach to propose an alternative treatment for anxiety. **Methodology** Three concentrations of a Stanhopea tigrina aerial extract (10, 50 and 100 mM) were tested in 3 groups of 10 mice of the CD1 strain, which were orally administered. After one hour of the extract administration, the mice were placed in 3 different unconditioned tests to

evaluate the possible anxiolytic effect of the orchid: Elevated plus maze, Light/dark box and Open field. The results were compared with a positive control of clonazepam (anxiolytic drug) and a negative control of saline solution. For the statistical analysis, the Graph Pad Prism 9.4.0 software was used, and the ANOVA test was performed with a post-hoc Tuckey test. **Results** In the elevated plus maze, the mice treated with the 50nM concentration stayed longer and entered the open arms area more times, compared to the vehicle. In this test we did not observe significant differences between any of the doses used for the compound. The 10 and 100nM didn't show a relevant effect. In the light/dark box the mice treated with the 100nM concentration showed a number of entries and duration time in the illuminated area similar to clonazepam. In the open field the mice traveled a total distance and explored the center area (anxious area) similar to or even more than clonazepam. The concentration the effect was most prominent was 10nM. **Discussion and conclusion** Since clonazepam-like behavior was observed, the results suggest that Stanhopea tigrina aereal parts extract induces anxiolytic effects in mice under unconditioned tests. It is important to determine the majority compound present in the aereal parts extract of Stanhopea tigrina, which is being done by our research group.

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Poster

400. Animal Models of Anxiety: Pharmacology and Neural Circuitry

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Topic: G.06. Anxiety Disorders

Support: FSU Jim and Betty Ann Rodgers Chair Fund

Title: Transgenerational transmission of aspartame induced anxiety associated with changes in amygdala transcriptome and glutamate-GABA signaling

Authors: *S. K. JONES[1], D. M. MC CARTHY[1], C. VIED[2], P. G. BHIDE[1];
[1]Biomed. Sci., [2]Translational Sci. Lab., Florida State Univ. Col. of Med., Tallahassee, FL

Abstract: C57BL/6 mice exposed to 0.03% aspartame, an artificial sweetener, in drinking water (11% of the FDA-recommended maximum human daily intake) daily for 12-weeks developed anxiety (open field test and elevated zero maze). A single i.p. administration of diazepam (3mg/kg), an allosteric modulator of the GABA-A receptor, alleviated the anxiety. RNA sequencing and the amygdala showed 1,467 differentially expressed genes, among which 1,073 were upregulated and 394 were downregulated. KEGG analysis showed upregulation of glutamatergic synapse and downregulation of GABAergic synapse pathways. Quantitative PCR confirmed upregulation of NMDA receptor subunit 2D (Grin2d) and metabotropic receptor 4 (Grm4) mRNA and downregulation of the GABA-A receptor associated protein (Gabarap) mRNA. Male and female F1 offspring produced by breeding aspartame-exposed males with

naïve females displayed anxiety, which was alleviated by diazepam. In addition, F1 mice showed upregulation of amygdala Grin2d and Grm4 mRNA and downregulation of Gabarap mRNA. Male and female F2 mice produced by breeding F1 males from paternal aspartame lineage with naïve females also displayed anxiety, which responded to diazepam treatment. Thus, aspartame may produce anxiety by altering amygdala excitation-inhibition balance in aspartame-consuming individuals and up to two generations of descendants.

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Poster

400. Animal Models of Anxiety: Pharmacology and Neural Circuitry

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Title: Caffeine affects adult male rats differently depending on their original level of anxiety

Authors: *S. FLORÉN LIND[1], F. STAM[1], S. ZELLEROTH[1], A. FRICK[2], A. GRÖNBLADH[1]; [1]Uppsala University, Dept Pharmaceut. Biosci., Uppsala University, Dept Pharmaceut. Biosci., Uppsala, Sweden; [2]Uppsala Univ., Uppsala, Sweden

Abstract: Anxiety disorders are one of the most common psychoactive disorders in the west world today. There are several different triggers for anxiety, including threatening situations, but also factors such as lack of sleep and stress. Furthermore, alcohol and certain drugs may trigger anxiety. In the present study our objective is to elucidate the effects of high doses of caffeine, which have been reported to induce anxiogenic effects for both humans and model organisms. Caffeine acts antagonistically on adenosine receptors, foremost on the subtypes A2a and A1. The receptors endogenous agonist is adenosine involved in several important functions in the body. This the specific aim of this study is to investigate the individual differences in male Wistar rats before and after caffeine treatment both in anxiety-like behavior and in general behavior, based on their naïve anxiety-like behavior, as well as the expression of relevant markers. Behavior tests suitable for anxiety and general behavior were performed with the rats separated based on their naïve anxiety state. Post behavior testing of gene expression was added as a compliment to the behavior. The screening behavior test produced two significantly separate groups, and these groups continued to distinguish themselves though out the behavior study and further in the biochemical examination of gene expression. These result supports the hypothesis that depending on individual variation, caffeine affects the individuals differently, resulting in some more prone to increase their level of anxiety when consuming caffeine while others remain unaffected. The

results aid in the understanding of how anxiety varies between individuals developing more individual specific treatments for anxiety disorders.

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Poster

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Title: Chronic exposure to cyclohexane induces stereotypic circling and anxiety-like behavior associated with atypical c-Fos expression in motor- and anxiety-related brain regions

Authors: *N. IBARRA CASTAÑEDA[1,2], T. CAMPOS-ORDOÑEZ[1,4], E. ALCALÁ[6], D. ZARATE-LOPEZ[3,5], J. BURITICÁ[4], O. GONZALEZ-PEREZ[1];
[1]Lab. of Neurosci., [2]Med. Sci. PhD Program, Sch. of Med., [3]Physiological Sci. PhD Program, Sch. of Med., Univ. of Colima, Colima, Mexico; [4]Ctr. de Estudios e Investigaciones en Comportamiento, [5]CUCS-Department of Neurosci., Univ. of Guadalajara, Jalisco, Mexico; [6]Res. Lab. on Optimal Design, Devices and Advanced Materials, Dept. of Mathematics and, ITESO, Jalisco, Mexico

Abstract: Cyclohexane (CHX) is a volatile solvent used as a substitute as safe organic solvents in several products, such as paint thinners, gasoline and adhesives. These products are used as drugs of abuse and can severely damage brain functioning; however, there is very little information on its effects on anxiety and brain activity upon drug removal. In the present study, we used CD1 adult mice to mimic an intoxication period of recreational drugs for 30 days. During the CHX exposure, we assessed biphasic and circling behaviors. After CHX removal (24 h or a month later), we analyze anxiety-like behaviors and quantified c-Fos cells in motor- and anxiety-related brain regions. Our data indicate that the repeated inhalation of CHX caused steady hyperactivity and decreases ataxia, sedation, and seizures as the exposure to CHX progressed. In the first week of CHX inhalation, a stereotypic circling behavior emerged and increased gradually. One month after CHX withdrawal, mice presented low activity in the open field and more buried marbles. Twenty-four hours after CHX removal, c-Fos expression was low in the dorsal striatum, ventral striatum, motor cortex, dorsomedial prefrontal cortex, basolateral amygdala, lateral hypothalamus, and ventral hippocampus. One month later, c-Fos expression continued low in the ventral striatum and lateral hypothalamus but increased in the dorsomedial

prefrontal cortex and primary motor cortex. This study provides a comprehensive behavioral characterization and novel histological evidence of the CHX effects on the brain when is administered in a recreational-like mode.

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Poster

400. Animal Models of Anxiety: Pharmacology and Neural Circuitry

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Title: Inhibition of fatty acid binding protein-5 alleviates stress-induced anxiety-like and depression-like psychopathology

Authors: *T. UZUNESER[1], M. JONES[1], H. WANG[3,4], I. OJIMA[3,4], W. J. RUSHLOW[1], S. R. LAVIOLETTE[1,2];
[1]Dept. of Anat. and Cell Biol., [2]Dept. of Psychiatry, Schulich Sch. of Med. and Dentistry, Lawson Hlth. Res. Inst., Univ. of Western Ontario, London, ON, Canada; [3]Dept. of Chem., [4]Inst. of Chem. Biol. and Drug Discoveries, Stony Brooks Univ., Stony Brook, NY

Abstract: Anxiety disorders and depression are the most frequently diagnosed neuropsychiatric disorders worldwide. There are not only distinct, but also overlapping pharmacological treatments for these disorders, yet limitations of these treatments in terms of efficacy and tolerability remain to be a key pharmacotherapeutic challenge. The endocannabinoid system (eCB) is a promising neurobiological system in which to target the development of novel pharmacotherapies for neuropsychiatric disorders. Previous findings have revealed that genetic and/or pharmacological manipulations altering eCB signaling modulate depression- and anxiety-related behaviours and neuronal transmission patterns in both humans and rodents. Fatty acid binding protein-5 (FABP-5) is a chaperone protein in the eCB system, responsible for the intracellular transport of anandamide for degradation by fatty acid amide hydroxylase. Previously, we have shown that acute pharmacological inhibition of FABP-5 within the prelimbic cortex of rats altered neuronal activity in key regions of anxiety-related neural circuitry, resulting in an anxiolytic behavioral phenotype in a cannabinoid CB2 receptor dependent fashion. In this study, we aimed to investigate the effects of systemic FABP-5 inhibition on anxiety- and depression-related behaviours using adult male Sprague Dawley rats. Following a 2-week long unpredictable stress paradigm, we assessed whether an acute intraperitoneal injection of SBFI-103, a selective inhibitor of FABP-5, could reverse stress-induced anxiogenic and depressive-like phenotype. Anxiety-related behaviour was examined

using elevated plus maze, light-dark box, open field and sociability tests whereas depression-related behaviour was investigated using sucrose preference and forced swim tests. Our behavioural results indicate that both low dose (2mg/kg) and high dose (20mg/kg) SBFI-103 ameliorate stress-induced anxiogenesis. Furthermore, high dose SBFI-103 restores stress-induced anhedonia. Neither dose of SBFI-103 seems to influence cognition or memory. Additionally, our ongoing examinations and preliminary findings indicate that behavioral effects of SBFI-103 are possibly mediated by altered activity of proteins in key signaling pathways of the eCB system within the limbic regions of the brain. In brief, our findings provide a promising role for FABP-5 inhibition as a novel pharmacotherapy with anxiolytic and antidepressant-like properties.

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Poster

400. Animal Models of Anxiety: Pharmacology and Neural Circuitry

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Title: Acid-sensing Ion Channels Mediate Distinct Neural and Behavioral Responses to Interoceptive Carbon Dioxide Threat

Authors: *A. CHAN, R. TAUGHER-HEBL, Y. KIM, N. NARAYANAN, J. WEMMIE; Univ. of Iowa, Iowa City, IA

Abstract: Panic disorder is characterized by spontaneous panic attacks, which can also be provoked by CO_2 inhalation. Thus, understanding mechanisms of CO_2 responses may shed light on the pathophysiology of panic disorder. Interestingly, CO_2 evokes exaggerated panic attacks in people with amygdala lesions, suggesting a critical role for the amygdala. In mice, CO_2 inhalation evokes a spectrum of defensive behaviors ranging from freezing to fight-or-flight, and mice with amygdala lesions also show increased flight responses to CO_2. Because CO_2 inhalation rapidly lowers brain pH, we hypothesized these different CO_2-evoked behaviors are mediated by acid sensing ion channels (ASICs) in the amygdala. We exposed wild-type and *Asic1a*$^{-/-}$ mice to various CO_2 concentrations and quantified freezing and flight-related jumping behaviors. We also delivered acidic pH directly into the amygdala of awake, behaving mice. Both CO_2 and acidic pH elicited freezing and jumping responses. In *Asic1a*$^{-/-}$ mice, freezing responses to both

CO_2 and acidic pH were impaired, whereas jumping responses to both stimuli were exaggerated. Furthermore, specifically disrupting *Asic1a* in amygdala reduced CO_2-evoked freezing but did not increase CO_2-evoked jumping, suggesting effects of ASIC1A on these two behaviors involve distinct cellular sites of action. Because *Asic1a*$^{-/-}$ mice behaved like mice with amygdala lesions, we examined CO_2 induction of c-Fos in amygdala. Surprisingly, CO_2 induced more c-Fos in *Asic1a*$^{-/-}$ mice, indicating behavioral effects of ASIC1A disruption were due to more than silencing the amygdala. To further investigate mechanisms of ASIC1A action, we recorded unit activity and local field potentials (LFPs) from amygdala in awake, behaving mice. CO_2 suppressed firing of some units and stimulated others; strikingly, proportions of both activated and suppressed neurons were increased in *Asic1a*$^{-/-}$ mice. LFPs also revealed interesting effects: CO_2 increased theta power but suppressed other frequencies, especially gamma. In *Asic1a*$^{-/-}$ mice, the magnitudes of both effects were significantly enhanced. These data suggest ASIC1A in the amygdala plays a central role regulating defensive behaviors and begins to delineate distinct circuit activity patterns corresponding with different behaviors.

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Poster

400. Animal Models of Anxiety: Pharmacology and Neural Circuitry

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 400.09

Topic: G.06. Anxiety Disorders

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Title: Cell-type-specific dopamine signaling in ventral hippocampus tracks anxiety-related behaviors

Authors: *A. GODINO[1], M. SALERY[1], A. M. MINIER-TORIBIO[1], V. PATEL[1], J. F. FULLARD[2], E. M. PARISE[1], C. MOREL[1], S. E. MONTGOMERY[1], P. ROUSSOS[2], R. D. BLITZER[1], E. J. NESTLER[1];
[1]Nash Family Dept. of Neurosci. & Friedman Brain Inst., [2]Dept. of Psychiatry & Friedman Brain Inst., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Despite accumulating evidence for both the ventral hippocampus (vHipp) and the mesocorticolimbic dopamine system in encoding anxiety-relevant information, surprisingly little is known about how dopamine signaling selectively affects vHipp representations of emotionally-salient stimuli to guide approach/avoidance behaviors. To address these shortcomings, we here study dopaminoceptive neurons in mouse vHipp - which can be segregated based on their expression of either the dopamine D1 or D2 receptor - to delineate a

model of dopamine action in vHipp. At the histological level, D1- and D2-expressing cells exhibit a precise topographical organization across vHipp subfields, which we further dissected using RNA-sequencing of single, sorted nuclei from D1 and D2 cells. We also confirmed dopamine's pharmacological effects on the electrophysiological properties of these cell types. Functionally, we found that anxiogenic environments and approach/avoidance conflicts trigger distinct patterns of calcium activity in D1 vs. D2 vHipp neurons in concert with dopamine release in in this region. Bidirectional chemogenetic and optogenetic manipulation of D1 or D2 vHipp neuron activity causally demonstrated their opposite roles in mediating anxiety and approach/avoidance behaviors. Together, we propose that dopamine dynamics in vHipp operate as a feedback loop that bidirectionally tracks anxiety levels to gate exploratory behaviors through differential recruitment of vHipp D1 and D2 neurons, which in turn mediate opposite approach/avoidance and anxiety-like responses. Intriguingly, vHipp dopaminoceptive mechanisms also contribute to cocaine-related behaviors, suggesting drug-induced plasticity in this circuit as well. This work paves the way for further studies of dopamine signal processing in limbic regions, and underscores the complexity of the circuit mechanisms that govern affective states.

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Poster

400. Animal Models of Anxiety: Pharmacology and Neural Circuitry

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Topic: G.06. Anxiety Disorders

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Title: Acquisition and expression of Social Familiarity-induced Anxiolysis (SoFiA) in male rats requires intact ventromedial prefrontal cortex and amygdala: understanding how a familiar social partner relieves anxiety.

Authors: *A. R. BURKE, S. MAJUMDAR, A. DIETRICH, K. D. ANDREWS, W. A. TRUITT;
Dept. of Anatomy, Cell Biology, & Physiol., Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Familiar social support attenuates anxiety and facilitates exposure-based therapy for anxiety disorders. We explore neurobehavioral mechanisms of this phenomenon with an animal model called Social Familiarity-induced Anxiolysis (SoFiA). SoFiA is a learned suppression of anxiety facilitated by familiar social cues that serve as social safety signals. Exposing male rats to repeated daily social interaction (SI) tests in the presence of an anxiogenic stimulus results in

sustained reductions in SI time indicative of high levels of anxiety. However, repeatedly exposing the experimental rat to the same conspecific rat during these anxiogenic daily SI tests results in a reduction of anxiety after about five SI sessions, demonstrating SoFiA. The neural mechanisms underlying acquisition and expression of SoFiA were investigated by manipulating glutamatergic neurons using chemogenetics. The DREADD virus, pAAV5-CAMKIIa-hM4D(Gi)-mCherry or a control virus, was infused into the infralimbic (IL) subdivision of the ventromedial prefrontal cortex or amygdala of male Wistar rats 6 weeks prior to any SI testing. Clozapine-n-oxide (CNO) or vehicle was injected (0.5 mg/kg, ip.) 30 mins prior to each SI test to stimulate the hM4D inhibitory receptor. While controls acquired SoFiA normally, inhibited DREADD rats continued to show low SI times in both the IL and amygdala DREADD groups, thus failing to exhibit SoFiA behavior. Both groups of these rats did gradually acquire SoFiA when vehicle was used in place of CNO on subsequent daily SI tests. These results suggest that both the IL and amygdala are key regions involved in the acquisition of SoFiA. Similar results were observed when bilateral infusions of muscimol (90 pmol), were injected into the basolateral amygdala 10 min prior to the SI tests. Intersectional chemogenetics targeting basolateral amygdala neurons that project to the IL failed to block SoFiA acquisition. Interestingly, CNO also inhibited expression of SoFiA in amygdala-DREADD rats, but not control virus rats, when CNO was administered following acquisition of SoFiA. Inhibiting the ventral hippocampus with muscimol did not alter acquisition of SoFiA. These results implicate the amygdala, in addition to the IL (past studies), in the expression of SoFiA. Overall, we discovered that the IL and amygdala are key sites of acquisition and expression of SoFiA. These results are impactful because deciphering how familiar social presence contributes to learning to alleviate anxiety will facilitate the development of novel and improved anxiety therapies.

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Poster

400. Animal Models of Anxiety: Pharmacology and Neural Circuitry

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 400.11

Topic: G.06. Anxiety Disorders

Title: Comparing effects of acute and chronic oral administration of Cannabidiol on anxiety-like behavior in rats

Authors: *C. MAESTAS-OLGUIN, T. CHANEL, N. PENTKOWSKI;
Univ. of New Mexico, Albuquerque, NM

Abstract: Anxiety disorders are a cluster of mental disorders that are characterized by excessive, debilitating worry or fear of ordinary situations. Although several psychological and pharmaceutical interventions are available to treat these conditions, less than two-thirds of patients achieve the clinical goal of remission defined as the absence or near absence of

symptoms and the return to premorbid functionality. Many of the commercially available pharmaceuticals face challenges related to long-term efficacy (benzodiazepines), delayed onset of action (SSRIs/SNRIs), risk of dependency (benzodiazepines, SSRIs), withdrawal/discontinuation syndrome (benzodiazepines, SSRIs, SNRIs), or harsh side effect profiles (TCAs, SSRIs, SNRIs) which all reduce patient adherence. Though relatively few, previous studies of general anxiety disorder, social anxiety disorder, and chronic pain induced anxiety have reported a potential anxiolytic effect of Cannabidiol (CBD), the non-psychoactive component of the *Cannabis* sativa plant. However, these studies have not explored the effects of varied administration schedules or sex on the anxiolytic effects of CBD. In this study, we aim to characterize the effects of acute or daily oral administration of CBD on the behavior of free-moving male and female rats within two unfamiliar, potentially threatening environments. Adult rats (N = 72) were randomly assigned to low-concentration full-spectrum CBD, high-concentration CBD isolate, or no CBD control groups. Rats were presented with either peanut butter alone or peanut butter laced with their assigned CBD condition for seven consecutive days. Following consumption on the first and seventh days, rats were placed in either the elevated-plus maze or the light/dark task. The order of tests was counterbalanced across all subjects. We found mixed effects of CBD on anxiety. For instance, a single administration of CBD reduced anxiety-like behaviors at low-concentration ($p=0.0087$) and high-concentration ($p=0.0088$) when compared to controls ($m = 34.6$; $SD = 12.11$, $m = 34.64$; $SD = 5.658$, and $m = 55.82$; $SD = 14.44$ respectively) in the EPM, but there were no observed differences in anxiety-like behaviors following seven days of chronic administration in the LDT. Results suggest that CBD may be a useful short-term addition to standard treatment plans for GAD. In fact, due to its relatively fast onset of effects and low risk of abuse, CBD may be the ideal intervention to compliment pharmaceutical treatments for GAD that typically take a week or more to exert their full pharmacodynamic effects. More research exploring possibly interactions with these treatments is required.

Disclosures: **C. Maestas-Olguin:** None. **T. Chanel:** None. **N. Pentkowski:** None.

Poster

400. Animal Models of Anxiety: Pharmacology and Neural Circuitry

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Title: The Effect of Atrazine on Anxiety and Fear Behaviors in Rats

Authors: *G. HERNÁNDEZ-BUSOT[1], M. RIVERA-LÓPEZ[2], M. CÁCERES-CHACÓN[2], A. FIGUEROA-PÉREZ[1], S. RODRÍGUEZ-ROSADO[1], O. MARTÍNEZ-GUZMÁN[2], D. SIERRA-MERCADO[2];
[1]Biol., Univ. of Puerto Rico Rio Piedras, San Juan, PR; [2]Anat. and Neurobio., Univ. of Puerto Rico Sch. of Med., San Juan, PR

Abstract: Atrazine (ATR) is a widely used herbicide in agriculture. Unfortunately, excessive use of ATR has led to the contamination of food and drinking sources (Mosquin et al., 2012; Whitmore and Chen, 2013). Subsequently, this has led to daily human exposure to ATR. Rodent studies have shown that ATR can influence emotionally relevant behaviors such as anxiety (Ma et al., 2018; Chavez[1]Pichardo et al., 2020). However, other emotional behaviors such as fear remain unexplored. Thus, we wanted to explore how ATR influences anxiety-like behaviors such as grooming (de Oliveira Mendes et al., 2018; Pu et al., 2020) and fear behaviors. To address this gap, rats were exposed to ATR in their drinking water at a dose considered safe for ingestion (0.035mg/kg; Environmental Protection Agency), whereas control animals received filtered water. Both groups had ad libitum access to water for four months. Following exposure, anxiety-like behavior was examined using the open field test and self-grooming behaviors. Furthermore, fear was assessed using Pavlovian fear conditioning where rats learn that a tone predicts a shock by exhibiting freezing behavior to the tone. We hypothesized that rats exposed to ATR would show increased fear expression compared to control rats. In the open field test, ATR rats trended towards having more center entries (ATR: 23.8, Controls: 14.8; n=10, p=0.0570) and had decreased time spent in corners (ATR: 70.5 s, Controls: 114.2 s; n=10, p=0.0248) compared to controls. For self-grooming, ATR rats had decreased bouts (ATR: 2.5, Controls: 1; n=10, p=0.0187) and decreased time grooming (ATR: 8s, Controls: 20s; n=10, p=0.0194) when compared to controls. During fear conditioning, no differences were observed in freezing behavior between groups (ATR: 58%, Controls: 59%; n=10; p=0.3714). These results suggest that ATR does not affect fear behaviors, but supports previous findings that it can decrease anxiety-like behaviors. Future directions aim at examining brain regions key to emotional behaviors, such as the amygdala, using immunohistochemistry. Our findings may help elucidate how prolonged ingestion of atrazine at a dose considered safe for humans may influence emotionally relevant behaviors

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Poster

400. Animal Models of Anxiety: Pharmacology and Neural Circuitry

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Program #/Poster #: 400.13

Topic: G.06. Anxiety Disorders

Support: Canadian Institutes of Health Research
Natural Sciences and Engineering Research Council

Title: Sex differences in mood and anxiety-related outcomes in response to adolescent nicotine exposure

Authors: *T. NG, M. SARIKAHYA, M. DEVUONO, H. J. SZKUDLAREK, T. UZUNESER, E. PÉREZ-VALENZUELA, S. R. LAVIOLETTE;
Anat. and Cell Biol., Univ. of Western Ontario, London, ON, Canada

Abstract: Electronic cigarette vaping has become more popular among teenagers in recent years. These products contain nicotine, which is the main psychoactive component in cigarettes. The increase in the teen vaping trend is particularly concerning since adolescence represents a critical period of brain development, where complex changes occur at the synaptic and network level. Clinically, nicotine addiction is associated with, and even causally linked to mood and anxiety disorders. In clinical studies, females are found to have increased prevalence of mood and anxiety disorders, experience greater difficulty quitting nicotine products, and are less likely to respond positively to smoking intervention treatments, suggesting a potential sex-specific response to nicotine. However, most pre-clinical studies only utilized male animal models, and sex differences in response to nicotine exposure are currently unclear in the literature. Thus, the objective of this study is to determine any sex differences in mood and anxiety-related outcomes using rodent model in response to adolescent nicotine exposure. To investigate the role of sex differences in these behavioural outcomes, adolescent male and female Sprague Dawley rats received either nicotine (0.4 mg/kg) or saline injections subcutaneously, 3 times daily for 10 consecutive days (post-natal day [PND] 35-44). Once all animals had matured into adulthood (PND 75 and later), they underwent a series of mood and anxiety-related behavioural testing, such as the open field test (OFT), light-dark box (LDB), elevated plus maze (EPM) and forced swim test (FST), followed by *in-vivo* electrophysiology recordings in the prefrontal cortex and ventral tegmental area. Meanwhile we are in the process of analyzing electrophysiological data, our preliminary behavioural data showed a trend in anxiety and depressive-like behaviours in the male nicotine group, including decreased central time in OFT, reduced open-arm time in EPM, decreased light-side time in LDB, increased immobility time in FST; whereas there were no significant differences between female treatment groups in these measures. These results indicate that estrogen or progesterone could play a protective role against mood and anxiety disorders following nicotine exposure. Furthermore, they could serve as a therapeutic starting point for developing novel smoking intervention treatments in a sex-specific manner.

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Poster

400. Animal Models of Anxiety: Pharmacology and Neural Circuitry

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Title: Screening of fast acting antidepressants in larval zebrafish using a tail-free giving up assay

Authors: *M. DUQUE RAMIREZ[1], A. B.-Y. CHEN[2], F. ENGERT[1], M. C. FISHMAN[1]; [1]Harvard Univ., Cambridge, MA; [2]Neurobio., Janelia Res. Campus, Ashburn, VA

Abstract: Major depressive disorder (MDD) is a complex disease with high prevalence. A significant number of patients show resistance to treatment with classic antidepressants, such as selective serotonin reuptake inhibitors. Recently, fast acting antidepressants including ketamine and psilocybin have shown promise to reduce MDD symptoms in treatment-resistant patients, but their mechanism of action is poorly understood and the repertoire of fast acting antidepressants is limited. Classical screening methods for new antidepressants in rodents, such as the forced swimming test and the tail suspension test, have been useful to discover new compounds in the past. However, these methods lack high-throughput and are not suitable for imaging or perturbation experiments needed to understand the mechanisms behind antidepressant action. Here, we present an assay in larval zebrafish, “tail-free giving up”, that could be useful to screen new fast antidepressants. After screening several compounds, we show that treatment with either ketamine or a 5HT2A receptor agonist, TCB-2, reduces percentage of passive time in tail-free giving up, recapitulating results observed in rodents. Moreover, co-treatment of ketamine with an adenylate cyclase inhibitor seems to reduce the effect of ketamine, suggesting a role for cyclic AMP in the fast antidepressant action of ketamine. Overall, these results suggest that tail-free giving up in larval zebrafish is a promising assay to discover new antidepressant compounds and understand the mechanism behind their action.

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Poster

400. Animal Models of Anxiety: Pharmacology and Neural Circuitry

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Program #/Poster #: 400.15

Topic: G.06. Anxiety Disorders

Support: Artelo Biosciences
MITACS Canada

Title: A novel fatty acid binding protein-5 mechanism in the basolateral amygdala regulates anxiety and fear behaviours

Authors: *M. JONES[1], T. UZUNESER[2], T. CLEMENT[4,5], H. WANG[4,5], I. OJIMA[4,5], S. R. LAVIOLETTE[2,3,6,7];
[1]Neurosci., [2]Anat. and Cell Biol., [3]Psychiatry, Univ. of Western Ontario, London, ON, Canada; [4]Inst. of Chem. Biol. and Drug Discoveries, [5]Chem., Stony Brook Univ., Stony Brook, NY; [6]Lawson Hlth. Res. Inst., London, ON, Canada; [7]Schulich Sch. of Med. and Dent., London, ON, Canada

Abstract: Anxiety disorders are a class of disorders characterized by excessive worrying, uneasiness, and stress symptoms, impacting one in three people throughout their lifetime. Current medications for treatment of anxiety-related symptoms are associated with significant side-effects, calling for the development of alternative pharmacotherapies. The endocannabinoid (eCB) system is a crucial emotional regulator within the brain, exhibiting large control over anxiety and fear reactions. We have previously reported that eCB transmission within the basolateral amygdala (BLA) regulates emotional sensory information salience via modulation of neuronal activity patterns in the prefrontal cortex (PFC). Given eCB ligands, such as anandamide, are water-insoluble lipids that have limited ability for diffusion, intracellular transportation for termination of action is largely reliant on Fatty Acid Binding Proteins (FABPs). Here, we investigated the effects of a novel FABP5 inhibitor (**SBF I-103**) when infused locally to the BLA of adult male Sprague Dawley rats on anxiety and fear behaviours, along with physiological and molecular impacts. Upon acute intra-BLA administration of SBF I-103, there was a clear anxiolytic effect across multiple behavioural assays (Light-dark box, Elevated-plus maze, Open-field test). Furthermore, we observed faster fear extinction in conditioned auditory fear expression when SBF I-103 was given prior to the first fear memory recall. On following test days, animals that had received SBF I-103 had reduced fear expression with no further drug infusions. Interestingly, these anxiolytic and fear memory effects were reversed upon administration of LEI-401 - a potent blocker of anandamide synthesis. In addition, *in vivo* neuronal recordings following intra-BLA infusion of SBF I-103 showed modulation of pyramidal neurons in the prefrontal cortex, as well as increases in gamma oscillatory patterns. Remarkably, these effects were reversed upon co-administration with either a CB2-receptor antagonist or LEI-401, allocating responsibility for the CB2-receptor system and the anandamide ligand. Finally, intra-BLA FABP5 inhibition was found to regulate phosphorylation states of numerous anxiety and stress-related proteins within the BLA. Our findings illustrate a novel FABP5-dependent mechanism within the BLA that regulates anxiety phenotypes, fear memory expression, and related neuronal circuitry. Along with advancing knowledge on neurobiological mechanisms of anxiety, this research illuminates FABP5 inhibition as a promising avenue for anxiolytic and post-traumatic stress pharmacotherapies.

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Poster

400. Animal Models of Anxiety: Pharmacology and Neural Circuitry

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Title: Glyphosate decreases exploration but not social interaction in male rats

Authors: *A. FIGUEROA-PÉREZ[1], M. CACERES-CHACON[2], S. RODRÍGUEZ-ROSADO[1], G. HERNÁNDEZ-BUSOT[1], M. RIVERA-LÓPEZ[2], O. MARTÍNEZ-GUZMÁN[2], D. SIERRA-MERCADO[2];
[1]Biol., Univ. of Puerto Rico Rio Piedras, San Juan, PR; [2]Anat. and Neurobio., Univ. of Puerto Rico Sch. of Med., San Juan, PR

Abstract: Glyphosate is the most common active ingredient in herbicides used in landscaping and agriculture. Glyphosate is thought to be safe for humans and animals because it acts by inhibiting a metabolic route almost exclusive to plants. However, recent studies in rodents have shown that glyphosate can increase the expression of anxiety-like behaviors. Moreover, studies have shown that emotional states influence exploration of novelty. The effect of glyphosate on the exploration has not been evaluated. Therefore, we aimed to evaluate the effect of pure glyphosate and a glyphosate-based herbicide (GBH) on the exploration of a novel object and caged rat. To achieve this, rats were treated with a target dose of 2.0 mg/kg of glyphosate daily (chronic references dose approved by the E.P.A.) in their drinking water. Control rats were treated in the same manner but received filtered drinking water. After 4 weeks of exposure, we assessed for exploration in an enhanced open field test that consisted of three segments, 1) empty open field, 2) open field with a novel object in the center, and 3) open field with a caged rat in the center. Here we examined the amount of time spent in the center and the amount of time exploring the object or caged rat. Neither glyphosate nor GBH affected the number of entries to the center ($F_{(2, 35)}$=0.2876; p=0.7518) nor the time spent in the center of the open field ($F_{(2, 35)}$=0.08574; p=0.9180). Interestingly, glyphosate but not GBH, decreased the number of approaches to the novel object ($F_{(2, 35)}$=3.970; p=0.0279) as well as the time exploring the novel object ($F_{(2, 35)}$=3.843; p=0.0310). However, neither glyphosate nor GBH affected the number of approaches toward the caged rat ($F_{(2, 35)}$=0.09876; p=0.9062), nor the time spent exploring the caged rat ($F_{(2, 35)}$=0.1516; p=0.8599). Given these results, it appears that glyphosate exposure decreases exploratory behaviors of inanimate object but not animate animals. Moreover, these effects seem to be limited to pure glyphosate, the active ingredient in GBH. Current work is evaluating for neuronal activity and markers of inflammation in brain regions such as the hippocampus and the anterior cingulate cortex that are involved in exploratory behaviors and social interaction.

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Poster

400. Animal Models of Anxiety: Pharmacology and Neural Circuitry

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Title: From an empty stomach to anxiolysis: molecular and behavioural sex differences in ghrelin axis

Authors: *S. BÖRCHERS[1,2], J.-P. KRIEGER[1,2], I. MARIC[1,3,2], J. CARL[1], M. ABRAHAM[1], F. LONGO[1,2], M. ASKER[1,2], J. E. RICHARD[1], K. P. SKIBICKA[1,3,2];
[1]Univ. of Gothenburg, Göteborg, Sweden; [2]Wallenberg Ctr. for Mol. and Translational Medicine,, Göteborg, Sweden; [3]Penn State Univ., University Park, PA

Abstract: Ghrelin, a stomach-produced hormone, is well-recognized for its role in promoting feeding, energy homeostasis, and glucoregulation, by acting on the growth-hormone secretagogue receptor 1A ($GHSR_{1A}$). It became clear only recently that its function in ensuring survival extends beyond that: its release paralleling that of corticosterone, together with anxiolytic and antidepressant effects of calorie restriction and ghrelin administration clearly suggest a role in stress and anxiety. Work examining sex differences in ghrelin's effect on ingestive behavior indicates intact males and ovariectomized female rats show a higher sensitivity to its orexigenic effects compared to intact females. To date, most studies of ghrelin's effects on anxiety have been conducted exclusively on male rodents. Here, we hypothesize that females are wired for higher ghrelin sensitivity. In comparison to males, we show that female rats have higher serum levels of acyl ghrelin, while expressing lower levels of the endogenous antagonist LEAP-2. Additionally, females express higher levels of $GHSR_{1A}$ in brain areas involved in feeding and anxiety-like behavior, such as lateral hypothalamus, hippocampus, and amygdala. Moreover, overnight fasting increased $GHSR_{1A}$ expression in the amygdala of females, but not males. To evaluate the expression of anxiety-like behavior, male and female rats were tested in the elevated plus maze (EPM), open field (OF), and acoustic startle response (ASR) test after three complementary ghrelin manipulations: increased endogenous ghrelin levels through overnight fasting, systemic administration of ghrelin, or blocking fasting-induced ghrelin signalling with a $GHSR_{1A}$ antagonist. We show that females exhibit a stronger anxiolytic response to fasting in the ASR, supporting our findings of sex differences in the ghrelin axis.

Most importantly, after antagonizing ghrelin's effects, females but not males show an anxiogenic response in the ASR, and a more pronounced anxiogenesis in the EPM and OF compared to males. Loss of gonadal steroids through gonadectomy decreased ghrelin levels and reduced the effect of ghrelin on anxiety-like behaviour in females only. Collectively, our findings suggest that, in the context of anxiety-like behavior, female rats appear to be wired for higher ghrelin sensitivity compared to males.

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Poster

400. Animal Models of Anxiety: Pharmacology and Neural Circuitry

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Program #/Poster #: 400.18

Topic: G.06. Anxiety Disorders

Support: MITACS Canada
Canopy Health Innovations

Title: Investigating the molecular mechanisms underlying cannabidiol - linalool synergistic anxiolytic effects: Evidence for the Entourage Effect

Authors: *M. RODRIGUEZ RUIZ[1], R. LEU[1], R. S. MANN[1], S. N. WHITEHEAD[1], S. SCHMID[1], W. J. RUSHLOW[1], S. R. LAVIOLETTE[2];
[1]Anat. and Cell Biol., [2]Dept. Anat. & Cell Biology; Dept. of Psychiatry; Lawson Hlth. Res. Inst., Univ. of Western Ontario, London, ON, Canada

Abstract: Anxiety disorders are the most prevalent neuropsychiatric syndromes and, even though there are multiple treatments available, most of them carry considerable side-effects. Given the urgent need for safer pharmacotherapies together with the emerging evidence showing cannabidiol (CBD) and specific monoterpene compounds to have promising potential for the treatment of anxiety-related syndromes, in the present work we aimed to determine if low doses of CBD in combination with the monoterpene linalool could be promoting an enhanced anxiolytic efficacy than the individual treatments (Entourage effect) and to explore the molecular mechanisms underlying such synergy. Our experiments were designed considering the main role of the mesocorticolimbic pathway and the endocannabinoid and GABAergic neurotransmitter systems in regulating anxiety. First, via olfactory and intra-Nucleus accumbens shell (NAcSh) routes of administration of linalool and CBD, respectively, we demonstrate the existence of an Entourage anxiolytic effect by subjecting treated adult male Sprague-Dawley rats to behavioural anxiety tests. Next, to explore the molecular mechanisms potentially underlying this synergy, we evaluated CBD and linalool formulations *in vitro* by kinetic cAMP assays performed in HEK293 cells expressing CB1R and our results indicate that CBD and linalool exert a synergistic negative

allosteric modulation at CB1R. Since CB1R-expressing synapses in NAcSh and basolateral amygdala have been demonstrated to specifically contact post-synaptic terminals expressing GABAARs, we evaluated CBD and linalool action on α1β3γ2-GABAARs by whole-cell voltage-clamp experiments in transfected HEK293 cells and discovered a differential positive allosteric modulation, by which CBD at low concentrations can increase decay time but does not affect amplitude, whereas linalool mainly increases amplitude at high concentrations. Finally, based on our *in vitro* results we hypothesized that linalool potentiation of CBD anxiolytic effect would be driven by both an increase in synaptic GABA levels through CB1R negative presynaptic modulation and ultimately by a positive modulation of post-synaptic GABAAR. To test this hypothesis, we repeated *in vivo* behavioural experiments including intra-NAcSh infusions with flumazenil, a GABAAR antagonist at the benzodiazepine site, which resulted in a complete suppression of CBD+linalool anxiolytic synergistic effect. Altogether, these findings support the existence of an Entourage effect and highlight the safe profile and efficacy of combined low doses of CBD+linalool as an alternative to conventional anxiolytic medications.

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Poster

400. Animal Models of Anxiety: Pharmacology and Neural Circuitry

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 400.19

Topic: G.06. Anxiety Disorders

Support: NARSAD 2019 Young Investigator Grant
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Title: Mast cells as novel modulators of brain serotonergic circuits driving anxiety

Authors: *N. DUQUE-WILCKENS[1], R. TEIS[1], E. SARNO[2], A. MOESER[3], A. J. ROBISON[1]; [1]Physiol., [3]Large Animal Clin. Sci., [2]Michigan State Univ., East Lansing, MI

Abstract: Anxiety disorders are among the most prevalent disorders worldwide, but the available treatments have suboptimal efficiency and tolerability. Mast cells (MCs), innate immune cells best known for their role in peripheral immunity, could represent a novel therapeutic target for anxiety disorders. MCs are ubiquitous residents of tissues throughout the body, including the brain; they express a diverse array of receptors for chemokines, cytokines, and neurotransmitters, including corticotropin releasing factor; and they can release a plethora of mediators that could affect neuronal, immune, and glial functions, including inflammatory cytokines and neurotransmitters such as histamine and serotonin. Together, these suggest that MCs are uniquely positioned to coordinate the multisystem responses to environmental and internal cues driving behavioral and psychological adaptation to stress, but their role in anxiety phenotypes is not well understood. We first found that, compared to wild type mice, MC

knockout c-Kit$^{w\text{-}sh}$ (sash) mice show sucrose avoidance, increased social vigilance, and are highly reticent to investigate open spaces. These results indicate that MC deficient mice display a basal hyperanxious phenotype, which is consistent with previous findings (Nautiyal *et al*, 2008) and suggests that MCs play a role in preventing exaggerated anxiety responses, but the underlying mechanisms remain unknown. We now report that reconstitution with wild type bone marrow derived mast cells (BMMCs) limited to central tissues is sufficient to normalize anxiety-like behaviors in sash mice, suggesting that brain MCs are involved in the anxiolytic effects. Next, we compared the expression of multiple genes in a variety of brain areas between wild type and sash mice and found that multiple genes associated with serotonergic signaling, as well as brain derived neurotrophic factor (BDNF), were reduced in sash vs. wild type dorsal raphe and hippocampus, while other genes, like inflammatory cytokines, were not differentially expressed. Further, using immunohistochemistry, we found that sash mice show reduced serotonin+ cells in the dorsal raphe, and preliminary data suggest that the anxiety-like behavior in sash mice can be reduced with only a single IP injection of either 1mg/Kg ketamine (NMDAR antagonist) or 20mg/Kg fluoxetine (serotonin transporter inhibitor). Together, these results suggest that brain MCs play a fundamental role in preventing exaggerated anxiety responses through modulation of serotonergic/BDNF signaling, providing a novel mechanism underlying anxiety-like responses to stress.

Disclosures: **N. Duque-Wilckens:** None. **R. Teis:** None. **E. Sarno:** None. **A. Moeser:** None. **A.J. Robison:** None.

Poster

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Title: Asprosin, a metabolic hormone, modulates cognition

Authors: *B. BASU, I. MISHRA, A. CHOPRA;
Case Western Reserve Univ., Case Western Reserve Univ., Cleveland, OH

Abstract: Obesity is associated with substantially increased risk for neurodegenerative and neuropsychiatric disorders, including anxiety and depression. The mechanistic link for the relationship between obesity and neuropsychiatric disorders, however, is unknown. In 2016, our lab discovered asprosin, a fasting-induced hormone that is highly expressed in adipose tissue. Upon secretion, asprosin stimulates appetite and hepatic glucose release. Asprosin is a ~30 kDa C-terminal cleavage product of fibrillin-1 that normally circulates in cerebrospinal fluid (CSF)

and plasma at nanomolar levels, and is significantly elevated in obesity. Importantly, we have demonstrated that anti-asprosin monoclonal antibodies (mAbs) are a dual-effect pharmacologic therapy that targets the two key pillars of metabolic syndrome (MS) - overnutrition and blood glucose burden with obesity. Recently we have also identified the neural target for asprosin, Protein tyrosine phosphatase receptor δ (Ptprd), which is highly expressed throughout the brain, in regions that have been implicated in cognition. This study suggests that elevated levels of asprosin alter cognitive functions, thereby providing a potential mechanistic link with obesity and a potential therapy for certain neuropsychiatric disorders. Specifically, my results indicate that (1) pharmacologic asprosin neutralization mitigates cognitive deficits in diet-induced obese mice, (2) asprosin neutralization in lean mice improves cognition in certain assays, despite not affecting metabolism, and (3) genetic inhibition of asprosin and its receptor leads to improvement of cognition. Future studies will delineate the central circuit necessary for asprosin-mediated modulation of neuropsychiatric disorders. My studies thus far suggest a novel function of asprosin in the control of cognition, and that the anti-asprosin mAb could be a novel cognitive agent that may have particularly enhanced benefits for individuals with obesity-associated neuropsychiatric disorders, significantly improving the quality of life in these individuals.

Disclosures: **B. Basu:** 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.; T32 GM007250. **I. Mishra:** None. **A. Chopra:** 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.; NIDDK (DK102529, DK118290). E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Atul Chopra has been awarded asprosin-related patents, and is a co-founder, director and officer of Vizigen, Inc., and Aceragen, Inc., and holds equity in both companies..

Poster

400. Animal Models of Anxiety: Pharmacology and Neural Circuitry

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Title: Glyphosate decreases exploration and increases threat response in male rats

Authors: *M. CÁCERES-CHACÓN[1], S. RODRÍGUEZ-ROSADO[2], A. FIGUEROA-PÉREZ[2], G. HERNÁNDEZ-BUSOT[2], M. RIVERA-LÓPEZ[1], O. MARTÍNEZ-GUZMAN[1], D. SIERRA-MERCADO[1];
[1]Anat. & Neurobio., Univ. of Puerto Rico Sch. of Med., San Juan, PR; [2]Biol., Univ. of Puerto Rico Rio Piedras, San Juan, PR

Abstract: Epidemiological studies have shown a link between environmental contaminants and mental health disorders such as anxiety. Glyphosate, a common herbicide, has been identified as an environmental contaminant, and has garnished attention due to its detection in food sources. Glyphosate was initially considered safe for mammals because it acts by inhibiting a metabolic route almost exclusive to plants. However, a correlation has been seen between the increased diagnosis of anxiety and the use of glyphosate. This highlights the need to evaluate the effect of glyphosate on the development of anxiety. A few studies have shown that high doses of glyphosate can affect emotional behaviors in rodents. However, few studies have evaluated how prolonged exposure to environmentally relevant doses can affect anxiety. Therefore, we aimed to assess the effect of glyphosate at the EPA established chronic reference dose, on anxiety-like behaviors. We also aimed to assess the effect of glyphosate on exploration and threat behaviors in response to novel neutral stimuli. To achieve this, rats were given water containing pure glyphosate or a commercial glyphosate-based herbicide (GBH) at a dose of 2.0 mg/kg of glyphosate. Control rats received filtered drinking water. Glyphosate and GBH did not affect bodyweight gain, water intake, or food intake. After 10 weeks, anxiety-like behavior was assessed in an elevated plus maze. Here, glyphosate and GBH decreased time spent in the open arms ($F_{(2, 35)}$=7.358; p=0.0021). After an additional 4 weeks, animals were assessed for exploration and threat response in an open field with a novel object in the center. Here, Glyphosate and GBH decreased time spent exploring the novel object ($F_{(2, 34)}$=8.164; p=0.0013) and increased time spent immobile ($F_{(2, 34)}$=7.896; p=0.0015). Lastly, after 16 weeks of exposure, we performed an auditory startle response test (5 repetitions of a tone in a familiar context). Threat response was measured as time spent immobile during the tone. We observed that glyphosate but not GBH increased immobility during the tone ($F_{(2, 34)}$=4.878; p=0.0135). Given the role of the prefrontal cortex (PFC) in the modulation of anxiety, animals were sacrificed, and we evaluated PFC for neuronal activity using c-Fos immunohistochemistry. Interestingly, there was no difference in neuronal activity in PFC. We conclude that glyphosate increases anxiety-like behaviors as well as negative valence interpretation of different types of neutral novel stimuli. Future directions include performing immunohistochemistry on brain regions involved in anxiety-like behaviors such as the basolateral amygdala and bed nucleus of the stria terminalis.

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Poster

400. Animal Models of Anxiety: Pharmacology and Neural Circuitry

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Title: The effects of glyphosate on locomotion, grooming, and rearing behavior in male rats

Authors: *M. A. GONZALEZ-PEDRAZA, H. G. HADDOCK-MARTÍNEZ, I. S. CASTRO-RIVERA, A. FIGUEROA-PÉREZ, G. HERNÁNDEZ-BUSOT, S. RODRÍGUEZ-ROSADO, M. RIVERA-LÓPEZ, M. CÁCERES-CHACÓN, O. MARTÍNEZ-GUZMÁN, D. SIERRA-MERCADO;
Anat. & Neurobio., Univ. of Puerto Rico Sch. of Med., San Juan, PR

Abstract: Glyphosate is a common herbicide that has been identified as an environmental contaminant. The use of glyphosate is correlated to increased incidence of anxiety disorders. Though initially considered safe for mammals, recent reports suggest that glyphosate increases negative valence emotional behaviors in rats. Studies evaluating how glyphosate affects spontaneous behavioral activity, such as locomotion, grooming and rearing, are lacking. Thus, we hypothesized that prolonged exposure to glyphosate will decrease locomotion, result in abnormal grooming, and decrease rearing behavior in rats. To test this hypothesis, male Sprague Dawley Rats (n=13) had access ad libitum to drinking water containing glyphosate for 16 weeks, whereas control rats received filtered drinking water. Glyphosate did not affect bodyweight gain nor water intake. After glyphosate exposure, rats were recorded in a home cage-like familiar cage for 10 minutes. Distance traveled was obtained using ANY-maze video tracking software. Additionally, videos were manually scored by blind experimenters for grooming bouts, time spent grooming, and number of rears. Data shows that glyphosate exposure decreased total distance traveled (glyphosate: 9.73, control: 15.8; p=0.0318). Interestingly, glyphosate exposure did not affect total grooming bouts (glyphosate: 2.46, control: 5.08; p=0.1039) nor percent time spent grooming (glyphosate: 5.14, control: 8.51; p=0.2269), but decreased rearing behavior (glyphosate: 16.8, control: 24.3; p=0.0383). Future directions include assessing these ethologically relevant behaviors across contexts to explore the context-dependent nature of these behaviors. Additionally, we will assess brain tissue for changes in biomarkers of neuronal activity in the hippocampus, a brain region implicated in context recognition and the expression of these ethologically relevant behaviors. The results of this study will allow us to elucidate the potential effects that glyphosate may have on the brain, and how these effects could manifest as aberrant behaviors in rats.

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Poster

400. Animal Models of Anxiety: Pharmacology and Neural Circuitry

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Topic: G.06. Anxiety Disorders

Support: US Department of Veterans Affairs Merit Review Grant I01 BX003377

Title: Testing the capacity of Interleukin-4 to attenuate traumatic brain injury-induced anxiety-like behaviors via regulating microglial responses in the limbic system

Authors: *H. PU[1], Y. WANG[1], Y. ZHAO[1], F. YU[1], W. ZHANG[1], R. B. PRICE[2], R. K. LEAK[4], T. K. HITCHENS[3], J. CHEN[1];
[1]Neurol., [2]Psychiatry, [3]Neurobio., Univ. of Pittsburgh, Pittsburgh, PA; [4]Pharm., Duquesne Univ., Pittsburgh, PA

Abstract: Objectives: Traumatic brain injury (TBI) is commonly followed by long-term symptoms of mental disorders. Anxiety disorder is an important type, which occurs in 9.1-23.8% of TBI patients. The present study sought to investigate whether repetitive intranasal delivery of interleukin-4 (IL-4) nanoparticles attenuated anxiety-like symptoms after TBI and the underlying mechanisms.
Methods: STAIR guidelines were followed, including investigator blinding. Adult male 10-12-week-old C57BL/6J mice were subjected to controlled cortical impact (CCI), then assessed by a battery of anxious neurobehavior tests after CCI. Long-term potentiation (LTP) of excitatory synaptic transmission will be performed to testify to the functional integrity of the hippocampus. White matter tracts' integrity in the limbic system will be evaluated using Diffusion Tensor Imaging (DTI) ex vivo. NeuN immunostaining will be performed to calculate the long-term neuron loss in the limbic system. mRNA and protein expression of M1/M2 markers (CD16/CD206) and PPARγ in Microglial/macrophage (Mi/Mϕ) will be assessed by RT-PCR and immunofluorescent staining. Mi/Mϕ-specific PPARγ conditional knockout (mKO) mice were constructed to determine the role of PPARγ in Mi/Mϕ polarization.
Results: IL-4-elicited mitigation of anxiety was associated with improvements in the integrity of the limbic system at the functional (LTP) and structural levels (neuronal loss, DTI). IL-4 increased the expression of PPARγ and CD206 within Mi/MΦ, thereby driving microglia toward a global inflammation-resolving phenotype. Notably, IL-4 failed to shift microglial phenotype after CCI in mKO mice, indicating an obligatory role for PPARγ in IL-4-induced Mi/MΦ polarization. Accordingly, IL-4 failed to improve the structural integrity of the limbic system or emotional functions in PPARγ mKO mice.
Conclusions: These results demonstrate that IL-4 stimulates PPARγ-dependent beneficial Mi/MΦ responses and promotes neuropsychological recovery after TBI. Thus, Il-4 may translate to effective clinical treatment for TBI.

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Poster

401. Auditory, Visual, Proprioceptive Perception

Location: SDCC Halls B-H

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Program #/Poster #: 401.01

Topic: H.02. Perception and Imagery

Title: The vivid lack of correlation between the vividness of mental imagery and music evoked emotions

Authors: V. TONG, J. YANG, S. HAFEZI, I. AMU, J. FREDA, *P. WALLISCH;
New York Univ., New York, NY

Abstract: Autobiographical memories are known to evoke vivid mental images. It has been proposed that they generate emotions that are associated with such memories. Music can also evoke strong and specific emotions. Thus, we wondered whether the emotions evoked by music - nostalgia in particular - are mediated by the vividness of mental imagery. To test this possibility, we used music to evoke emotions and measured the vividness of mental imagery with the VVIQ in a high powered sample (n = 944). We found that - contrary to our expectations - all music evoked emotions (including nostalgia) were uncorrelated with the vividness of mental imagery. This has interesting and important implications for the of role of the vividness of mental imagery in cognition, as well as for the question as to what does underlie the strong emotions evoked by music.

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Poster

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Topic: H.02. Perception and Imagery

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Title: Neural dynamics of listened and imagined musical sound sequences

Authors: *D. R. QUIROGA-MARTINEZ[1], L. BONETTI[2], R. T. KNIGHT[3], P. VUUST[4]; [1]UC Berkeley, UC Berkeley, Berkeley, CA; [2]Linacre Col. & Dept. of Psychiatry, Ctr. for Eudaimonia and Human Flourishing, University of Oxford, United Kingdom; [3]Univ. of California Berkeley, California Clin. Trials, Berkeley, CA; [4]Univ. of Aarhus, Univ. of Aarhus, Aarhus, Denmark

Abstract: Imagine a song you know by heart. With low effort you could play it vividly in your mind. However, little is known about how the brain represents and holds in mind such musical "thoughts". Here, we leverage time-generalized decoding from MEG brain source activations to show that listened and imagined melodies are represented in auditory cortex, thalamus, middle cingulate cortex and precuneus. Accuracy patterns reveal that during listening and imagining sounds are represented as a melodic group, while during listening they are also represented individually. Opposite brain activation patterns distinguish between melodies during listening compared to imagining. Furthermore, encoding, imagining and retrieving melodies enhances delta and theta power in frontopolar regions, and suppresses alpha and beta power in parietal and sensorimotor regions. Our work sheds light on the neural dynamics of listened and imagined musical sound sequences.

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Poster

401. Auditory, Visual, Proprioceptive Perception

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Program #/Poster #: 401.03

Topic: H.02. Perception and Imagery

Support: GRAMMY Museum Research Award

Title: Expectation in music listening: tracking the neural representation of surprisal

Authors: *E. B. ABRAMS, E. M. VIDAL, C. PELOFI, P. RIPOLLÉS; Psychology, NYU, New York, NY

Abstract: Music - an abstract reward - is known to engage a complex range of emotions and plays a crucial role in mood regulation, making it a useful tool in investigating the cognitive neuroscience of emotion and reward. Various features, from low-level acoustics to statistical regularities to memory associations, contribute to the experience of musical reward and emotion. Recent work suggests that surprisal, or, the unexpectedness of events over time, may directly predict listeners' experiences of reward while listening to a piece of music. Thus, understanding the neural basis of surprisal is crucial in generating a more complete picture of reward elicited by value-neutral and abstract stimuli.

To investigate whether and how the brain tracks surprisal during music listening, we validate a new computational model, called Dynamic Regularity Extraction (D-REX), that uses Bayesian inference to predict the continuous surprisal listeners experience while listening to music. Twenty participants listened to thirty one-minute-long musical excerpts while magnetoencephalography (MEG) activity was recorded. Surprisal was calculated using D-REX for each excerpt. To determine how well D-REX's surprisal output was represented in the neural activity, we used a decoding algorithm which calculates Temporal Response Functions (TRFs). A TRF describes the mapping between a stimulus feature and the neural data and is then trained and tested on an unseen part of the data using leave-one-out cross-validation, resulting in a correlation value for each channel between the real and predicted neural data.
In our first analysis, we tested the distribution of r-values for the actual D-REX surprisal against a random distribution using permutation tests. We found a significant enhancement of r-values obtained from the real model as compared to the null model, which suggests that D-REX surprisal is encoded in the neural data. We then examined the TRFs estimated for each MEG channel for a model trained either on the acoustic envelope or the surprisal output and found significantly higher peaks at what may be considered P1, P2, and P3 ERP components for the TRFs derived from the surprisal input. The third component, a well-characterized neurophysiological marker of syntactic processing, strongly suggests that D-REX is capturing higher-level expectations.
The present study finds that the brain tracks statistical regularities and expectations during music listening as modeled by D-REX. Given the crucial role that musical prediction plays in reward, our results lay the groundwork for assessing the prediction-to-reward relationship using neural data in a computationally well-defined framework.

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Poster

401. Auditory, Visual, Proprioceptive Perception

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Topic: H.02. Perception and Imagery

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Title: Neural representations of rhythm and beat perception

Authors: *J. D. HODDINOTT[1], J. A. GRAHN[2];
[2]Psychology, [1]Univ. of Western Ontario, London, ON, Canada

Abstract: Humans spontaneously perceive an underlying pulse, or "beat," arising from the rhythmic structure in music. Functional magnetic resonance imaging (fMRI) studies show that motor regions of the brain, such as the basal ganglia, supplementary motor area (SMA), and

premotor cortices have increased activity when people listen to rhythms with a strong beat (Grahn & Brett, 2007). However, previous fMRI studies have generally used univariate analyses, which investigate activity averaged over voxels in a region, whereas multivariate techniques can identify patterns of covariance across voxels that allow greater sensitivity and better identification of stimulus features that predict brain-behavior relationships. Thus, here we use multivariate pattern analysis (MVPA) to compare neural activity patterns elicited by acoustic rhythms with strong, weak, or no beat. By measuring the dissimilarity between activity patterns associated with each of our 12 rhythms, MVPA reveals which neural regions are sensitive to beat strength, and which regions are 'tuned' to individual acoustic rhythms with unique activity patterns. We predicted that auditory areas would be tuned to individual rhythms, and exhibit highly dissimilar activity patterns between each pair of rhythms. We also predicted that motor areas would be tuned to beat strength, either with highly dissimilar activity patterns elicited by individual strong-beat rhythms, and low dissimilarity between patterns elicited by individual weak- and non-beat rhythms, or by activating in highly-correlated patterns for strong-beat rhythms, with high-dissimilarity occurring between rhythms of different beat strength conditions. Our data (n = 26) show that auditory regions indeed encode rhythms via multivariate patterns: Auditory cortical regions, including the superior temporal gyrus, exhibit significantly dissimilar activity patterns between all rhythm pairs. Additionally, we show evidence that motor cortices, such as the SMA, activate in correlated patterns for strong-beat rhythms, but have high dissimilarity between strong-beat and nonbeat rhythms, suggesting this area is sensitive to beat strength. Our findings build on previous univariate work by showing motor cortices may be tuned to beat strength in acoustic rhythms.

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Poster

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Topic: H.02. Perception and Imagery

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Title: Distinctive cortical networks underlying oddball and omission responses during sequential auditory perception

Authors: ***J. SHIN**[1], Y.-H. WU[2], L. FAES[3], Z. YU[4], M. A. CLOOS[5], S. DEVORE[1], W. K. DOYLE[6], P. DUGAN[1], D. FRIEDMAN[1], A. SEEDAT[1], O. DEVINSKY[1], E. S. YACOUB[7], F. DE MARTINO[3], L. MELLONI[8];
[1]Neurol. Dept., [2]Neurosci. Inst., New York Univ. Sch. of Med., New York, NY; [3]Dept. of Cognitive Neurosci., Maastricht Univ., Maastricht, Netherlands; [4]Dept. of Med., Univ. of Hawai'i, Honolulu, HI; [5]Ctr. for Advanced Imaging, Univ. of Queensland, Brisbane, Australia; [6]Dept. of Neurosurg., NYU, New York, NY; [7]Dept. of Radiology, Univ. of Minnesota,

Minneapolis, MN; [8]Neural Circuits, Consciousness and Cognition Res. Group, Max Planck Inst. for Empirical Aesthetics, Frankfurt am Main, Germany

Abstract: There is substantial evidence that auditory processing is influenced by the prediction of upcoming sounds. Yet, there remain long-standing questions regarding the local circuit and network-level mechanisms that implement predictive processing including which specific cortical areas and layers reflect the active calculation of prediction errors vs automatic stimulus specific adaptation. In this study, we combine non-invasive high-resolution laminar fMRI at 7 Tesla with intracranial electrocorticography (ECoG) to investigate the laminar profile of responses to predicted and unpredicted auditory stimuli, as well as the circuit level responses. We adapted an auditory oddball paradigm where syllables were repeated four times in a sequence. In 25% of trials, we replaced the last repetition with either a deviant syllable (12.5%) or silence (12.5%) in order to examine oddball and omission responses, respectively. This paradigm enabled us to simultaneously investigate auditory predictions errors (i.e., unexpected syllables) as well as unexpected omissions. Using ECoG in treatment-resistant epilepsy patients, we found that electrodes in the superior temporal gyrus (STG) and frontal areas show distinctive oddball and omission responses. Consistent with previous reports, deviant syllables triggered stronger responses in these electrodes. Omission trials also induced responses; however, the omission responses did not always correlate with the oddball responses at the single electrode level. Importantly, neither prediction errors nor omission responses were caused by motor preparation as we also observed these responses in a no-report paradigm under passive listening conditions. We also performed laminar functional imaging in the STG using 7 Tesla MRI to elucidate the laminar origin of oddball and omission responses. Our results suggest that different types of auditory prediction signals are generated by distinct cortical networks.

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Poster

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Topic: H.02. Perception and Imagery

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Title: The Beholder's Share: Cross-subject Variability in Responses to Abstract Art

Authors: *C. DURKIN, B. PETERS, C. BALDASSANO, E. KANDEL, D. SHOHAMY; Columbia Univ., New York, NY

Abstract: Subjective experience of art emerges from an interaction between external input, which is shared across individuals, and internal associations, which vary across individuals and

give art its personal meaning. In art theory, the Beholder's Share refers to the contribution a viewer makes to the meaning of a painting by drawing on a set of unique prior experiences. A key tenet of the Beholder's Share is that a viewer brings more personal meaning to abstract art than to representational art. Here, we empirically test this theory. Specifically, we use fMRI to examine the extent to which abstract vs. representational art elicit different patterns of brain activity across individuals. We reason that more personal meaning brought to a painting should manifest in variability across subjects in responses to the same painting. Thus, we predicted that both neural and behavioral variability should be greater in response to abstract paintings than representational paintings. To test this, we scanned participants with fMRI while they viewed a series of paintings that were either abstract or representational. To determine whether subjects respond more subjectively to abstract vs. representational paintings, we measured cross-subject variability in patterns of BOLD activity. We found that abstract paintings elicited more variable patterns of BOLD activity. Moreover, this enhanced cross-subject variability for abstract paintings was found throughout the Default Mode Network, but not in low-level visual regions, such as the primary visual cortex. This pattern is consistent with the idea that abstract paintings evoke more subjective high-level responses despite common visual input. Next, we leveraged neural networks to model how differences in individuals' prior visual experiences could drive the variability in high-level responses to abstract art. We simulated individual differences in visual experience using instances of the same neural network (ResNet50) trained on different visual data sets and compared across-network variability in activations for abstract and representational paintings. We found that representations varied across networks more for abstract paintings than for representational paintings. Complementing the fMRI results, this pattern was found specifically in higher layers of the network, while remaining similar in early layers of the networks. Overall, these studies provide insight into a possible neural instantiation of the Beholder's Share and how it may emerge from individual differences in prior experience.

Disclosures: C. Durkin: None. **B. Peters:** None. **C. Baldassano:** None. **E. Kandel:** None. **D. Shohamy:** None.

Poster

401. Auditory, Visual, Proprioceptive Perception

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Program #/Poster #: 401.07

Topic: H.02. Perception and Imagery

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NSF Grant 1734887

Title: Attentional focus while viewing Italian Renaissance paintings

Authors: *R. G. ALEXANDER[1], A. VENKATAKRISHNAN[2], J. CHANOVAS[2], S. L. MACKNIK[1], S. MARTINEZ-CONDE[3];

[1]SUNY Downstate Hlth. Sci. Univ., [2]SUNY Downstate Hlth. Sci. Univ., Brooklyn, NY; [3]State Univ. of New York Downstate Med. Ctr., State Univ. of New York Downstate Med. Ctr., Brooklyn, NY

Abstract: We conducted a psychophysical and eye-tracking study aimed at quantifying gaze dynamics during the free viewing of Rubens' version of Titian's "Fall of Man," as well as during the viewing of other similar paintings. Rubens' copy of Titian's masterpiece is very similar to the original in many ways, and virtually identical in some regards. By comparing gaze behavior during viewing of the two paintings, presented on a computer screen, we assessed the effects of Rubens' changes to Titian's composition on the viewer's experience. An EyeLink 1000 was used to record gaze binocularly from 33 participants as they viewed each of the images for 45 seconds. We analyzed multiple gaze parameters, including fixation duration, saccade and microsaccade production, fixation consistency across subjects, and others, as well as the natural image statistics of each painting. We found that participants gazed for longer durations at Eve's face in the Rubens' version of the painting than in Titian's original. In addition, gaze positions were more dispersed for the Titian painting than for the Rubens painting, indicating different allocations of viewer interest for each painting. Our combined results suggest that the above differences in gaze behavior can be attributed to critical variations in composition between the two paintings. Notably, Rubens changed the depicted characters' body arrangement and gaze direction, providing powerful joint attentional cues for viewers to focus their central vision on Eve's face. Thus, joint attention may be a key element in the viewer's experience of "The Fall of Man" as imagined by Rubens—though not by Titian.

Disclosures: R.G. Alexander: None. **A. Venkatakrishnan:** None. **J. Chanovas:** None. **S.L. Macknik:** None. **S. Martinez-Conde:** None.

Poster

401. Auditory, Visual, Proprioceptive Perception

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Program #/Poster #: 401.08

Topic: H.02. Perception and Imagery

Support: NRF Korea 2022R1I1A2067731

Title: Pictorial cues are superior to binocular cues for size perception when they are in conflict

Authors: *S.-A. YOO, S. LEE, S. J. JOO;
Psychology, Pusan Natl. Univ., Busan, Korea, Republic of

Abstract: The visual system seems to optimally integrate multiple depth cues based on their reliability to represent objects' size. To understand the visual cue integration process, we examined how the congruency between binocular disparity and pictorial cues affects size perception using the Ponzo illusion in three-dimensional (3D) settings. We presented a Ponzo figure which consisted of the left, middle, and right walls. The pictorial cues in the figure made

the left wall look closer to a participant compared to the right wall. Binocular disparity was applied to the figure using virtual reality settings. It could provide the same depth information as pictorial cues (congruent) or the opposite information (incongruent). Each of the two red bars was superimposed on the left and right walls, respectively and participants (n = 50) judged which bar appeared longer. We used an adaptive staircase method to measure each participant's point of subjective equality (PSE). On a given trial, while the size of the right bar was kept constant (standard), the size of the left bar was adaptively adjusted. The PSE was estimated and the illusion magnitude was defined by (PSE - standard)/standard x 100. In Experiments 1 and 2, the 3D depth of the red bars was manipulated so they were completely embedded in the Ponzo figure or separated from the figure. When binocular and pictorial cues were congruent, the illusion was stronger than the typical Ponzo illusion. However, depth separation of the red bars substantially reduced the illusion magnitude. When the depth cues were incongruent, the illusion magnitude was similar to that of the typical Ponzo illusion. An additional analysis suggested that participants relied more on pictorial cues for size judgment. Interestingly, depth separation did not reduce the illusion magnitude as much as in the congruent condition. The results demonstrated that the effects of depth cues are cumulative if they provide the same information. They also suggested that pictorial cues may have a higher processing priority than binocular disparity if they are in conflict. This is probably due to the higher uncertainty of binocular disparity in our Ponzo figure that sometimes went against typical visual experiences. Experiment 3 tested if our findings in the incongruent condition were indeed derived from depth cue processing or not. The illusion magnitude decreased to a greater degree after removing linear perspective, suggesting that depth cue processing was the main factor of size perception in our study. To sum, the visual system puts more weight on pictorial cues with higher reliability to optimize size perception.

Disclosures: S. Yoo: None. **S. Lee:** None. **S.J. Joo:** None.

Poster

401. Auditory, Visual, Proprioceptive Perception

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Program #/Poster #: 401.09

Topic: H.02. Perception and Imagery

Title: From "Impossible" to "Possible": Investigating Perceptual Inference and Neural Activity on 3D Impossible Objects

Authors: *Y.-C. HSIEH[1], S. KOKICHI[4], C.-L. TSAI[5], W.-S. LAI[1,2,3];
[1]Dept. of Psychology, Natl. Taiwan Univ., Taipei City, Taiwan; [2]Grad. Inst. of Brain and Mind Sci., [3]Neurobio. and Cognitive Sci. Ctr., Natl. Taiwan Univ., Taipei, Taiwan; [4]Meiji Inst. for Advanced Study of Mathematical Sci., Meiji Univ., Tokyo, Japan; [5]Natl. Palace Museum, Taipei, Taiwan

Abstract: As claimed by Dr. Rudolf Arnheim, a Gestalt Psychologist, "*The inborn capacity to understand through the eyes has been put to sleep and must be reawakened*". We are all endowed with the sensory processing ability and are inherent to match incoming sensory inputs with experienced top-down expectations. Our brain tends to make various predictions to facilitate this sensory processing; however, the brain could commit errors—illusions. An illusion is a distortion of our senses, which can reveal how our brain normally organizes and interprets sensory stimulation. In contrast to the various research on 2-dimensional (2D) optical illusory stimuli, there is hardly any empirical neural investigation of 3D illusions. The second author has turned the well-explored 2D illusions into 3D impossible objects, whose appearance from the front view is so incongruent with their reflection in the mirror. In collaboration with the second author, we first conducted a spatial aptitude test to select 30 suitable 3D objects for this study based on two levels of configuration. We then created a set of video clips of authentic 3D impossible objects and designed two experimental tasks: the peekaboo task and the perceptual inference task. The peekaboo task is used to investigate the matching process between prior prediction and observed sensory data and, importantly, the sensory prediction error signaling. The perceptual inference task is used to explore the perceptual inference process on novel objects and also its potential neural correlates. Through the tasks, we investigated the incongruent perceptions and the neural dynamics of the Bayesian inference process of illusion with the electroencephalogram (EEG). The result of the peekaboo task revealed significant differences between impossible objects and their counterparts in the time segment after the expected or unexpected sensory data inputs, which reflected the predictive error signals and also affective arousal. Applying multivariate pattern analysis (MVPA) to simultaneous EEG recordings, we found that the impossible objects can be discriminated from the possible ones in the perceptual inference task, even between two configuration levels. With these exploratory results, this study highlights the neural dynamics underlying illusory perception and shed the light on the sensory processing of 3D illusions.

Disclosures: Y. Hsieh: None. **S. Kokichi:** None. **C. Tsai:** None. **W. Lai:** None.

Poster

401. Auditory, Visual, Proprioceptive Perception

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Program #/Poster #: 401.10

Topic: H.02. Perception and Imagery

Title: Peripheral contrast sensitivity predicts the preview effect at idiosyncratic preferred performance field

Authors: *X. LIU, D. MELCHER;
New York Univ. Abu Dhabi, Abu Dhabi, United Arab Emirates

Abstract: Peripheral vision serves to guide saccades and facilitates subsequent foveal processing through trans-saccadic integration. An example is the preview effect, in which glimpsing a target

in the periphery enhances the speed and accuracy of post-saccadic processing of that object. Importantly, human peripheral vision has been shown to consist of iso-eccentric areas with varied visual acuity (performance field; PF), raising the question of how these differences in peripheral acuity influence trans-saccadic perception in natural viewing. We investigated this question with an extrafoveal preview paradigm. Twenty observers (21.23 ± 4.48 years old; 5 females) were presented with a 300 ms Gabor patch (±3° to vertical) at one of four iso-eccentric locations (8 visual degree; upper, lower, left, and right meridians) while fixating at the center. In the baseline session, observers were then prompted to indicate the tilt direction (counterclockwise/clockwise) of the peripheral Gabor. In a separate eye-movement session, the peripheral preview was followed by a cue to make an immediate saccade to the Gabor in order to make the orientation judgment after foveating the target. On invalid preview trials (50%), the Gabor tilted to an opposite direction during the saccade and stayed on the screen for another 100 ms, followed by the same tilt discrimination task. The Gabor contrast in different conditions (four PFs × three viewing conditions) was determined by the PEST procedure, with threshold set to 70% performance level. Individual contrast sensitivity (i.e., the reciprocal of contrast threshold estimate; CS) for each location in the baseline session (peripheral CS) was used to determine the preferred PFs for each participant. As predicted, repeated measures ANOVA reveals a preview effect where the CS was higher in the valid than invalid preview condition ($F(2, 30) = 22.58$, $p < .001$). A linear mixed model with preferred PFs, preview validity, and scaled peripheral CS as predictors suggests that the magnitude of the preview effect is predicted by peripheral CS ($X^2(1) = 6.11$, $p = .013$). This suggests that the visual system "knows" and takes into account its own idiosyncratic variation in PFs, consistent with an optimal integration strategy in which more reliable peripheral inputs contribute more to the post-saccadic percept. There was a significant correlation between peripheral CS and preview effect for the most preferred PF ($R^2 = 0.26$, $p = .042$). These results suggest that preview effects in trans-saccadic perception take into account individual variations in performance fields, showing that performance fields may have perceptual consequences in natural viewing settings.

Disclosures: **X. Liu:** None. **D. Melcher:** None.

Poster

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Topic: H.02. Perception and Imagery

Support: ANR-14-ACHN-0023
ANR-16-CONV-0002

Title: Body-ownership illusions in virtual reality reveal that internal representations supporting bodily-self consciousness are more malleable in early adolescence

Authors: L. RAOUL, C. GOULON, F. SARLEGNA, ***M.-H. GROSBRAS**;
Aix Marseille Univ., Marseille, France

Abstract: How do the neurophysiological bases of bodily-self consciousness adjust during adolescence when the body shape and size change massively? This question can be addressed indirectly using full-body-ownership illusions. These are induced by applying tactile or proprioceptive stimulation to an observer and simultaneously on an avatar presented visually in first-person perspective. We tested the robustness of bodily-self consciousness either by manipulating the synchronicity between the visual display and the sensorial stimulations or by changing the avatar's size (increased or decreased Body Mass Index relative to that of the observer). The illusion was measured by the observers' ratings of the sense of ownership and agency over the avatar. A stronger full-body illusion in the more naturalistic (compatible) conditions, i.e. synchronous stimulation on a matched-size avatar, compared to the incongruent conditions, is a signature of robust own-body representations sustaining bodily-self consciousness. In contrast, a tolerance for deviation from the optimal conditions with respect to the full-body-ownership illusion indicates more plastic representations. We tested two groups of girls: early adolescents, aged 10-13, beginning of puberty, n= 35, and mid-adolescents, age 14-17, advanced puberty, n=34. The two groups reported similar high sense of ownership and agency over the matching avatar (main effect of age $F(1,67)=0.007$; $p=.93$). The older adolescents only were impacted by the incongruent conditions. They reported significantly lower ownership when the avatar size differed from their own ($F(1,67)= 5.89$, $p=0.018$). And lower sense of agency when the visual and sensory stimulations were asynchronous ($F(1,67)=11.019$, $p=0.001$). This could result from weaker multisensory integration in early- compared to mid-adolescence. We describe for the first time the full-body illusion in adolescents. Our results indicate that younger adolescents have more facility than older adolescents to embody an avatar of a different size or with visuo-sensory incongruency. This points towards greater malleability of bodily-self consciousness at the beginning of adolescence. This has implications for characterizing this age as a period of vulnerability for disorders of body representation.

Disclosures: L. Raoul: None. **C. Goulon:** None. **F. Sarlegna:** None. **M. Grosbras:** None.

Poster

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Topic: H.02. Perception and Imagery

Support: EEC-1028725

Title: The rubber hand illusion in virtual reality

Authors: *S. LOWE-HINES[1], E. TANUMIHARDJA[2], C. PASCHALL[3], B. L. GRANNAN[1], A. KO[1], J. S. HAUPTMAN[1], J. G. OJEMANN[1], R. P. RAO[2], J. A. HERRON[1];

[1]Dept. of Neurolog. Surgery, [2]Paul G. Allen Sch. for Computer Sci. and Engin., [3]Dept. of Bioengineering, Univ. of Washington, Seattle, WA

Abstract: In the present study, we replicate the experimental structure of the Rubber Hand Illusion (RHI) in virtual reality (VR) to further embodiment research and help bring a new flexible RHI experimental setup into the clinical intracranial population. The RHI is a classic perceptual illusion used to induce a feeling of body ownership over a non-self object, and is used to examine the mechanisms underlying embodiment. Traditionally, the illusion is induced by synchronously stroking a visible rubber hand and the subject's own hand which is hidden. The strength of the illusion is evaluated qualitatively by questionnaire and quantitatively by a proprioceptive drift metric. The RHI questionnaire uses a Likert scale to subjectively quantify three distinct components of embodiment: body-ownership, self-localization, and agency. Proprioceptive drift is the difference between pre- and post-induction self-localization measurements.

We present here a behavioral evaluation of a novel virtual reality rubber hand illusion (VR-RHI) designed for use with healthy human subjects as well as human patients undergoing clinical, intracranial neural recording. Our implementation of the VR-RHI experiment was able to achieve real-time induction of the illusion. This is a significant improvement over previous virtual implementations that used synchronized pre-recorded animations and audio cues to coordinate experimental delivery of touch during induction. In addition, we implemented a new gaze-based proprioceptive drift metric that is uniquely available within the VR setting. Originally, subjects selected the location of their hand by either pointing at a ruler by hand in the classic experiment, or at a virtual ruler by controller in our virtual adaptation. We leveraged built-in eye tracking on the VR headset to allow subjects to use gaze to identify the location of their hand, which may elicit new results. Initial trails of the experiment have shown that the combination of concordant visual and tactile cues heavily influences self-localization and body ownership. Our VR-RHI experiment enables RHI embodiment research within the hospital setting and facilitates adaptable approaches to RHI induction and evaluation that may further elucidate the elements of embodiment.

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Poster

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Topic: H.02. Perception and Imagery

Support: Royal Society and Wellcome Trust Sir Henry Dale Fellowship 211200/Z/18/Z).

Title: Object processing is shaped by expectations about the environment

Authors: *A. KRUGLIAK, Y. GU, A. CLARKE;
Psychology, Univ. of Cambridge, Cambridge, United Kingdom

Abstract: We live in a complex environment in which context shapes expectations about the kind of things we might see. For example, we would be startled to encounter a donkey inside a house but not when we see one in a field. But in both cases, we know it's a donkey - yet the neural processes that allow us to recognise the item as a donkey might be different. Research has established that objects which occur in a congruent context are recognized faster and more accurately than objects which occur in an incongruent context. However, it is still not known in what way context impacts object recognition. Does the preceding environment modulate low- or higher-level visual features, or even semantic object properties? Here, we use Magnetoencephalography (MEG) and computational modelling to address the critical question of how expectations elicited by a scene affect the subsequent processing of an object. We recorded MEG while (n = 32) participants performed a speeded object recognition task. On each trial a scene and an object were presented separated by a blank screen. In total, 150 everyday objects were presented in consistent, inconsistent, and neutral contexts. We calculated the similarity between objects for brain responses, a model of object vision (Cornet-S) and a model of semantics, and related them using Representation Similarity Analysis (RSA). The model of object vision reliably distinguished when a scene and when an object image was presented. However, only the highest-order layer of the visual model showed a significant modulation by context. Between approximately 150-250 ms the model fit was significantly stronger for consistent objects compared to inconsistent objects. The semantic model showed the opposite pattern, inconsistent objects were represented more strongly than consistent objects. The current findings demonstrate that objects are processed differently in the brain depending on our expectation. The contexts in which we encounter objects impacts multiple level of perception, from visual features to semantic processing.

Disclosures: A. Krugliak: None. **Y. Gu:** None. **A. Clarke:** None.

Poster

401. Auditory, Visual, Proprioceptive Perception

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Program #/Poster #: 401.14

Topic: H.02. Perception and Imagery

Title: The Effect of Metacognitive Introspection On Neural Signatures of Perceptual Evidence Accumulation

Authors: *W. DOU, S. AFRAKHTEH, K. CALLWOOD, A. FEGHHI, J. SAMAHA;
Univ. of California, Santa Cruz, Santa Cruz, CA

Abstract: The ability to introspect about the accuracy of one’s performance plays an essential role in adaptive behavior. This metacognitive capacity has been studied as confidence in the

outcome of a choice. On the one hand, confidence has been demonstrated as a direct readout of evidence that leads to our choices. On the other hand, some studies also suggest that subjective confidence is estimated based on evidence continually accumulated after the decision commitment. Our previous research revealed that confidence is informed by the same evidence underlying one's decision, as the centro-parietal positive (CPP), an event-related potential (ERP) component representing sensory evidence signal, predicts participants' confidence in the process of decision formation, independent of evidence strength, reaction time, and accuracy. It remains unclear, however, if humans first accumulate evidence for their decision and then decide on their confidence level afterwards, or if the act of introspecting about confidence alters the way evidence is actually accumulated for the first-order decision. Our current study measures neural signatures of evidence accumulation under conditions where participants do or do not have to report confidence in their decision. In the experiment, participants perform a two-alternative dot-motion discrimination task with three motion coherence levels while electrical brain activity (via EEG) is recorded. On half of the trials, participants will be asked to only report their decision about the motion. On the other half of the trials, participants will report the decision and rate their confidence in it sequentially. The order of the presentation of decision-only and confidence-rating trials will be counterbalanced across twenty participants. We will examine whether there is a difference in the buildup rate of CPP between decision-only trials and confidence-rating trials, which could reflect the change in evidence accumulation in the two distinct processes. Our study seeks to offer insight into the neural mechanism of metacognition and uncover neural correlates of the human decision process to inform models of how choice and confidence are computed from sensory inputs.

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Poster

401. Auditory, Visual, Proprioceptive Perception

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Program #/Poster #: 401.15

Topic: H.02. Perception and Imagery

Title: Measuring shape-based object recognition performance

Authors: *J. W. NAM, A. S. RIOS, B. W. MEL;
USC, USC, Los Angeles, CA

Abstract: Object recognition in humans is based primarily on shape (Grill-Spector et al., 2001; Biederman, 1987; Biederman and Ju, 1988; Hoffman, 1998; Kourtzi, 2001). In contrast, deep networks (DNs) trained on conventional datasets such as ImageNet have only a weak grasp of global shape, instead classifying images mainly based on color, texture, local shape, and context cues (Baker et al., 2018; Brendel and Bethge, 2019; Geirhos et al., 2019). The lack of a "shape bias" is a likely contributor to the various un-biological performance characteristics of DNs,

including their susceptibility to adversarial inputs (Goodfellow et al., 2015); their propensity to confidently classify random noise patterns as specific objects; their poor generalization behavior; and their inability to recognize objects based on line drawings (Brendel and Bethge, 2019; Geirhos et al., 2019; Russakovsky et al., 2015).A poor grasp of global object shape does not prevent conventional DNs from performing well on benchmark tasks, however: state-of-the-art top-5 performance on ImageNet is approaching 99% (Pham et al., 2021). It therefore seems that ImageNet and other similar benchmarks do not test, and are evidently not well suited for training, the basic representational capability that underlies human object and scene vision.We describe a new system for measuring shape-based recognition performance called ShapeY, including its image set, measures of task performance, and methods used to control task difficulty. ShapeY is based on the idea - similar to that motivating some contrastive learning approaches (Chen et al., 2020) - that the core competence of a 3D recognition system is to produce a similar internal visual code when an object or scene is viewed from a different perspectives, under different lighting conditions, and/or with different backgrounds. On the other hand, to state the obvious, the recognizing system should produce different codes for different objects regardless of viewing conditions. The ability to perform well at this basic matching task is, in our view, a prerequisite for performing well and generalizing well in real-world object recognition tasks. We used ShapeY to test 26 DN architectures ranging in size from 5.5 to 660 million parameters. Even the best performing among these (XCiT) performed poorly, for example, committing ~40% errors when asked to match 2 views of the same object that differed by ~27° in both pitch and roll. Error rates were even higher (~60%) when match candidates were both reoriented and contrast reversed. ShapeY provides a new tool for charting the progress of artificial vision systems towards human-level shape recognition capabilities.

Disclosures: **J.W. Nam:** None. **A.S. Rios:** None. **B.W. Mel:** None.

Poster

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Topic: H.02. Perception and Imagery

Support: College of Social and Behavioral Sciences Summer Research competition funding award, CSUN

Title: Take a Good Look at Yourself... or Don't: Self-Viewing During Videoconferencing Predicts Eyestrain.

Authors: *D. A. VIDAMUERTE[1], B. ACEITUNO[1], E. BENCHEK[1], J. GLUCK[1], A. GUERRA LOPEZ[1], P. M. SANTOS[2], L. E. KNOX[1], S. A. DREW[1];
[2]Psychology, [1]California State University, Northridge, Northridge, CA

Abstract: The neuropsychological field of perception research invariably measures participants' visual systems and as such, it is imperative to study the relationship between virtual conferencing behaviors and their potential physiological effects on ocular health. The increase of digital communication has led to greater reports of "Zoom Fatigue", a term coined to describe a group of symptoms including exhaustion and eyestrain following videoconferencing. One explanation for this phenomenon is the unique nature of virtual meetings; unlike in-person interactions, these engagements entail a loss of non-verbal cues (e.g. less perceivable body language) and an additional element permitting continuous self-viewing during a meeting. Additional consideration must also be given to the degree to which allergies contribute to eyestrain. The inter-relationship between these factors has been minimally investigated and warrants additional research. Therefore, this study sought to examine the relationship between the frequency of self-viewing and eyestrain during virtual meetings. We conducted a study in which students from California State University, Northridge (N = 453) completed an online survey that included measures of allergy symptoms, virtual-meeting fatigue, self-viewing, and eyestrain. We hypothesized that increased frequency of self-viewing during video meetings would be positively associated with increased eyestrain symptoms controlling for reported allergy symptoms and virtual-meeting fatigue. A hierarchical multiple regression revealed that increased self-viewing significantly predicted increased eyestrain after controlling for virtual-meeting fatigue and allergy-related eye symptoms. These data suggest that continuously looking at oneself during a virtual meeting may contribute to visual discomfort, beyond what is accounted for by viewer allergies and virtual-meeting fatigue. These findings better inform the understanding of the relationship between eyestrain, self-viewing, and virtual-meeting fatigue, as well as identify a potential avenue to reduce eyestrain through a decrease in self-viewing practices, though further experimental study is warranted.

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Poster

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Program #/Poster #: 401.17

Topic: H.02. Perception and Imagery

Title: The neural mechanisms of afterimages: A model of illusory conscious perception

Authors: M. HOLNESS, T. MORGAN, J. TEVES, D. A. HANDWERKER, P. BANDETTINI, *S. I. KRONEMER;
Natl. Inst. of Mental Hlth., NIH, Bethesda, MD

Abstract: A common approach to study the neural mechanisms of conscious perception is to present participants with external sensory stimuli. However, not all conscious perceptions originate from external input. Memory, imagination, and hallucinations are examples of internal

or introspective conscious perceptions without simultaneous real-world correspondence. Much remains unknown regarding overlapping versus distinct neural mechanisms for external and internal sensory conscious perceptions. Studying internal conscious perception is challenging because the experimenter does not know the perceptual characteristics of the evoked image. Previous studies contrasting external and internal sensory conscious perceptions can be confounded by unknown phenomenological differences in experience. To address this concern, we developed a novel visual paradigm where real face stimuli are contrasted with perceptually identical face afterimages - illusory percepts induced by preceding real face stimuli. Healthy, adult participants (N = 21) were asked to match the blur, opacity, and duration of their afterimages with a real, self-controlled stimulus. We then constructed a mock afterimage - a real stimulus that perceptually matched with each participant's average reported afterimages. Unbeknownst to the participants, we presented the mock afterimage among real afterimages. Subjects were asked to report the on-screen afterimage location and onset/offset times. Concurrent eye-tracking and pupillometry were recorded with EyeLink 1000 Plus (1000Hz; SR Research, Inc.). A post-task survey and the vividness of visual imagery questionnaire (VVIQ) were administered to inquire on insight to the mock afterimage and mental imagery clarity, respectively. We found that all participants reported perceptual similarities between their real and mock afterimages. And, a majority of participants (18 out of 21 participants) believed that the mock afterimage was an illusory, real afterimage. EyeLink recordings revealed that real and mock afterimages induced similar pupillary dynamics and blink rate changes. We found a significant positive correlation ($p < 0.05$) between VVIQ ratings and afterimage opacity, but no relationship to afterimage duration. These results hint that afterimages may share a mechanism with mental imagery. These preliminary findings demonstrate the opportunity for using afterimages as a model of illusory conscious perception in healthy participants. Future directions include implementing this novel afterimage paradigm with high-field fMRI to evaluate the neural mechanisms of afterimages versus perceptually-matched real stimuli.

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Program #/Poster #: 401.18

Topic: H.02. Perception and Imagery

Support: 202153

Title: Difference between performed and imagined timed up and go test: a new way to assess different cognitive states

Authors: *F. LI[1], D. HAO[2], W. QIN[2], X. WEN[2], J. HUANG[2];
[1]Neurol., [2]Fu Xing Hospital, Capital Med. Univ., Beijing, China

Abstract: BackgroundMotor imagery (MI) includes imaging a given action without actuallyexecuting it, which offers a novel path for investigations of higher-levelcontrol of body movement. It is confirmed that the actual performance andimaging processes have similar physiological bases and nerve conductionpathways. The most used method for examinations of MI is the imaginedtimed up-and-go (iTUG) test. Previous studies found that the timedifference between the performed timed up and go test (pTUG) and iTUGwas higher in patients with impaired cognition, not physical fitness. Wehypothesized it can identify the different stage of dementia spectrum aswell.Methods All participants were recruited from the Department of Neurology, theMemory Clinic of Fu Xing Hospital and the communities around it, fromSeptember 2016 to January 2018, including 74 NC; 42 MCI patients, and33 mild Alzheimer's disease (AD) patients. The participants were evaluatedby battery of neuropsychological tests assessing global functions and fivecognitive domains: memory, executive function, attention, language, andvisuospatial skills. The time difference between the pTUG and the iTUG,delta TUG, was used as the main outcome. This study was approved by theMedical Ethics Committee of Fu Xing Hospital, Capital MedicalUniversity, Beijing, China. All participants or their legally acceptable representatives signed the informed consent forms.ResultsNo differences in sex, age, and years of education, BMI, and number ofdaily prescribed medications were found among the three groups (Ps >0.05). The delta TUG increased with the degree of cognitive impairmentand was significantly associated with age and visuospatial ability. TheAUC distinguishing among NC and MCI, NC and mild AD, and MCI andmild AD of the delta TUG were 0.721 ($P < 0.0001$), 0.843 ($P < 0.0001$),and 0.667 ($P = 0.012$), respectively. The cut-off score of delta TUG fordistinguishing NC, MCI, mild AD was 40 and 58, respectively.ConclusionThe delta TUG can contribute to the effective distinction among patientswith NC, MCI, and mild AD: <40 indicates NC, and >58 indicates mildAD; the range between these two scores correlates with MCI. The deltaTUG may be a useful tool to quickly screen patients with differentcognitive status.

Disclosures: **F. Li:** None. **D. Hao:** None. **W. Qin:** None. **X. Wen:** None. **J. Huang:** None.

Poster

401. Auditory, Visual, Proprioceptive Perception

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 401.19

Topic: H.02. Perception and Imagery

Title: Testing the other-race effect with generative adversarial networks

Authors: *M. SHOURA[1], A. NESTOR[2], D. B. WALTHER[3];
[1]Psychology, Univ. of Toronto, Scarborough, Scarborough, ON, Canada; [2]Psychology, Univ. of Toronto Scarborough, Scarborough, ON, Canada; [3]Psychology, Univ. of Toronto, Toronto, ON, Canada

Abstract: The other-race effect (ORE) refers to the advantage of recognizing faces of one's own race relative to faces of other races. This is a well-documented effect behaviorally, but a neurocomputational account of this effect is still largely missing from the field. The current work utilizes style-based generative adversarial networks (styleGANs), a state-of-the-art deep learning technique for generating photorealistic synthetic images (Karras et al., 2020), to explore the nature and characteristics of the ORE. Specifically, the emergence of ORE characteristics was examined in styleGANs as a function of training set as well as face representation similarities regarding the ORE between GANs and human participants. To this end, a family of styleGANs have been trained while manipulating the ratio of face images in the training set based on race (i.e., Asian/Caucasian) and gender. Then, pairwise visual similarity of different GAN-generated face images was assessed by healthy Caucasian and Asian adults with different levels of ORE. And last, the structure of face space in the latent space of the GANs was assessed and related to human participants' face space. The results point to linear separability of race and gender information in styleGANs as well as to the emergence of ORE characteristics (e.g., a relative compression of representational space for one race) as a function of training set. Next, the representational face similarity of styleGANs and human participants was investigated as a function of training set bias, in styleGANs, and ORE size, in human participants. Thus, the present work serves to, first, establish the presence of ORE characteristics in GANs by varying the structure of the training datasets, second, account for face space structure and ORE in humans using a GAN-based computational model and, third, highlight the utility of GAN-manipulated images with respect to face perception investigations.

Disclosures: M. Shoura: None. **A. Nestor:** None. **D.B. Walther:** None.

Poster

401. Auditory, Visual, Proprioceptive Perception

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 401.20

Topic: H.02. Perception and Imagery

Support: FRQSC Grant B2Z
Canada Research Chairs program
NSERC Discovery Grant

Title: Divergent perception: Linking creativity with perception of ambiguous visual stimuli

Authors: *A. BELLEMARE[1,2], Y. HAREL[2], J. O'BYRNE[2], G. MAGEAU[2], A. DIETRICH[3], K. JERBI[2];
[1]Concordia Univ., Montreal, QC, Canada; [2]Dept. of Psychology, Univ. de Montréal, Montréal, QC, Canada; [3]American Univ. Beirut, Riad El-Solh Beirut, Lebanon

Abstract: Creativity is a highly coveted and multifaceted skill. Empirical research typically probes creativity by estimating the potential for problem solving and novel idea generation, a

process known as “divergent thinking”. Here, by contrast, we explore creativity through the lens of perceptual abilities by asking whether creative individuals are better at perceiving recognizable forms in ambiguous stimuli, a phenomenon known as pareidolia. We designed a visual perception task in which 50 participants, with various levels of creativity, were presented with ambiguous stimuli and asked to identify as many recognizable forms as possible. The stimuli consisted of cloud-like images with various levels of complexity, which we controlled by manipulating fractal dimension (FD). Furthermore, MEG data were collected while participants performed the same pareidolia task. We found that pareidolic perceptions arise more often and more rapidly in individuals that are more creative. Furthermore, the emergence of pareidolia in high-creatives happened more constantly across the span of FD values, suggesting a wider repertoire for perceptual abilities in creative individuals. MEG data revealed that pareidolia is characterized by increase in hurst exponent, a measure of long-term memory of the signal. Our behavioral findings suggest that pareidolia may be used as a perceptual proxy of idea generation abilities, a prerequisite for creative behavior. Moreover, our MEG results constitute a first step towards the characterization of the neural scaling dynamics associated with pareidolia, as a marker of divergent perception. In particular, our Hurst exponent findings suggest putative links between brain criticality and creativity. These findings expand our understanding of the perception-creation link and open new paths in studying creative behavior in humans.

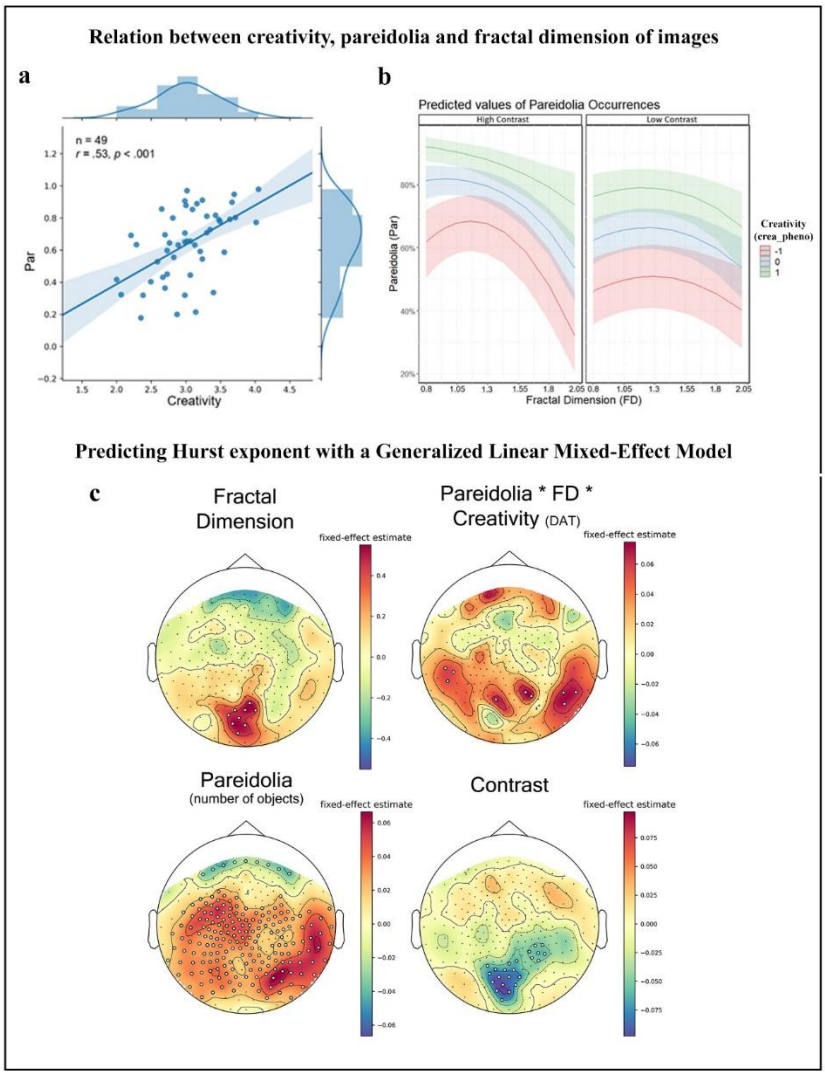

Fig 1 | **(a)** Regression between creativity and pareidolia occurrences (Par). Pareidolia scores are averaged across all trials. **(b)** Predicted probabilities of the interaction between FD, Contrast and Creativity on pareidolia occurrences. The green line corresponds to high-creative individuals and the red line to low-creative individuals. **(c)** Fixed-effects of the GLMM predicting Hurst exponent.

Disclosures: **A. Bellemare:** None. **Y. Harel:** None. **J. O'Byrne:** None. **G. Mageau:** None. **A. Dietrich:** None. **K. Jerbi:** None.

Poster

401. Auditory, Visual, Proprioceptive Perception

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 401.21

Topic: H.02. Perception and Imagery

Support: NSF Award 2043637

Title: Effects of scene complexity on visual attention during visual search in naturalistic virtual environments

Authors: *I. J. LACHICA, J. M. FINLEY;
Div. of Biokinesiology and Physical Therapy, USC, Los Angeles, CA

Abstract: The processing of visual information is vital in performing everyday tasks and relies on the proper allocation of visual attention to task-relevant targets. However, our ability to allocate visual attention properly may become impaired as a scene's complexity increases. Specifically, prior work has indicated that we are less able to ignore irrelevant stimuli as their number increases during visual selective attention tasks. However, studies demonstrating these effects used simple stimuli that are not representative of the real world. Thus, we used a custom-designed virtual reality (VR) application to determine how scene complexity affects visual attention during visual search in naturalistic environments. Participants performed a VR-based visual search task derived from the Trail Making Test-B. This task required participants to search for and select targets that alternated between letters and objects whose names start with those letters (e.g., A-Ant-B-Butterfly). In addition, the task was performed in naturalistic virtual environments with three levels of increasing visual complexity. Gaze data from eye trackers were used to identify the focus of each fixation. Fixations were then classified based on their task relevance and we also determined the proportion of total fixation duration on each class. We processed each frame recorded during the task to compute the salience of each pixel, and these values were converted to percentile ranks, with 100% being most salient while 0% being least salient. As the visual complexity of the environment increased, we expected that participants would reduce the time spent fixating on task-relevant objects and that there would be an increase in the salience at points of fixation. Preliminary data from 5 participants indicated that the salience at points of fixation was generally consistent as the visual complexity of the environment increased (low: M = 87%, SD = 1.6%; medium: M = 82%, SD = 1.9%; high: M = 82%, SD = 1.9%). Similarly, the proportion of time spent fixating task-relevant objects remained largely unchanged even as the complexity of the environment increased (low: M = 0.87, SD = 1.6; medium: M = 0.86, SD = 1.9; high: M = 0.86, SD = 1.9). These results indicate that the participants fixated on task-relevant objects most of the time during the task regardless of the visual complexity of the naturalistic virtual environment. This suggests that, in contrast to what has been previously demonstrated, we are less vulnerable to being distracted by irrelevant stimuli when performing visual selective attention tasks in naturalistic contexts.

Disclosures: I.J. Lachica: None. **J.M. Finley:** None.

Poster

401. Auditory, Visual, Proprioceptive Perception

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 401.22

Topic: H.02. Perception and Imagery

Support: NIH Grant NS122333

Title: Integration of proprioceptive and tactile information to achieve haptic object perception

Authors: *E. DOGRUOZ[1], N. SHELCHKOVA[1], S. J. BENSMAIA[2];
[2]Dept. of Organismal Biol. and Anat., [1]Univ. of Chicago, Chicago, IL

Abstract: Stereognosis, the sense of the 3-dimensional shape of objects held in hand, requires the integration of somatosensory signals about local features -such as edges and surface curvature- with proprioceptive signals about the conformation of the fingers on the object. Here, we investigate how information about the global shape of the object is integrated with information about its local features at each point of contact to give rise to a wholistic percept of the object. To this end, we have human observers judge the dissimilarity of pairs of objects that vary only in their global shape, only in their local features, or both. We then compare the dissimilarity ratings when both global shape and local features change to ratings when only global shape changes or when only local features change. We can then investigate the degree to which the same feature changes contribute to perceived dissimilarity in different global shape conditions and vice versa. We explore the implications about the neural mechanisms that underlie stereognosis.

Disclosures: E. Dogruoz: None. **N. Shelchkova:** None. **S.J. Bensmaia:** None.

Poster

401. Auditory, Visual, Proprioceptive Perception

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 401.23

Topic: H.02. Perception and Imagery

Support: NIMH ZIAMH002958

Title: Perceptography: taking pictures of the perceptual events induced by brain stimulation

Authors: *E. SHAHBAZI[1], T. MA[2], A. AFRAZ[3];
[1]NIH, Bethesda, MD; [2]NIMH, Natl. Inst. of Hlth., North Bethesda, MD, MD; [3]NIH/NIMH, Bethesda, MD

Abstract: Perceptography: taking pictures of the perceptual events induced by brain stimulation. Local stimulation in high-level cortical visual areas perturbs the contents of visual perception. Here we utilized a machine learning structure in combination with high throughput behavioral optogenetics in order to take pictures of the visual perceptual events induced by local stimulation in macaque inferior temporal (IT) cortex. Two adult macaque monkeys were trained to behaviorally detect and report a brief optogenetic excitatory impulse delivered to their central IT cortex. The animals started each trial by fixating on a computer-generated image. Halfway through the 1-second image presentation, we altered the image features for 200ms. A ~1x1mm

area of the IT cortex was optogenetically stimulated in half of the trials at random for 200ms using an implanted LED array. By looking at one of the two subsequently presented targets, the animals reported whether or not the trial included a cortical stimulation impulse and received liquid reward for correct reports. We hypothesized that image alterations that share common features with the stimulation-induced perceptual event increase the chance of behavioral false alarms (FA: nonstimulated trials that were reported as stimulated) . Under the hood, two learning systems were deployed to increase the probability of FA by perturbing the image that was visually presented to the animals. Ahab, our feature extraction deep network, used the animals' behavioral responses to guide DaVinci (a generative adversarial network) to achieve this goal. This closed-loop system created altered images that induced a 55-85% FA rate, dramatically higher than the baseline of 3-7% (cross-validated, $p<0.01$). Since the animals are unable to discriminate between the state of their IT cortex being stimulated and the state of perceiving the images, we call these images "perceptograms". We hope that precise, pictorial and parametric recording of the perceptual events induced by stimulation of high-level visual cortex, provides the building blocks for making the bridge between neuronal activity and visual perception.

Disclosures: E. Shahbazi: None. **T. Ma:** None. **A. Afraz:** None.

Poster

401. Auditory, Visual, Proprioceptive Perception

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 401.24

Topic: H.02. Perception and Imagery

Title: Uncovering the time course of perceiving spatial locations

Authors: *Y. CHUNG, V. STOERMER;
Psychological & Brain Sci., Dartmouth Col., Hanover, NH

Abstract: How do we know where objects are by looking? In most cases the physical location of objects corresponds to the perceived location. This makes it difficult to disentangle the neural processes associated with the physical location from the processes that reflect the perceived location. Here we are using a special case of a visual illusion, the so-called frame-induced position shift, where the relationship between the physical stimulus location and their perceived location is dissociated: When a frame is moved left to right and two probe stimuli are flashed inside the frame at the same physical location before and after the frame moves, participants report these probes to appear at separate locations (Özkan et al., 2021). This is a particularly strong example where perception differs from the physical stimulus input, providing an opportunity to investigate when and where localization of objects arises along the visual processing stream. Using this novel illusion, we here examine at what point in time the perception of location arises using EEG. Specifically, we capitalize on the fact that early visually-evoked responses are sensitive to the physical position of stimuli in the visual field, such that a stimulus presented in the left visual field elicits a larger amplitude over right visual cortex

and vice versa. We measured event-related potentials (ERPs) to probe stimuli that were physically at the same location but perceived at different locations due to the moving frame, and compared them to probe-elicited ERPs of stimuli that were physically (and perceptually) at separate locations. Our results show that early visually evoked ERPs starting at ~70ms reflected the physical locations of stimuli while later ERPs at ~140ms showed a lateralized effect that corresponds to the perceived locations due to the illusion (N=18). Using multivariate pattern decoding analyses, we also show that the probe locations could only be reliably decoded across physical and perceived locations during the later time interval (140~180ms) but not during the earlier time interval (70~110ms). Overall our findings suggest that the perception of spatial location arises around 140ms and that the visual processing of the illusory position shares neural activity patterns with the matched physical location during this later time interval. Broadly, this suggests that physical location information is coded in the initial feedforward sweep of visual information processing and that later recurrent processes are involved in the perception of location.

Disclosures: Y. Chung: None. **V. Stoermer:** None.

Poster

401. Auditory, Visual, Proprioceptive Perception

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 401.25

Topic: H.02. Perception and Imagery

Support: JSPS Kakenhi 20H00235

Title: Possibility for better perception using partial face over full face stimulus

Authors: *I. CHANPORNPAKDI[1], Y. WONGSAWAT[2], T. TANAKA[1];
[1]Electronic and Information Engin., Tokyo Univ. of Agr. and Technol., Tokyo, Japan; [2]Biomed. Engin., Mahidol Univ., Nakhon Pathom, Thailand

Abstract: Objective: Since 2019, when the SARS-CoV-2 pandemic happened, many countries live with the masks on. However, people can recognize people's faces correctly although half of the face is covered with the mask. Therefore, we raised the hypothesis that the partial face could also be used in face recognition and provided the performance as the full-face cognition. This study aimed to identify how partial face affects face cognition mechanism.
Method: The full and part face; faces with eyes covered, nose covered, mouth covered, eyes and nose covered, eyes and mouth covered, and nose and mouth covered, of unfamiliar faces were used to identify the effect of each part of the face on face cognition. We performed the experiment on 16 healthy volunteers, 6 male and 10 female (mean age 27.89 ± 2.77, min 22 and max 31). During the experiment, we recorded an electroencephalogram (EEG) along with the eye tracker. The experimental task contained seven blocks (one full face and six partial faces) of cognition conditions. Each image was presented for 200 ms with the fixation of 500 ms at the

beginning, in between each stimulus, and at the end of each block. Each block contained one target face, which is trained priorly, and six unknown faces. Each face was presented 100 times and the subjects were asked to report the number of target faces shown after each block. The obtained EEG was filtered with a bandpass filter of 0.5 to 30 Hz and extracted the epoch from -0.1 to 0.7 s. The grand average across the subjects was calculated to explore the changes in the event-related potential (ERP) component.
Results: We have found that the partial face with the eye component elicited a larger amplitude of the P300 component of ERP than the partial face without the eye component. Among all the conditions, the partial face with nose covered (eyes and mouth were visible) yielded the largest P300, including the full-face condition. We also confirmed using the eye gaze data that the participants focus on the eye component the most.
Discussion: The above result suggested that the eye component was the most crucial feature in face cognition and using partial face with the eye component presented might provide better cognition for face perception.

Disclosures: I. Chanpornpakdi: None. **Y. Wongsawat:** None. **T. Tanaka:** None.

Poster

401. Auditory, Visual, Proprioceptive Perception

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 401.26

Topic: H.02. Perception and Imagery

Support: Mind Science BrainStorm

Title: Uncovering the mechanism of intentional binding using visual masking

Authors: *A. HOPKINS[1,2], A. SCHURGER[2], T. LAN[2];
[1]UCLA, Los Angeles, CA; [2]Inst. for Interdisciplinary Brain and Behavioral Sci., Chapman Univ., Orange County, CA

Abstract: If two events occur sequentially in time, a causal relationship can be perceived between them. If a causal relationship is perceived between two events, and the first event is a self-initiated movement, then the time interval between the first event (the cause) and the one that follows (the effect) can appear shorter than it would be otherwise. This perceived contraction of time between action and effect is known as *intentional binding.* The mechanisms responsible for intentional binding remain unclear. It could be that (1) the neural events corresponding to the processing of the cause and effect occur at the same time as they would otherwise, but the time interval seems shorter because intentional binding affects time perception; or (2) the neural response to the effect is precipitated, bringing the neural events corresponding to the processing of the cause and effect closer in time. We can refer to these two possibilities as an effect on (1) the perception of time or (2) the timing of perception. We set out to distinguish between these two possible explanations of intentional binding in terms of their

respective effects on visual perception using a combined intentional binding and visual backward masking paradigm. We tested the hypothesis that intentional binding increases the effectiveness of a visual mask when that mask is perceived as the effect in a cause-effect relationship.

Disclosures: A. Hopkins: None. **A. Schurger:** None. **T. Lan:** None.

Poster

401. Auditory, Visual, Proprioceptive Perception

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 401.27

Topic: H.02. Perception and Imagery

Title: Modest effect of knowledge on bistable perception

Authors: *B. ZHANG, J. W. BRASCAMP;
Michigan State Univ., Michigan State Univ., East Lansing, MI

Abstract: Ambiguous visual input can result in multiple perceptual interpretations of the same input, and spontaneous reversals between those interpretations. Such multi-stable perception of ambiguous input has been interpreted in the context of two broad classes of theories: top-down theories that emphasize dependence on higher-level cognitive factors such as knowledge, and bottom-up theories that suggest more vital involvement of aspects of lower-order information processing such as adaptation in the visual system. Most modern-day accounts hold that both top-down and bottom-up processes play a role, such that perceptual switches arise inevitably due to factors like adaptation, yet can also be delayed or hastened by higher-level cognitive influences. Surprisingly, some existing literature also suggests that the occurrence of perceptual reversals is vitally dependent on the observer's knowledge that the input is, indeed, ambiguous: without such knowledge many observers in that work did not experience any reversals, in apparent conflict with the idea that reversals are inevitable, stimulus-driven occurrences. Motivated by this conflict we re-evaluated the impact of knowledge on multi-stable perception. We used an ambiguous animation that allowed observers to report perceptual reversals without realizing the ambiguity. A total of 800 subjects were randomly assigned to an uninformed condition or an informed condition. Subjects in both conditions continuously reported their perception of the animation for a total of 180 seconds, but only in the informed condition was the ambiguity of the stimulus explained prior to the experiment. Comparing perceptual reversal dynamics between conditions, we found that subjects who had been informed of the animation's ambiguity reported slightly more perceptual reversals than subjects who had not. This between-condition difference was modest, however, compared to knowledge effects reported in the literature. Moreover, it was dwarfed by inter-observer variability within each group. These findings suggest that knowledge of ambiguity can influence perception of ambiguous stimuli, but only mildly, in keeping with most modern-day accounts.

Disclosures: B. Zhang: None. **J.W. Brascamp:** None.

Poster

401. Auditory, Visual, Proprioceptive Perception

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 401.28

Topic: H.02. Perception and Imagery

Support: NSF Grant BCS-1844144

Title: The conjunction fallacy in rats

Authors: *A. P. BLAISDELL[1], V. V. GONZALEZ[2], S. SADEGHI[3], L. TRAN[3];
[1]UCLA, [2]Psychology, [3]UCLA, Los Angeles, CA

Abstract: Humans and other animals have shown to be capable of reasoning. Nevertheless, there are overwhelming examples of errors or anomalies in reasoning. In two experiments, we studied if rats, like humans, evaluate the conjunction of two events as more likely than each event independently, a phenomenon that has been called the conjunction fallacy. In both experiments, rats learned through food reinforcement to press a lever under some cue conditions but not others. Sound B was rewarded, whereas sound A was not. However, when B was presented with visual cue Y (BY) it was not rewarded, whereas when A was presented with visual cue X (AX) it was rewarded (i.e., A-, AX+, B+, BY-). Both visual cues were presented on the same bulb. After training, rats received test sessions in which A and B were presented with the bulb explicitly off or occluded by an opaque metal shield. Thus, in the occluded condition, it was ambiguous whether the trial consisted of the presentation of an element alone (A or B) or of the compound (AX or BY). Rats responded on the occluded condition trials as if the compound cues were present, thus demonstrating the conjunction fallacy. The second experiment investigated if this conjunction fallacy could be attenuated by increasing the ratio of element/compound trials from the original 50-50 to 70-30 and 90-10. The 50-50 condition replicated the results of Experiment 1. The 70-30 condition also showed a strong conjunction fallacy. Only in the 90-10 condition (where 90% of the training trials were of just A or just B) did rats fail to show a conjunction fallacy. Nevertheless, the conjunction fallacy emerged in the 90-10 condition following additional training. These findings open new avenues for exploring the psychological and neural mechanisms of the conjunction fallacy effect.

Disclosures: A.P. Blaisdell: None. **V.V. Gonzalez:** None. **S. Sadeghi:** None. **L. Tran:** None.

Poster

402. Neural Mechanisms of Decision Making: Choice

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 402.01

Topic: H.03. Decision Making

Support: Toyota Central R&D Labs., Inc.

Title: Cognitive flexibility associated with respiratory cardiopulmonary dynamics

Authors: *A. NARITA[1], H. KANAZAWA[2], S. YONEKURA[3], Y. KUNIYOSHI[3];
[1]The Univ. of Tokyo, The Univ. of Tokyo, Tokto, Japan; [2]The Univ. of Tokyo, [3]The Univ. of Tokyo, Tokyo, Japan

Abstract: Introductions: Respiration is a cyclic movement that is essential for human life. Several studies have reported that there is a positive association between aerobic exercise and cognitive flexibility. Another study found that moderate-intensity aerobic cycling selectively increased synchrony among brain regions. The purpose of this study is to verify the possibility of regulating cognitive flexibility by changing breathing rhythm through human psychophysiological studies.
Methods: Binocular rivalry was chosen as a method of observing cognitive flexibility for the following two reasons. First, binocular rivalry arises from the interaction of many brain regions, and therefore the number of perceptual alterations can be used to indirectly measure cognitive flexibility. Second, since binocular rivalry cannot be consciously controlled, it is easy to check the influence of respiration.10 participants (male; mean age 25.1 ± SD 4.3) worked on a button-pushing task to signal the timing of perceptual alternation in a sitting position. Respiration and ECG of the participants were measured during the task. The experiments are conducted in 3 conditions; (i) normal state (ii) slow breathing (iii) fast breathing. Participants are verbally instructed to adjust breathing pace. We then analyzed the total number of button pressing for each respiratory speed and for each respiratory phase. In order to examine the differences between 3 conditions, bootstrap estimates of the average number of pushing was used.
Results: It was found that the number of switchings increased when breathing was fast compared to when breathing was slow, consistent with the previous study (Yousef, 2020). Furthermore, perceptual switching was less likely to occur near the transition from inspiration to expiration, and more likely to occur with inspiration. With fast breathing, the graph of the frequency of perceptual alternation with respect to respiration phase exhibited double-peaks. This characteristic did not appear in the case of slow/normal speed breathing.
Conclusions: This study revealed that frequency of perceptual alternation (i.e. cognitive flexibility) is not uniform for respiratory phases, but is specific.This finding extends insights into the physiological foundations of cognition considering the integration of dynamically interacting with the body.

Disclosures: A. Narita: None. **H. Kanazawa:** None. **S. Yonekura:** None. **Y. Kuniyoshi:** None.

Poster

402. Neural Mechanisms of Decision Making: Choice

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 402.02

Topic: H.03. Decision Making

Support: Weizmann-TASMC joint research program

Title: Two mechanisms of exploration by single neurons in the human brain

Authors: *T. REITICH-STOLERO[1], F. FAHOUM[2], I. STRAUSS[3], R. PAZ[1];
[1]Dept. of Brain Sci., Weizmann Inst. of Sci., Rehovot, Israel; [2]Neurol., [3]Functional Neurosurg. Unit, Tel Aviv Sourasky Med. Ctr., Tel Aviv, Israel

Abstract: To cope in uncertain environments, humans and animals have to balance their actions between utilizing current resources and searching for new ones. This exploration-exploitation (EE) tradeoff has been studied extensively in paradigms involving positive outcomes, with a focus on maximizing rewards, but it is just as important for survival when trying to avoid negative outcomes (punishments). Here, we examined the difference in exploration rates when humans act in reward or punishment environments. We used single-neurons to study the coding mechanisms that drive exploration and underlie the observed differences. We recorded the spiking activity of single neurons while 22 epileptic patients implanted with clinical depth electrodes were engaged in a probabilistic two-armed bandit task with intermixed gain and loss trials. Subjects exhibited higher exploration rates in the loss condition, and this was accompanied by higher sensitivity to the overall uncertainty in the loss condition. We identified a population of neurons in the temporal cortex and the amygdala that code for a trial-specific signal for exploration by increasing firing rates (FR) prior to an exploratory action. In accordance with a general valence-independent exploration-directing signal, these neurons responded similarly before an exploratory action in the gain and in the loss condition, and training a decoder to classify exploration-trials based on one valence yielded successful decoding when tested on the other valence. Interestingly, we found that noise levels in the activity of amygdala neurons were higher prior to loss-related choices compared to gain related choices. These noise levels were reduced as trials progressed and uncertainty declined, in accordance with information-driven exploration. Moreover, noise levels were positively correlated with the behavioral exploration rates, specifically in loss trials. Therefore, noise levels in amygdala neurons are consistent with a random-exploration signal when learning to avoid losses. Together, our results suggest two mechanisms of exploration in single neurons: one that is driven by trial specific changes in the FR of single neurons and is similar across gain- and loss-related learning, and a second that is driven by a more global increase in noise levels in amygdala neurons and likely contributes to the higher exploration in loss-related environments.

Disclosures: T. Reitich-Stolero: None. **F. Fahoum:** None. **I. Strauss:** None. **R. Paz:** None.

Poster

402. Neural Mechanisms of Decision Making: Choice

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Program #/Poster #: 402.03

Topic: H.03. Decision Making

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Title: Single unit correlates of visual reasoning in the human lateral occipital complex

Authors: *J. GARCIA RAMIREZ[1], M. VANHOYLAND[1], A. ZERROUG[3,4], M. VAISHNAV[3,4], T. SERRE[3,4], P. JANSSEN[2], T. THEYS[1];
[1]Res. Group Exptl. Neurosurg. and Neuroanatomy, [2]Res. Group Neurophysiol., KU Leuven, Leuven, Belgium; [3]Artificial and Natural Intelligence Toulouse Inst., Univ. de Toulouse, Toulouse, France; [4]Cognitive Linguistic and Psychological Sci., Brown Univ., Providence, RI

Abstract: Making sense of the visual world requires comprehending complex relations between objects within a scene. However, our understanding of the underlying neural computations remains limited. Recent computational work has suggested that making judgments about the sameness of two or more items on a screen requires additional neural computation compared to judging their spatial relations. Here, we aim to compare the neural correlates for the assessment of these two elemental visual relationships. We had the unique opportunity to record intracortical neuronal activity (96-electrodes Utah array) from the lateral occipital complex (LOC) in one epilepsy surgery patient while they performed a visual reasoning task. In this paradigm, the patient had to assess whether three items presented on the screen were positioned in a particular spatial configuration (aligned or not; Spatial Relation or SR condition) and/or whether they all belonged to the same or a different category (faces and bodies, based on neural selectivity; Same Category or SC condition). At the beginning of each trial, a black fixation point appeared at the center of the display for 300ms, after which the three objects were displayed for 250ms. Then, 500ms after stimulus offset, the fixation marker turned green, cueing the participant to respond with a button press. We employed linear decoding techniques to characterize differences in the neural population activity between the two conditions. We found that we could reliably predict which task the patient was engaged in directly from the spontaneous neural activity (from 150ms prior to target onset, $p<0.01$) which indicates preparatory activity from the LOC. After target onset, decoding for the two conditions remained at chance for 250ms, after which the task could again be significantly decoded until the end of the trial ($p<0.01$). This late discriminability between tasks was most likely related to the higher sustained firing found in the SC task in a relatively large number of channels (25% of visually selective channels, $p<0.05$) compared to the SR task. This suggests additional processing in the SC vs. SR task consistent with our hypothesis. To our knowledge, this study is the first attempt to investigate the neural correlates of basic visual reasoning at the single unit level in the human visual cortex.

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Poster

402. Neural Mechanisms of Decision Making: Choice

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Program #/Poster #: 402.04

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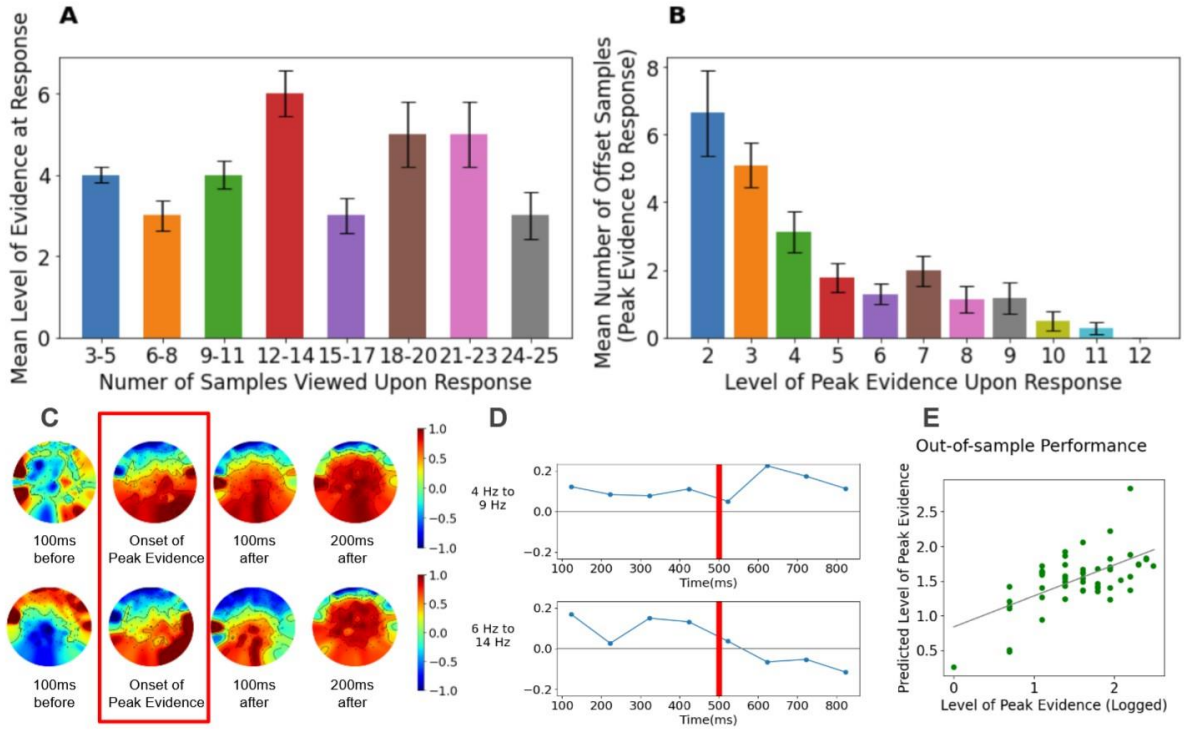
NSF 2051186

Title: Novel Probabilistic Perceptual Decision Making Task To investigate Trial-Level Computation of Evidence Accumulation in EEG and Behavior

Authors: *Q. SUN[1,2], J. O. GARCIA[2], J. VANDEKERCKHOVE[1], J. ROUDER[1], R. SRINIVASAN[1,2];
[1]Univ. of California, Irvine, Irvine, CA; [2]Humans in Complex Systems Div., US DEVCOM Army Res. Lab., San Francisco, CA

Abstract: Sequential sampling frameworks hypothesize that patterns of choice response times are produced by integrating noisy sensory information until a threshold is reached. We introduce here a novel probabilistic Perceptual Decision Making (pPDM) task to examine how and when noisy evidence leads to a decision on a trial level. The task involves presenting stimuli as a succession of random samples with a known probability biased towards one of the two alternatives. Subjects were asked to indicate which category was more frequently observed. We present a chain of evidence to experimentally control the hypothesized random walk of decision variables in the brain. ISI of the evidence chain is manipulated at three different levels: 0.05s, 0.1s, 0.25s. The pPDM task provides trial-level ground truth about the evolution of evidence prior to response to evaluate behavior and track evidence in the brain.
The participants do not appear to accumulate evidence to a fixed threshold to respond (Fig A). Instead, the criterion for the Decision Variable to trigger a response depends on both the magnitude of peak level of evidence, and the timing of the peak. When evidence obviously favors one of the choices after a large number of samples, subjects respond seemingly at the peak of evidence. When evidence peaks early, subjects wait for more samples to respond, even when they are uninformative, producing an offset between the peak of evidence accumulation and response (Fig B). We hypothesize that the brain tracks the peak of evidence to inform decisions, but not to trigger responses. We used a novel interpretable neural network model to use EEG to predict the magnitude of evidence following the display at which the evidence peaks. The model can automatically identify the signals that are critical to predict level of peak evidence (Fig C, D), showing theta enhancement and alpha suppression at peak evidence and can predict peak evidence in new test data (Fig E). These results show that while evidence accumulation is central to decision making, integration of noisy evidence to a bound does not by itself account for response times.



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Poster

402. Neural Mechanisms of Decision Making: Choice

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Topic: H.03. Decision Making

Support: NRF-2018M3C7A1022317
NRF-2020R1A6A3A13052912

Title: Decision-making modulation through direct cortical stimulation on inferior parietal lobule

Authors: D. LEE[1], J. KIM[2], C. CHUNG[1,3];
[1]Brain and Cognitive Sci., [2]Seoul Natl. Univ., Seoul, Korea, Republic of; [3]Seoul Natl. Univ. Hosp., Seoul, Korea, Republic of

Abstract: The modulation of the perceptual decision-making process by brain stimulation in humans has been tried in many studies(Green et al. 2013; Mansouri et al. 2017; Filmer et al.

2017). However, no studies have yet clearly identified whether the behavioral changes caused by brain stimulation affected sensory perception or decision-making. We used the vibrotactile discrimination task to solve this problem which represents the perceptual decision-making process(Romo and Salinas 2003). We recorded electrocorticography (ECoG) in 3 subjects with intractable epilepsy (1 male and 2 females; average age, 24.3 ± 4.0 years; all right-handed) and used direct cortical stimulation (DCS) for brain stimulation. To find a specific brain area to modulate perceptual decision-making with DCS, we instructed the subjects to perform two tasks (discrimination task, passive task). In the discrimination task, subjects compared two sequentially presented vibrotactile stimuli and responded to which of the two stimuli had a higher frequency. In the passive task, subjects did not compare two sequential vibrotactile stimuli and responded when they felt the second stimulus. We compared the brain activities from the two tasks and selected the electrode to which DCS would apply. We found the gamma band power (30-200 Hz) in the inferior parietal lobule (IPL) represented the decision-making process. IPL showed a significant gamma power difference between the discrimination task and the passive task. We also found that DCS affected the decision-making process, leading to behavioral change. We did not observe any behavioral changes when DCS was applied during the first tactile stimulus period but observed significant response speed changes and correct answer rate increase when DCS was applied during the second tactile stimulus period. Since decision-making occurred in the second tactile stimulus period and sensory perception occurred in the first tactile stimulus period(Schall 2001), this result represented that DCS applied to IPL affected the decision-making. We propose that a specific cognitive process can be artificially modulated through DCS and brain stimulation to the association area affects the high-level cognitive process.

Disclosures: D. Lee: None. **J. Kim:** None. **C. Chung:** None.

Poster

402. Neural Mechanisms of Decision Making: Choice

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Topic: H.03. Decision Making

Support: NSF Grant 1835202
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Title: Humans and non-human primates show similar sampling and choice behavior during multi-attribute decision making

Authors: *Y.-P. YANG[1], A. L. SAMPSON[1], D. LEVY[3], M. USHER[4], E. NIEBUR[2], V. STUPHORN[2];
[1]The Zanvyl Krieger Mind/Brain Inst., [2]Neurosci., Johns Hopkins Univ., Baltimore, MD;
[3]Marketing Dept., [4]Psychology Dept., Tel-Aviv Univ., Tel-Aviv, Israel

Abstract: In the real world, we make many complex multi-attribute decisions that require the integration and comparison of multiple pieces of information. This complexity is thought to tax limited cognitive resources since an agent can only pay attention to a limited set of information and then use it to dynamically construct preferences. Previous evidence supports the role of attention in constructing subjective value and guiding choice. However, the cognitive and neural processes that are at the heart of preference formation are still poorly understood. An animal model that allows the tracking of attention during the decision process is of critical importance.Here, we developed a multi-attribute decision-making task, which allows us to observe the focus of attention of subjects as they sequentially inspect each spatially separated and masked attribute (amount or probability), determine the value of reward options, and finally select one of them by moving a joystick. Two important aspects make it well suited to investigating the dynamic preference formation of risky choices. First, our task is a free-viewing task. Thus, the subject's eye movements directly indicate which decision-related variable is attended, to and in what order. Second, the information about the value of the two functionally different attributes is parametrically encoded using two different symbolic cues. The cue shape explicitly indicates the attribute types, which are weighted deferentially by the subject in computing subjective value.We observed behavior in the task in both humans (n=34) and macaques (n=2). Monkeys' choice behaviors indicate that they understand the meaning of the attribute cues. They can integrate this information into a single subjective value estimate for each option and compare the value of each option before choosing. The risk attitude of individual subjects can be explained by their relative weighing of the two attributes. In most trials, both humans and monkeys inspected all available information before making a choice, and first fully inspect one option, before inspecting the other. Late fixations are strongly correlated with choice. Our new attention-guided sequential sampling task will allow the study of option value computation and comparison in a primate model of multi-attribute choice.

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Poster

402. Neural Mechanisms of Decision Making: Choice

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NIH Grant R01DC020123
NIH Fellowship 5K00NS105204-05

Title: Pre-supplementary motor area activity indicates a parallel, not serial, choice process during multi-attribute decisions

Authors: *A. L. SAMPSON[1], Y.-P. YANG[1], D. LEVY[3], M. USHER[4], E. NIEBUR[2], V. STUPHORN[2];
[1]Zanvyl Krieger Mind/Brain Inst., [2]Zanvyl Krieger Mind/Brain Inst. and Solomon Snyder Dept. of Neurosci., Johns Hopkins Univ., Baltimore, MD; [3]Sagol Sch. of Neurosci. and Coller Fac. of Mgmt., [4]Sch. of Psychological Sci., Tel Aviv Univ., Tel Aviv-Yafo, Israel

Abstract: Real-life decisions typically require the consideration of multiple options, each with multiple attributes. To investigate the neuronal mechanism underlying the dynamic preference formation, we recorded pre-supplementary motor area (preSMA) activity in two macaques engaged in a multi-attribute decision-making task. Options in this task were defined by two attributes each: amount of water reward and probability of receiving that reward. Each attribute was revealed only while fixated and monkeys indicated their choices via arm movement after freely inspecting the options. Neuronal activity was aligned to both the information-gathering and option-selection stages of the task and analyzed using a generalized linear model (GLM) and de-mixed principal components analysis (dPCA). We found preSMA neurons encoded action value signals that reflected the sequentially sampled attributes of the options. During each fixation, preSMA activity changes according to value of each option as indicated by the currently fixated attribute. These value signals were gradually accumulated over multiple fixations, until the preSMA activity strongly indicated the choice. The current focus of attention lead to shifts in firing rates and the gain of value information, but the relative value of both available options was continuously represented independent of which option was currently fixated. Thus, our results are not compatible with recent models of value-based choice as a series of accept/reject decisions. Instead, they suggest a parallel evaluation and comparison process that is driven by an attention-guided sequential sampling of value information.

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Poster

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Support: NIH Grant R01 DA040990
NIH Grant R01 DA049147

Title: Attentional Sampling Strategies in Multi-Attribute Decision Making

Authors: J. ELSEY[1], D. LEVY[3], M. USHER[4], E. NIEBUR[2], ***V. STUPHORN**[2];
[1]Psychological and Brain Sci., [2]Neurosci., Johns Hopkins Univ., Baltimore, MD; [3]Sagol Sch. of Neurosci. and Coller Fac. of Mgmt., Weizmann Inst. of Sci., Rehovot, Israel; [4]Psychological Sci., Tel Aviv Univ., Tel-Aviv-Yafo, Israel

Abstract: Many everyday decisions require us to consider multiple options, each comprising of a number of shared or dissimilar attributes. In such situations, optimal decisions require the simultaneous integration and comparison of a large amount of information. This complexity is thought to tax limited cognitive resources in humans, who therefore use attention to select a limited subset of information, on which the decision is then based. To better understand the role of attention in decision making, we conducted a series of experiments in which we systematically varied the complexity of the choice menu by increasing the number of options, or attributes, or both. We recruited cohorts of subjects to participate in variations of a multi-attribute decision making task that differed in the number of options and attributes presented. Our task design utilized eye position as an overt measure of attention while subjects made decisions between menus of gambles that systematically covered the decision space. Each attribute's magnitude was masked by its corresponding color cue, and only revealed when fixated. Subjects freely inspected the attributes with no time constraint, before indicating their choice with a keypress. The resulting eye movements provide temporal and spatial information about subjects' focus of attention during the attribute sampling process. We find that human participants mainly switch between two strategies for sampling decision-related information. When only few options are available, participants most frequently use an *exhaustive strategy* to sample all attributes of each option independently, before examining the next one (2-Option, 2-Attribute: 68% of subjects, 2-Option, 4-Attribute: 66% of subjects). This is a more optimal strategy, because all information is examined. However, when more options are available, participants most commonly employ a *filtering strategy* to first compare the most important attribute across all options to identify the one or two best ones, followed next by sampling of all attributes of the remaining candidate options (4-Option, 2-Attribute: 75% of subjects, 4-Option, 4-Attribute: 68% of subjects). This strategy reduces the amount of information that needs to be processed, but can lead to sub-optimal choices. Importantly, which strategy is used depended on the number of options, but not the number of attributes. These findings elucidate the differences in the strategic direction of attention and its role in the option comparison process when evaluating increasingly complex decision problems.

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Poster

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Topic: H.03. Decision Making

Support: NIH Grant R01MH122513

Title: The role of insula in perceptual decision making and performance monitoring

Authors: *S. GHERMAN[1], N. MARKOWITZ[2], G. TOSTAEVA[2], E. ESPINAL[2], A. D. MEHTA[3], S. KELLY[4], R. G. O'CONNELL[5], S. BICKEL[6];
[1]The Feinstein Inst. for Med. Res., Manhasset, NY; [2]The Feinstein Inst. for Med. Res., Manhassett, NY; [3]Neurosurg., Hofstra North Shore LIJ Sch. of Med., Great Neck, NY; [4]Sch. of Electrical and Electronic Engin., Univ. Col. Dublin, Dublin, Ireland; [5]Trinity Col. Inst. of Neurosci., Trinity Col. Dublin, Dublin, Ireland; [6]Neurosurg. / Neurol., Hofstra Northwell Sch. of Med., Manhasset, NY

Abstract: Human neuroimaging has previously implicated the anterior insular cortex (AIC) in accumulating abstract sensory evidence during perceptual decision making. Concurrently, neuroimaging and iEEG work implicates the AIC in performance monitoring processes (i.e., selective responses to negative feedback and/or self-detected errors). It is currently unclear whether the same or distinct areas of the AIC might be involved in these separate processes, and if so, what spatiotemporal dynamics support their role in these functions.
Here, we capitalize on the high spatiotemporal precision of invasive intracranial EEG recordings in humans to investigate the neural dynamics of the anterior insula (AIC) during perceptual decision making. We recorded data from presurgical epilepsy patients while they made speeded perceptual categorizations. In two separate tasks, subjects judged either the direction (up vs. down) of random-dot stimuli (N=22), or the pitch (high vs. low) of tone cloud stimuli (N=14), and reported their choice with a button press. Feedback on the accuracy of the responses was provided after each trial. We analyzed high frequency activity (70-170Hz) in the insular cortex, time-locked to the presentation of sensory evidence, choice commitment, and performance feedback. Neural responses to sensory evidence were found across both anterior and posterior regions of the insula. However, we observed distinct activity profiles between these two regions. The posterior insula exhibited activity consistent with sensorimotor-related processes, peaking at or after motor response in an effector-selective manner (i.e., showing effector-dependent hemispheric lateralization), and showed no response to task feedback. In contrast, activity in the AIC during evidence presentation resembled a possible abstract evidence accumulation signal, showing a gradual ramping response which scaled positively with the strength of sensory evidence, increased at a rate that predicted reaction time, was independent of motor effector, and peaked just before commitment to choice. Intriguingly, at the time of feedback, the same sites showed stronger responses to error than correct choices (i.e., negative vs. positive feedback), consistent with the known role of the AIC in feedback monitoring. A preferential response to error trials was also observed earlier, immediately after commitment to choice (and before feedback was provided). Our preliminary results are in line with the known anterior-posterior dichotomy of functions in the insular cortex, and suggest that the AIC may be flexibly recruited during both decision-related and performance monitoring processes.

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Poster

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Support: NSF Grant 1732963

Title: Poor reputation increases anterior insula activity and decreases interpersonal investment

Authors: *H. WANG[1], D. P. CHRISTIANO[2], J. TSAI[1], B. D. KNUTSON[3];
[1]Stanford Univ., STANFORD, CA; [2]Psychology, Stanford, Stanford, CA; [3]Dept Psychology, Stanford Univ. Dept. of Psychology, Stanford, CA

Abstract: In the social neuroscience literature, increased Anterior Insula (AIns) activity has been associated with perceptions of unfairness and suboptimal sharing of resources, consistent with a mediating role of negative aroused affect (Sanfey et al. 2003). The role of anterior insula activity in positive social interactions involving trust, however, is less clear. To examine the role of anterior insula activity in choices to trust a partner, we assessed brain activity using FMRI in 22 healthy adults as they played 72 trials of one-shot Trust Games. On each trial, subjects saw pictures of a target face (4s), followed by information about previous reciprocation history of the target (i.e., the percentage of previous players who rated the target as trustworthy; 4s), and finally a prompt to choose how much to invest in the trustee (out of a $6 endowment; 4s). Target faces varied by race (White, Asian), sex (male, female), and expression (excited, calm, neutral). We predicted targets with poor reputations would elicit more AIns activity and would subsequently receive less investment from subjects. As predicted, repeated-measure ANOVAs revealed a main effect of Trustworthiness on investment ($F = 78.84$, $p < .001$), with subjects giving less to targets with poor relative to moderate ($p < .001$) and high ($p < .001$) reputations. Neurally, targets with poor reputations elicited the most AIns activity ($ps < .001$). AIns also was higher prior to low investments ($p = .008$). Finally, AIns activity during choice also partially mediated the relationship between reputation and investment ($\beta = .029$, 95% CI = [.013, .04], $p = .002$). These findings specifically extend previous work on sharing (in the context of Dictator Games) to investment (in the context of Trust Games). The pattern of results more generally suggests that poor reputation can decrease investment partially by as a function of increasing anterior insula activity. These findings may have implications for a chilling effect of negative aroused affect on investment in the context of interactions that rely upon trust.

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Poster

402. Neural Mechanisms of Decision Making: Choice

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Topic: H.03. Decision Making

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NIH R01DC020123
NIH D-SPAN fellowship 5K00NS105204-05

Title: Theta-gamma phase-amplitude modulation in human orbitofrontal cortex, hippocampus, and amygdala during value-based decision making

Authors: *V. SUBRITZKY KATZ[1,4], A. L. SAMPSON[1], E. EMERIC[1], W. J. LIPSKI[5], S. MOREIRA GONZALEZ[5], J. A. GONZÁLEZ-MARTÍNEZ[6], S. SARMA[2], V. STUPHORN[1,3], E. NIEBUR[1,3];
[1]Zanvyl Krieger Mind/Brain Inst., [2]Biomed. Engin., [3]Solomon Snyder Dept. of Neurosci., Johns Hopkins Univ., Baltimore, MD; [4]Neurosci., Univ. of Pennsylvania, Philadelphia, PA; [5]Cortical Systems Lab., [6]Neurosurg., Univ. of Pittsburgh Med. Sch., Pittsburgh, PA

Abstract: Most real world decisions require a choice between multiple alternatives, typically each defined by several attributes. This process requires that participants gather and integrate information about available alternatives, make a decision of which one to choose, anticipate the outcome, and collect rewards. We developed a novel multi-attribute decision making task that allows for the monitoring of the attentional state of participants during multi-attribute decision making. We recorded behavioral and neuronal data from patients undergoing epilepsy monitoring through stereotactic electroencephalogram (sEEG) recordings. It has been hypothesized that different brain regions communicate by slow oscillations (like Theta) modulating more localized activity reflected in higher frequency components (like Gamma). To test this hypothesis, a cross-frequency coupling analysis was performed on the sEEG data. We developed a new approach for identifying significant modulation of the amplitude of gamma oscillations (40- 200 Hz) by the phase of theta oscillations (4-8 Hz). This revealed hippocampus, amygdala, and orbitofrontal cortex as key regions that displayed significant theta phase-gamma amplitude modulation in either the option inspection, outcome anticipation, or feedback time periods of the task. These results help shed light on how synchrony in the theta band coordinates activity between brain regions during complex decision-making tasks.

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Poster

402. Neural Mechanisms of Decision Making: Choice

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Support: U01 NS094368

Title: Cortical processing of reward dynamics during visually-guided foraging behaviorin freely moving non-human primates

Authors: A. G. BUCKNER[1], M. FRANCH[1], X. PITKOW[2], V. DRAGOI[1,2];
[1]Neurobio. and Anat., McGovern Med. Sch. at The Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX; [2]Electrical and Computer Engin., Rice Univ., Houston, TX

Abstract: Foraging is a pervasive and natural behavior that animals exhibit while searching for food. To make efficient foraging decisions, animals must integrate sensory data, memory, and predictions to continually evaluate the decision about location and duration of foraging and how to move between food locations. Historically, foraging has been studied with the use of head-fixed animals performing foraging tasks on a computer or through observing freely moving animals without recording neural information, limiting our understanding of the neural underpinnings of sensory integration of environmental cues during foraging. To bridge this gap in knowledge, we utilize a novel approach using high-yield wireless electrical recordings and wireless eye tracking to study the neural activity in the prefrontal cortex and primary visual cortex while animals engage in an ethologically relevant foraging task. In this task, animals could freely move between two food patches that each have a monitor to display a color reflecting the reward probability at the given patch. Over time, animals' behavioral strategy changed in response to the changing certainty of the visual stimuli during foraging. Additionally, neural activity in dorsolateral prefrontal cortex (dlPFC) and V4 showed sensitivity to reward dynamics, such as cue information or reward probability during foraging. Finally, the dlPFC predicted the animals' decision to change foraging patches. Overall, this work will allow us to uncover neural computations that orchestrate complex decision involving sensory integration, memory, and prediction in a freely moving foraging task.

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Poster

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Topic: H.03. Decision Making

Support: ERC consolidator grant ERC/DYNERFUSION/865003 to Marios Philiastides

Title: Distinct neural representations of implicit and explicit feedback drive perceptual learning

Authors: *T. BALSDON[1], M. A. PISAURO[2], M. G. PHILIASTIDES[1];
[1]Univ. of Glasgow, Glasgow, United Kingdom; [2]Ctr. for Human Brain Hlth., Univ. of Birmingham, Birmingham, United Kingdom

Abstract: Metacognitive estimates of decision accuracy (confidence) have been proposed to support learning in the absence of explicit feedback. We examined how humans might learn

from this implicit feedback in direct comparison with that of explicit feedback, using simultaneous EEG-fMRI. Participants (N=23) performed a classic random dot motion discrimination task in which they gained or lost points according to their performance on each trial. Participants were given the opportunity to bet on their responses to double the points gained (or lost), which we took as a proxy for confidence. On intermixed trials, participants received either explicit feedback on the points scored, or a no-feedback cue, in which case the reward could only be inferred from implicit estimates. During the experiment, performance was maintained while task difficulty increased (decreased dot motion coherence), suggesting learning improved sensitivity. Patterns of response perseveration consistent with learning from feedback were observed following both explicit and (to a lesser extent) implicit feedback trials. A linear discriminant analysis identified EEG components around the time of the response that predicted whether the participant would bet, and which yielded a representation that scaled with a typical signature of decision confidence, reaction times on correct decisions. Around the time of feedback, these components again dissociated bet trials, especially when no explicit feedback was given, suggesting a regeneration of confidence representations to serve as implicit feedback. A separate discriminant analysis revealed distinct EEG components associated with explicit feedback. The EEG derived trial-wise predictions of explicit feedback were associated with changes in BOLD in regions consistent with the valuation system, notably, ventromedial prefrontal cortex and the ventral striatum. An adjacent, more dorsal, region of the striatum was associated with the EEG derived trial-wise predictions of confidence on no-feedback trials, though these trials were extrinsically identical. These findings suggest the presence of spatial gradients within the valuation system, giving nuances to the role of different subregions of the striatum in motivated behaviour. Together, this evidence indicates distinct neural mechanisms are recruited for learning from explicit and implicit (metacognitively driven) feedback.

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Poster

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Program #/Poster #: 402.14

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Support: the McKnight Foundation

Title: The role of self-efficacy and response-efficacy during perceived control

Authors: *Y.-Y. YANG, M. DELGADO;
Psychology, Rutgers Univ., Newark, NJ

Abstract: An individual's perception of control, which refers to the belief that one's own behavior can lead to a desirable outcome, is an important factor that contributes to decision-making. Indeed, participants often prefer situations where they are able to exert their choices

(e.g., picking an airline seat) over not being in control (e.g., letting the computer assign them a seat) even if they have to incur a cost to maintain control - a process thought to be mediated by the ventromedial prefrontal cortex (vmPFC; Wang et al., 2019). Importantly, perceived control can be further distinguished into two components (Bandura, 1977): self-efficacy, referencing the belief of an individual in successfully executing a behavior, and response-efficacy, regarding the belief that the behavior itself will lead to an expected outcome. However, prior studies have not often taken this distinction into account or explored whether different neural systems may differentially contribute to such aspects of perceived control. Here, we investigate how self-efficacy and response efficacy are evaluated to form perceived control and influence decisions. Participants completed a novel task where they had to make a perceptual decision about whether they could hit a moving target on the screen (self-efficacy). Participants were also presented with a card that highlighted the probability of that trial being worth a reward (response-efficacy). Both conditions varied in terms of their level (low to high). We scanned participants while they were presented with these two pieces of information simultaneously and asked them to rate their confidence in a) performing the behavior and b) the trial leading to a reward. Preliminary results suggest that participants were more sensitive to changes in self-efficacy than response efficacy information. That is, participants valued self-efficacy more than response efficacy information while making choices. In addition, while participants were asked to rate their confidence based on only one piece of information (e.g., the particular level of self-efficacy), they demonstrated the integration of both self-efficacy and response-efficacy when making behavioral ratings. Using fMRI, we observed activity in the vmPFC which positively correlated with self-efficacy, whereas activity in the striatum positively correlated with response efficacy. Further analysis will examine how self-efficacy and response-efficacy are integrated to influence decision making.

Disclosures: Y. Yang: None. **M. Delgado:** None.

Poster

402. Neural Mechanisms of Decision Making: Choice

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 402.15

Topic: H.03. Decision Making

Support: HSC R&D (STL/5540/19)
MRC (MC_PC_20020)

Title: Dynamic causal modelling reveals signal propagation of decision confidence in a perceptual decision-making task

Authors: A. ASADPOUR, *K. WONG-LIN;
Intelligent Systems Res. Centre, Ulster Univ., Derry~Londonderry, United Kingdom

Abstract: Perceptual decisions often entail a subjective level of confidence. Previous studies have investigated the neural representations of confidence based on behavioural measurements

as well as their spatial representation in the human brain. However, large-scale neural circuit dynamical mechanisms underlying decision confidence and the effective connectivity of active brain regions for decision confidence remain unclear. In this study, we implemented dynamic causal modelling (DCM) on a previously acquired dataset (Gherman & Philiastides, 2020) to determine the dynamics of neural population activities and signal propagation during the early stage of a visual motion discrimination decision-making task. The task consisted of the discrimination of the coherent direction of random dot kinematograms and rating the confidence of each choice on a trial-by-trial basis in two blocks of 160 trials acquired by a simultaneous electroencephalogram and functional magnetic resonance imaging (EEG-fMRI) method. The stimulus-locked epochs with the correct responses were extracted from the EEG data, and the epochs of low confidence (less than 50%) and high confidence (greater than 60%) of 19 participants were selected as conditions. Based on Gherman & Philiastides (2018) and previous studies, we included eight brain regions whose activity is positively correlated with confidence. However, unlike Gherman & Philiastides (2018), we neurally modelled the EEG-based dynamics, informed by fMRI, using the DCM approach. Using SPM12, Bayesian model selection was used to determine the best model fit among four different event-related potential forward-backward candidate models (David et al., 2006) between the two conditions. Our findings showed the activity of left and right posterior parietal cortices (pPC) in addition to the extracted active brain regions from fMRI in Gherman & Philiastides (2018), while complementing the latter with higher temporal resolution. Importantly, our results revealed not only the temporal flow of information from the occipital lobe (OL) to the lateral orbitofrontal cortex (lOFC) through the pPC, but also the underlying temporal responses of excitatory and inhibitory neural populations. Using the parametric empirical Bayes approach, the relatively stronger connections with a 100% probability of occurrence among the participants were forward and backward connections from the OL to right pPC, lateral connection from right lOFC to left lOFC, forward connection from OL to left pPC, and forward connection from right striatum to anterior cingulate cortex, respectively.

Disclosures: **A. Asadpour:** None. **K. Wong-Lin:** None.

Poster

402. Neural Mechanisms of Decision Making: Choice

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 402.16

Topic: H.03. Decision Making

Title: Response to Transcranial Direct Current Stimulation and Transcranial Magnetic Stimulation on Craving Behaviors in a Food Preference Task

Authors: J. L. SCHOLL, M. AHRENHOLTZ, E. NIELSON, C. FOX, T. J. BOSCH, ***L. A. BAUGH**;
Basic Biomed. Sci. & Ctr. for Brain and Behavior Res., Univ. of South Dakota, Vermillion, SD

Abstract: Prevalence of obesity and weight-related health issues are on the rise, and approximately 30-million Americans have suffered from an eating disorder at a point in their lifetime. Mortality rates for diagnosed eating disorders can range from 2 to 6 times that of the general population, and recovery rates are rarely greater than 50% overall. Non-invasive techniques, including repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS) of the dorsolateral prefrontal cortex (DLPFC) have shown promise in the treatment of maladaptive eating behaviors and eating disorders. However, efficacy in clinical trials has been varied, highlighting the need for improvements in treatment design. Our study aims to quantify the use of rTMS and tDCS on food choice preference and impulsivity. We predicted that targeted stimulation of the left DLPFC via both rTMS and tDCS would reduce craving for high calorie/low nutrition food options and would have a positive effect on impulsivity. Participants in this study (N = 20, 10 Female, Age Range 19-31) were randomly assigned to either the rTMS (N = 9) or tDCS (N = 11) and participated in three sessions. During the first session participants completed a series of general and psychological health assessments followed by sham stimulation. The latter 2 sessions consisted of active treatment. To minimize carryover effects, sessions were separated by a minimum of 2 days. Further, participants were scheduled for the same time of day across each session to remove variability in natural hunger cycles. Following active/sham stimulation, participants completed a food preference test and pre/post food craving questionnaires (FCQ-S). As predicted, when compared to the sham stimulation session, participants reported a significant decreased desire to eat in both the tDCS and TMS conditions. Further, this effect was driven by a significant reduction in desirability for high calorie vs. low calorie foods in a food ranking task. When examining responses to the state food craving questionnaire, participants reported an increase in control related to food craving, suggesting the decreased appeal of high calorie foods could be a result of increased control over current craving state following DLPFC stimulation. Taken together, results from this pilot study may be used in the future to optimize outcomes in the treatment of eating disorders.

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Poster

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Program #/Poster #: 402.17

Topic: H.03. Decision Making

Support: NIH Grant ZIA-NS003144

Title: Medial-inferior temporal regions contrast memory and perceptual inputs to guide visual decision

Authors: *W. XIE[1], K. A. ZAGHLOUL[2];
[1]NIH, NIH, Bethesda, MD; [2]NINDS, Bethesda, MD

Abstract: Many of our everyday decisions are based on the memory of individual past events. For example, when identifying a suspect, a witness needs to contrast his/her memory with a lineup in order to render a proper judgment. Despite this behavioral intuition, how the human brain contrasts memory and perceptual inputs to guide a visual decision is not well understood. Here, we develop a new task to probe the neurocomputational processes underlying visual decision as participants' choice unfolds over time. In this task, observers are asked to remember and reproduce customized humanoid robot faces that are drawn from a circular face space. On each trial, participants first tried to remember the association between a reward value (high vs. low) and each one of the two sequentially presented faces. Afterward, they were cued to recall the high-reward face and then try to reproduce it as precisely as possible based on the method of adjustment in order to earn a reward. As participants were continuously adjusting a randomly selected humanoid robot face to match their memory, we recorded intracranial EEG signals from the brain. We found that the divergence of an observer's perceptual choice from the remembered item is associated with an increase in 70-150 Hz power in the medial-inferior temporal areas. This neural signature is predictive of the observer's subsequent change of course in the choice trajectory to reproduce the remembered high-reward face. These results suggest that the medial-inferior temporal regions compute the discrepancy between memory and perceptual inputs, which is directly used to adjust subsequent choice behavior.

Disclosures: W. Xie: None. **K.A. Zaghloul:** None.

Poster

402. Neural Mechanisms of Decision Making: Choice

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

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Topic: H.03. Decision Making

Support: R01HD097619

Title: Fatigue reflects an affective response to dyshomeostasis and is part of an allostatic strategy

Authors: *A. CASAMENTO-MORAN, A. KIM, J. LEE, V. S. CHIB;
Biomed. Engineering, Sch. of Med., Johns Hopkins Univ., Baltimore, MD

Abstract: Fatigue is one of the most prevalent debilitating symptoms in neurological illnesses but remains poorly understood. Different frameworks have been developed to conceptualize fatigue, but these lack direct empirical support. The somatosensory attenuation framework proposes that fatigue reflects heightened effort perception due to altered sensing of neuromuscular information, while the metacognitive framework of dyshomeostasis proposes that fatigue reflects an affective response to dyshomeostasis. Here we examined whether the somatosensory attenuation framework or the metacognitive framework of dyshomeostasis better explains fatigue. Participants performed a fatiguing grip task that required maintaining a specified level of effort or electromyographic (EMG) in two non-consecutive sessions. We found

that fatigue increased similarly in both conditions, while effort perception did not increase and was also similar between conditions, providing empirical support to the metacognitive framework of dyshomeostasis. A key assumption of this framework is that fatigue is part of a regulatory process that aims to maintain homeostasis, namely 'allostasis'. We hypothesized that, if fatigue is part of an allostatic response, both biofeedback conditions would induce changes in subjective effort cost. However, we also hypothesized that optimal allostasis would occur when interoceptive information - regarding dyshomeostasis - is integrated with sensorimotor information - regarding the compensatory increase in neuromuscular activity - to adjust participant's subjective effort cost. If this hypothesis is correct, then effort biofeedback would result in a greater increase in subjective effort cost. As hypothesized, we found that participants' subjective effort cost increased during both biofeedback conditions, but it increased the most with effort biofeedback. These results suggest that fatigue reflects an affective response to dyshomeostasis and is part of an allostatic strategy that aims to regain homeostasis.

Disclosures: A. Casamento-Moran: None. **A. Kim:** None. **J. Lee:** None. **V.S. Chib:** None.

Poster

402. Neural Mechanisms of Decision Making: Choice

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Program #/Poster #: 402.19

Topic: H.03. Decision Making

Title: Depression Severity Relates To SmallerAffect-Modulated Reward Positivity Amplitude

Authors: *G. SINGH[1], T. JACKSON[1], M. LAVELLE[1], D. BROWN[2], J. F. CAVANAGH[1]; [1]Dept. of Psychology, Univ. of New Mexico, Albuquerque, NM; [2]Pitzer Col., Claremont, CA

Abstract: The Reward Positivity (RewP) is a positive deflection in the EEG sensitive to reward receipt. Recent evidence suggests that the RewP is modulated by both reward probability as well as affective valuation ("liking"). We hypothesize that this latter "liking" feature is specifically affected in major depression. We recruited 69 participants (MDD =35, Control= 34) who completed a reinforcement learning task (green or red screen feedback) with concurrent affective images. We specifically examined the modulation of the RewP when paired with hedonically preferred images (puppies) vs. less-preferred images (cows). There was no group difference in "liking" ratings of puppy or cow pictures, nor were there differences in RewP between groups. Across all participants, there was a significant negative correlation between BDI score and RewP amplitude difference (puppy-cow; r= -0.246, p=0.041), confirming our hypotheses. Within MDD group alone, there was a significant negative correlation between depression severity (BDI score) and RewP amplitude difference (puppy-cow; r=-0.321, p=.030), indicating an inter-individual influence of self-reported depression on hedonically-related RewP amplitudes. These findings suggest a domain-specific diminution of hedonic responsiveness in severely depressed people.
Keywords: Reward Positivity, MDD, affective imagery

Disclosures: **G. Singh:** None. **T. Jackson:** None. **M. Lavelle:** None. **D. Brown:** None. **J.F. Cavanagh:** None.

Poster

402. Neural Mechanisms of Decision Making: Choice

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 402.20

Topic: H.03. Decision Making

Title: Effect of emotional congruency and task complexity on decision-making

Authors: *P. CORTES ESPARZA, J. GARCÍA-HERNÁNDEZ, F. IRIBE-BURGOS, M. HERNÁNDEZ-GONZÁLEZ, M. GUEVARA;
Inst. De Neurociencias, Inst. De Neurociencias, Guadalajara, Mexico

Abstract: The heuristic approach to decision-making (DM) holds that the selection process becomes more efficient when part of the information available is ignored. One element involved in information selection is emotional valence (positive or negative). Some works have reported an emotional bias towards options that are similar to the individual's mood or emotional state. For purposes of this study, this correlation between emotional states (in terms of valence) and outcomes is defined as emotional congruency. If emotional congruency plays a role in simplified decision-making strategies, then an interaction of this factor with task complexity should exist; that is, emotional congruency should simplify the selection process. To evaluate this interaction in terms of behavioral responses and brain activity in the cortices associated with DM, we explored how emotional congruency and task complexity influence decision-making execution and electroencephalographic (EEG) activity. We hypothesized that emotional congruency would have a positive effect on task execution and that the magnitude of that effect will increase with task complexity as the amount of information to be processed increases, suggesting that a heuristic approach to the problem is more efficient. The EEG activity of 20 young men was recorded during performance of a DM task that was divided into 3 conditions, each with 60 trials, 30 of lower complexity and 30 of higher complexity: 1) direct congruency: participants had to select positive stimuli to earn high scores; 2) inverse congruency: participants had to select negative stimuli to earn high scores; and 3) null congruency: there was no relation between stimuli valence and scores. Results showed that the direct and inverse conditions improved execution (higher scores), though distinct types of emotional congruency had differential effects on behavior; that is, while direct congruency enhanced overall decision-making performance, the inverse condition interacted with task complexity to modify the pace at which task feedback affected behavior. The prefrontal cortex showed higher absolute power in the higher complexity condition. No between-factor interaction was found. Results suggest that both emotional congruency and its type have a differential effect on DM execution. Task complexity was mainly associated with changes in EEG activity, but the effect of emotional congruency on this parameter appeared to be minimal. Emotional congruency seems to improve DM execution, not

by reducing the quantity of information to be processed, but due to an emotional bias towards relevant stimuli.

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Poster

402. Neural Mechanisms of Decision Making: Choice

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 402.21

Topic: H.03. Decision Making

Title: Gaze behaviour reflects computational complexity of decisions in humans

Authors: *E. A. BOWMAN[1], K. ROTARU[2], J. FRANCO[1], C. MURAWSKI[1];
[1]The Ctr. for Brain, Mind and Markets, Univ. of Melbourne, Parkville, Australia; [2]Monash Business Sch., Monash Univ., Caulfield, Australia

Abstract: This project examined how computational complexity and cognitive load interacted during complex decisions, and if these interactions were detectible in gaze behaviour.
73 participants aged between 18 and 35 years (mean age 22.7 years, 52 were female, 21 male) completed 72 trials of the decision knapsack task. Before each trial, participants were required to memorise either a 1-digit or a 6-digit number displayed for 5 seconds. Each trial consisted of a 5- to 7-second grey fixation screen, followed by the knapsack decision task where participants were given up to 25 seconds to decide if a combination of any of the six items displayed on the screen could be found to satisfy a both minimum value and maximum weight constraint. After the knapsack decision screen, participants were required to enter the number they were asked to memorise at the start of the trial. Participants were paid according to the number of trials in which they both correctly recalled the memorised number and made the correct knapsack decision.
Knapsack task instances varied in computational complexity (as measured by a control parameter defined by the ratio of normalised capacity and normalised profit), but the number of satisfiable and unsatisfiable instances was the same. Gaze and pupil recordings were performed during the task, sampling at 300Hz with Tobii Pro Spectrum video eye trackers.
Mixed-effects modelling of participant decision responses found a strong effect of instance complexity, and of satisfiability, on trial performance. However, only an inconsistent effect of memory load was found. Participants also spent significantly less time solving knapsack trials of lower instance complexity, and the relationship between instance complexity and time taken significantly predicted performance on a trial. Gaze fixation rate of Knapsack items during a trial decreased significantly in satisfiable trials ($p = 0.003$), however there was no significant effect of memory load condition, or of instance complexity. Additionally, rate of fixation on Knapsack items also did not demonstrate a relationship with the correct decision performance of a Knapsack trial. There was, however, an interaction between instance complexity and fixation

dwell time on decision performance (p = 0.03).
These results suggest that although the rate of gaze fixation on individual knapsack items varies with certain aspects of a problem's complexity, changes in fixation rate have not been found to be reflected in decision performance.

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Poster

402. Neural Mechanisms of Decision Making: Choice

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COSMAX

Title: fMRI outperforms marketing surveys in forecasting cosmetic sales

Authors: *M. KWON[1], Y. LEE[1], J.-H. LEE[1], J. J. IM[1], E. RHO[3], Y. JO[4], W.-Y. AHN[2]; [1]Dept. of Psychology, [2]Dept. of Psychology; Dept. of Brain and Cognitive Sci., Seoul Natl. Univ., Seoul, Korea, Republic of; [3]Grad. school of education, Univ. of California, Berkeley, Berkeley, CA; [4]Electric and electronic engineering, Yonsei Univ., Seoul, Korea, Republic of

Abstract: "Will this new product become popular?" is a question worth our attention due to its high demands in business and marketing. Recent neuroimaging studies suggest that neural signals from participants in a laboratory setting may forecast consumer behavior, but whether they can predict actual sales is less clear. We tested if functional magnetic resonance imaging (fMRI) responses to cosmetic images could forecast their actual sales amount. Inside the fMRI scanner, 53 female participants viewed a series of 8 different lipstick images and answered whether they would like to buy the product or not. Based on the sales data for the past 2 years, those 8 products were classified into four popular and four unpopular categories. The general linear modeling (GLM) revealed that brain regions in the reward circuit (e.g. striatum, medial prefrontal cortex, dorsolateral prefrontal cortex, and insula) are activated when participants viewed popular products compared to unpopular products. We then computed an fMRI forecast value based on a previous neuroforecasting study (Kuhn et al., 2016, Neuroimage), by aggregating neural signals from the brain regions in the reward circuit. The actual sales amount was significantly correlated with the fMRI forecast value ($R = 0.77$, $p < 0.05$). In contrast, self-reported marketing surveys collected outside the scanner did not ($R = 0.33$, $p = 0.427$) even if

preference, willingness to purchase and subjective estimate of popularity were explicitly asked while viewing the same cosmetic images. The finding was replicated with larger samples collected online (N = 300, R = -0.12, p = 0.79). Our findings suggest that fMRI data might allow us to predict consumer behavior beyond traditional survey measures and highlight the potential utility of neuroforecasting.

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Poster

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Program #/Poster #: 402.23

Topic: H.03. Decision Making

Support: NIMH Grant R01 112775

Title: Challenges in fMRI-based Lie Detection: Heterogeneous Neural Correlates

Authors: ***S. LEE**[1], L. ZHU[2], P. H. CHIU[3], B. KING-CASAS[4], A. S. KAYSER[5], M. HSU[6]; [1]Univ. of California, Berkeley, Berkeley, CA; [2]Peking Univ., Beijing, China; [3]Virginia Tech. Carilion Res. Inst., Virginia Tech. Carilion Res. Inst., Roanoke, VA; [4]Virginia Tech., Roanoke, VA; [5]Univ. of California San Francisco, Univ. of California San Francisco, San Francisco, CA; [6]Univ. of California, Berkeley, California Clin. Trials, Berkeley, CA

Abstract: Can we detect lies using fMRI? Previous studies have used instructed lying paradigms to identify neural correlates of deception, which involved heightened activity in the insula, inferior parietal lobule, middle frontal gyri, etc. Here we sought to extend these findings in a more naturalistic setting where participants were incentivized, but not instructed, to lie. We used a messaging task in which the participants play the role of the 'signaler' who sends a message to the 'recipient' about which monetary outcomes the recipient should pick (e.g., much like the role of a financial advisor advising the client which option to choose). In the preference condition, participants tell the recipient that they would 'prefer the recipient choose option A/B', while in the deception condition, participants tell the recipient that 'option A/B will earn you more money'. Hence, a selfish message in the preference condition does not involve a lie but a selfish message in the deception condition does. Using meta-analysis ROIs from previous instructed lying studies, we were able to predict whether the participants were lying or not on a given trial, but only for those participants who showed aversion to lying. Regions such as the insula and cingulate cortex showed increased activity to lying only if the participants were averse to lying, while participants who were comfortable with lying showed dampened activity in these regions. Furthermore, those who were comfortable with lying showed neural responses in the deception condition that were similar to their responses in the preference condition.

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Poster

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Title: Unethical amnesia brain: Memory and metacognitive distortion induced by dishonesty

Authors: X. J. XU[1], D. MOBBS[2], ***H. WU**[1];
[1]Ctr. for Cognitive and Brain Sci. and Dept. of Psychology, Univ. of Macau, Macau, China;
[2]Humanities and Social Sciences,, California Inst. of Technol. (caltech), Pasadena, CA

Abstract: To preserve moral self-image, people's memory of unethical actions gradually become obfuscated, a cognitive process known as unethical amnesia. This self-serving dishonesty may change human memory from two aspects: memory accuracy and metacognitive confidence. To test this, we conducted 7 experiments to investigate how repeated dishonest responses over time distorts memory and memory confidence. These included fMRI in an information passing task, and pre-task memory and post-task memory testing combined with mouse-tracking. Our study shows three main findings here: (1) memory and confidence decreased for enhance dishonesty condition; (2) people show adaptation in the moral decisions, which effect could be manifested over cognitive control and moral ROI from Neurosynth; (3) the multi-attribute, time-varying DDM model suggest people put a relatively bigger weight on money reward and a relatively smaller weight on consistency in enhance dishonesty condition; (3) the ISRSA results provided further evidence that individual with similar a after-effect on memory decrease had similar BOLD patterns in IFG, MFG. Together, with a complementary manipulation of pre-task and post-task measures, the present study dissected brain regions involved to random dishonesty and consistent dishonesty adaption, and how consistent dishonesty caused memory distortion.

Disclosures: **X.J. Xu:** None. **D. Mobbs:** None. **H. Wu:** None.

Poster

402. Neural Mechanisms of Decision Making: Choice

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Program #/Poster #: 402.25

Topic: H.03. Decision Making

Title: Product endorser myth-- does celebrity endorsement effect truly increase consumers' purchase intention?

Authors: *Y.-H. KO[1], Y.-T. WU[1], R.-T. HUANG[1], C.-J. LIN[1,2,3], C.-H. LIN[1,2];
[1]Psychology, [2]Res. Ctr. of Nonlinear Analysis and Optimization, [3]Col. of Nursing, Kaohsiung Med. Univ., Kaohsiung, Taiwan

Abstract: Previous research found consumers tended to pay more attention to celebrity endorsers' advertisements due to the Celebrity Endorsement Effect (CEE). Although celebrities helped consumers to recall the products, Nistoreanu et al., (2019) study showed that celebrities in advertising can probably distract consumers' attention from the logo or the product itself. Therefore, this study aims to investigate whether the CEE enhances consumers' attention, memory, preference, and final purchase intention of the advertising products. Here we conducted the behavior test combined with an eye-tracker to monitor the difference in participants' responses between fame-endorsed products (FEP) vs. non-fame-endorsed products (non-FEP).The pre-test recruited 100 participants to rate the reputation, preference, and familiarity of the endorsers and all products to build the baseline in this study. In the formal test, 60 newly recruited participants (30 females, 30 males) were assigned to the FEP or non-FEP group to watch 2D advertisements videos including 12 categories of products (e.g. hair care). Simultaneously, participants' gaze frequency on the advertisements had been recorded by the eye-tracking system (Tobii Eye Tracker 4C) to be the indexes of attention. In the post-test phase, participants' memory, preference, and final purchase intention of the advertising products were measured.The product-recognition accuracy between the FEP and non-FEP groups was non-significance, but the confidence level of product recognition, preference, and purchase intention significantly differed. Also, within-group comparison, among the product categories, the main effect of the confidence level of recall, preference level, and purchase intention were significant. However, there were no significant interaction effects of group and confidence level of recall, preference level, and purchase intention. The eye-tracker data showed a significant difference in gaze frequency toward the body area of FE/non-FE between the two groups.This study did not replicate the CEE here. Interestingly, some non-FEPs even possessed slightly higher preference and purchase intention rather than FEPs in certain categories of products. Even more, according to various categories of products, if the celebrity endorser did not have the representative style and reputation of certain products (Parmar et al., 2014) as the consumers identified, there could be probably no further enhancement in the marketing efficacy. In the Net Generation, it should be very careful to use the celebrity endorser advertising strategy for certain product marketing.

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Poster

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Program #/Poster #: 402.26

Topic: H.03. Decision Making

Title: Idle hands, better grades? Extracurricular impacts on the rise in online academic performance

Authors: *J. SOSA[1], N. MOHAMMED[2], R. LUCIN[2], A. G. LOPEZ[2], P. S. ZABZDYR[1], L. E. KNOX[3], S. A. DREW[3];
[1]Psychology, [2]California State Univ. - Northridge, Northridge, CA; [3]California State University, Northridge, Northridge, CA

Abstract: The onset of the COVID-19 pandemic saw a surge in online learning, the effects of which must be better understood. Recent literature suggests increases in academic performance since the pandemic's onset. Although contributing factors are unclear, the allocation of time to study, as characteristic of metacognitive control, seems relevant. Whereby their time, decision-making, and attention in these self-directed learning environments can have effects on student focus. This exploratory study sought to identify elements of student life that significantly changed pre- to post- pandemic onset that relate to changes in academic performance. Students from California State University, Northridge (N = 198) answered questions pertaining to GPA and daily activities prior to and after the onset of the pandemic. These activities included: a) hours spent working at an on-campus job, b) hours spent working at an off-campus job, c) perceived effect of job hours on academic performance, d) hours spent on organization or team activities, and e) the number of co-curricular activities in which they participated. Given the literature, we hypothesized that we would observe a significant increase in GPA pre- to post-pandemic onset. Similarly, we anticipated observing a significant decrease in time spent on the aforementioned activities during this time span due to the "safer at home" measures. Furthermore, we anticipated a significant interaction between the changes in GPA and in time spent on activities where an increase in GPA would be accompanied by a decrease in time spent on activities. Initial paired sample *t*-tests revealed a significant increase in GPA pre- to post-pandemic onset, as well as a significant decrease in hours spent on organization or team activities across that time. All other paired sample *t*-tests were non-significant. A repeated measures ANOVA revealed a significant interaction between GPA and activity hours, with no significant main effects. These data provide insight into potential contributors to increased performance during the pandemic era and better inform our understanding of learning under these conditions. Further empirical research is needed to elucidate which specific aspects of activities most contribute to changes in academic performance.

Disclosures: J. Sosa: None. **N. Mohammed:** None. **R. Lucin:** None. **A.G. Lopez:** None. **L.E. Knox:** None. **S.A. Drew:** None.

Poster

402. Neural Mechanisms of Decision Making: Choice

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 402.27

Topic: H.03. Decision Making

Support: NIH Grant R01MH119086

Title: The Effects of Cognitive Fatigue on Effort-Based Choice in the Human Brain

Authors: *G. E. STEWARD[1], V. S. CHIB[1,2];
[1]Biomed. Engin., Johns Hopkins Univ., Baltimore, MD; [2]Kennedy Krieger Inst., Baltimore, MD

Abstract: Cognitive fatigue has a dramatic impact on our choices to engage in effortful activity. For example, after a particularly challenging day thinking about data analysis, we might feel too tired to engage in our regular exercise class. In a clinical setting, cognitive fatigue is a common symptom in many psychiatric disorders. While feelings of cognitive fatigue are ubiquitous, an understanding of its behavioral and neural mechanisms is limited. In this study, 28 healthy, human volunteers (9 Male, M_{Age}= 24.5, SD_{Age} = 4.1) made choices to either undergo a default, low-effort cognitive task for $1, or a higher-effort cognitive task for a larger reward. The effort and reward levels of the non-default options were varied to characterize participants' subjective value of cognitive effort. A working memory N-Back task was used as a proxy for cognitive effort, with increasing N representing increasing cognitive load. Participants performed this effort choice task in a rested/baseline state and after bouts of cognitively fatiguing exertion. The choice paradigm was designed to be robust, with considerations for balanced order randomization and balanced choice difficulty over time. Additionally, the choice battery was designed to span the range of effort and reward value regardless of differences in inter-subject preference. Sex differences have not been assessed at this time. Paradigm and imaging data were stored in the BIDS format for enhanced scientific rigor and reproducibility. Behaviorally, participants made choices indicating that their subjective value of an offer varied linearly with prospective reward (R) and the square of prospective effort (E^2). In examining the results of individual Generalized Linear Models, participants increased their value of reward ($p = 0.002$) and increased the cost of prospective effort ($p = 0.032$) between the baseline and fatigue phases. Generalized Linear Mixed-Effects Models showed no significant fixed-effect interaction of trial number, a proxy for fatigue, on the value of effort in the baseline phase ($p = 0.943$) while trial number did have a significant fixed-effect on the value of cognitive effort during the fatigue phase ($p = 0.031$). Using these behavioral models of value to inform imaging analysis, we found that activity in the right Insula, vmPFC, and ventral striatum associated with chosen value increased significantly in the fatigue phase compared to baseline (uncorrected, $p < 0.005$, $k = 10$). In conclusion, increased cognitive fatigue increases the subjective value of cognitive effort and rewards. These changes are correlated with changing activation in the network of brain regions associated with decision-making.

Disclosures: G.E. Steward: None. **V.S. Chib:** None.

Poster

402. Neural Mechanisms of Decision Making: Choice

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 402.28

Topic: H.03. Decision Making

Title: Neural mechanisms of political preferences

Authors: *B. B. LU, B. SHEN, J. J. VAN BAVEL, P. W. GLIMCHER;
New York Univ., New York, NY

Abstract: It is widely accepted that partisan affiliations have a significant impact on the political preferences of individuals. Many US voters, for example, show strong political preferences over important issues that appear to be more tied to their political affiliations than to their direct self-interest. We recently developed a behavioral task designed to measure the strength and structure of how partisan affiliations influence preferences. Our behavioral data showed that for many individuals, on both the left and the right, learning whether their political party supported a given law could rapidly and significantly shift their preferences for that law. Similarly, knowing whether the opposing political party supported that law could also strongly impact preferences. In addition to partisanship, the degree and direction of these shifts were notably influenced by a subject's traits related to social and interpersonal relationships, such as desire for social esteem and approval of social hierarchies. In this study, we examined the neural activations associated with this rapid partisan-evoked shift in political preferences. Forty subjects viewed short synopses of real proposed laws that have appeared before US Congress. They were additionally asked to report their preference for the law based on the synopsis. In later blocks, they viewed and evaluated the laws again after learning the true percent of members of Congress in each political party who voted in favor of the law. This allowed us to search the neural data not only for the loci at which subjects' political preferences were represented, but also for neural activations that correlated with the degree of partisanship expressed by individual subjects. Areas that represent in-group vs. out-group social signals were of particular interest. Functional activity that tracks political preference in areas known to represent other forms of value would indicate that these preferences follow known patterns and rules about decision-making in other domains. We found that areas in the medial prefrontal cortex do indeed track political preference. Additional analyses provide further insight into the nature of this neural computation.

Disclosures: **B.B. Lu:** None. **B. Shen:** None. **J.J. Van Bavel:** None. **P.W. Glimcher:** None.

Poster

402. Neural Mechanisms of Decision Making: Choice

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 402.29

Topic: H.03. Decision Making

Support: Universidad del Desarrollo Proyecto de Investigación Interno 2020-102 UDD
Clínica Alemana Santiago Dpto. Científico Docente Proyecto ID 1033

Title: Patients recovered from COVID-19 present alterations in their brain functioning assessed with a reversal learning decision-making task

Authors: *L. KAUSEL[1,3], X. STECHER[4,5], P. SOTO-ICAZA[1], A. FIGUEROA-VARGAS[1], F. J. ZAMORANO-MENDIETA[1,4], P. CARVAJAL[1], M. ASPÉ-SÁNCHEZ[1], R. HENRÍQUEZ-CH[6], P. MUÑOZ-VENTURELLI[2], R. URIBE[7], P. BILLEKE[1];
[1]Ctr. de Investigación en Complejidad Social, [2]Ctr. de Estudios Clínicos, Facultad de Medicina, Univ. del Desarrollo, Santiago, Chile; [3]Ctr. de Estudios en Neurociencia Humana y Neuropsicología, Univ. Diego Portales, Santiago, Chile; [4]Dept. de Imágenes, [5]Dept. de Radiologia, Neuroradiología, Clinica Alemana de Santiago, Santiago, Chile; [6]Lab. de Neurociencias Cognitivas, [7]Facultad de Medicina, Pontificia Univ. Católica de Chile, Santiago, Chile

Abstract: Coronavirus disease 2019 (COVID-19) is emerging as one of the greatest public health crises of our times. While COVID-19 primarily affects the respiratory system, other organs including the brain can be involved. Increasing evidence indicates that recovered COVID-19 patients present neuropsychiatric alterations and thinning of certain cerebral cortex areas, especially those connected to the primary olfactory cortex. Also, patients with COVID-19 that have anosmia, a highly reported symptom, show affection of orbitofrontal regions. Given this background it is highly relevant to evaluate the possibility of alterations at the brain level in recovered COVID-19 patients. In order to investigate how COVID-19 affects brain functioning, 62 (30 females) recovered COVID-19 patients (age 43±12; 35 with anosmia; 28 hospitalized because of respiratory symptoms) were asked to resolve a Reversal Learning Task (RL) while their brain activity was measured with fMRI. In the task, the participants had to choose between two options that were presented with different probabilities of obtaining reinforcement (0.8 vs 0.2 and 0.7 vs 0.3). Through trial and error, participants were able to learn the most advantageous option. After a certain number of repetitions, the probabilities associated with each option were reversed, and the subject had to learn the new association. This process is called reverse learning, and is related to orbitofrontal and dorsolateral prefrontal activity. Behavior was modeled using cumulative prospect theory. This model uses a multiplicative approach to calculate the expected utility that is used to calculate the probability of choosing one of the options. Brain activity associated with the decision-making period was analyzed in regard to the expected utility, controlled by reaction time as a proxy of difficulty. We found two major results. On one hand, there was a decrease of activity in the right temporal region in those patients that had anosmia, regardless of the severity of respiratory symptoms. On the other hand, there was an increase in orbitofrontal activity over time (months elapsed post contagion), regardless of whether the patients had anosmia or the severity of respiratory symptoms. This indicates that recovered COVID-19 patients have an functional alteration in their brain activity regarding value processes, that seems to be partly recovered over time. Our results suggest that COVID-19 could have an impact on brain functioning involved in decision-making. This should be further investigated incorporating a control group. Our study supports the evidence that COVID-19 can have an impact on the functioning of the central nervous system.

Disclosures: **L. Kausel:** None. **X. Stecher:** None. **P. Soto-Icaza:** None. **A. Figueroa-Vargas:** None. **F.J. Zamorano-Mendieta:** None. **P. Carvajal:** None. **M. Aspé-Sánchez:** None. **R. Henríquez-Ch:** None. **P. Muñoz-Venturelli:** None. **R. Uribe:** None. **P. Billeke:** None.

Poster

402. Neural Mechanisms of Decision Making: Choice

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 402.30

Topic: H.03. Decision Making

Title: How do we select our own ideas? A study of the neurocognitive mechanisms of creative choices

Authors: *S. MORENO RODRIGUEZ, A. LOPEZ-PERSEM, E. VOLLE;
Paris Brain Inst., Paris, France

Abstract: Creativity is formally defined as the ability to produce an idea that is both original and adequate. This definition implies an evaluative process of adequacy and originality, operating on candidate ideas and leading to the selection of a response. Research has also reported that creative ideas are often discarded, possibly the result of a cognitive bias against novel concepts during the evaluation and selection steps of the creative process. At the neural level, extensive research has led to the finding that creativity relies on two main networks: the default mode network (DMN) and the executive control network (ECN), which are thought to respectively support the generation of spontaneous ideas, and their evaluation and selection.
In parallel however, neuroscience of decision-making has identified that another network, the Brain Valuation System (BVS), represents how much an item is liked (or desired) by an individual, i.e., subjective preferences. It corresponds to the reward system previously identified in animal studies and is thought to implement item evaluation. Interestingly, the BVS and the DMN partially overlap in terms of anatomy, and the BVS is often coupled with the ECN when choices are made. Despite these convergences, very little research has tackled the role of preference in creativity. Therefore, the aim of this study is to offer a neurocognitive perspective of the decision-making processes of creativity and how they are implemented in the usually described networks: DMN, ECN, and for the first time BVS. 40 individuals performed creativity and decision-making tasks in an MRI scanner. Using computational analysis of behavioral data, we showed how adequacy and originality were involved in the evaluation and selection of ideas and revealed that individuals could be biased against uncommon ideas. By looking at parametric modulation of brain activity by ideas' subjective values, we demonstrated that the BVS reflected ideas' subjective values, not only when evaluating ideas, but also when generating them. These results not only offer new evidence for the theory that the BVS computation is generic (it applies to any kind of item) and automatic (it is at work even when engaged in another task) and for the first time extend this theory to the evaluation of ideas, but they are also the first steps towards a neurocognitive account of the decision-making mechanisms underlying the creative process. By

replicating and extending previous results, this study suggests that using neuroscience of decision-making framework and methods is a valuable approach to study creativity.

Disclosures: S. Moreno Rodriguez: None. **A. Lopez-Persem:** None. **E. Volle:** None.

Poster

403. Prefrontal Mechanisms of Working Memory: Nonhuman Primate Studies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 403.01

Topic: H.05. Working Memory

Title: Conserved and divergent patterns of alternative splicing across orthologous cell types in the mouse and human neocortex

Authors: *M. DAVIE[1], D. HOWARD[1], Y. CHEN[1], S. TRIPATHY[1,2,3,4];
[1]Krembil Ctr. for Neuroinformatics, Ctr. for Addiction and Mental Hlth., Toronto, ON, Canada; [2]Dept. of Psychiatry, [3]Dept. of Physiol., [4]Inst. of Med. Sciences, Temerty Fac. of Med., Univ. of Toronto, Toronto, ON, Canada

Abstract: Splicing of protein-coding genes can vary between neuronal cell types and alter features such as protein localization and binding activity, yet most alternative splicing events produce functionally identical proteins. Moreover, given the high rate of alternative splicing among mammals, most gene isoforms are species-specific. However, a number of isoforms show very high sequence similarity across species, suggesting the potential functional relevance of their proteins. Here, we hypothesized that examining between-species conservation of splicing expression patterns may highlight cell type-specific isoform switches that are potentially functionally relevant, suggesting mechanisms behind diverse neuronal functions beyond those implicated in gene expression data alone. We assessed patterns of alternative splicing among four major interneuron classes (PVALB, SST, VIP, LAMP5) using the Brain Initiative's single-nucleus SMART-seq data from the human middle temporal gyrus and mouse primary motor cortex. Among exons with a high degree of sequence similarity between species (>0.80), we found that orthologous exon expression levels were positively but weakly correlated across interneuron types (mean correlation ± SD: 0.20 ± 0.49). Several genes with orthologous exons demonstrated particularly highly correlated exon expression between species, including MEF2C, SMARCA2, NRXN1, PBRM1, ANK2, and KCNMA1. In contrast, we found other genes such as ZEB2 and MYO5A with highly uncorrelated expression between species. These results may prioritize gene isoforms of interest for studies aiming to elucidate the functional role of alternative splicing in the context of neuronal cell types.

Disclosures: M. Davie: None. **D. Howard:** None. **Y. Chen:** None. **S. Tripathy:** None.

Poster

403. Prefrontal Mechanisms of Working Memory: Nonhuman Primate Studies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 403.02

Topic: H.05. Working Memory

Support: CIHR
NSERC
DFG
NSF

Title: Characterizing Neuronal Diversity in Intrinsic Membrane Properties Across Non-Human Primate Species and Cortical Areas.

Authors: *M. FEYERABEND[1], S. POMMER[3,4], M. JIMENEZ-SOSA[1], J. RACHEL[3], J. SUNSTRUM[1], F. PREUSS[3], S. MESTERN[1], S. MATOVIC[1], M. WIEDERMAN[1], S. EVERLING[1,2], G. GONZALEZ-BURGOS[5], D. LEWIS[5], A. NEEF[4], J. STAIGER[3], W. INOUE[1], J. MARTINEZ-TRUJILLO[1];
[1]Schulich Sch. of Med. and Dent., [2]Dept. of Physiol. and Pharmacol., Univ. of Western Ontario, London, ON, Canada; [3]Inst. for Neuroanatomy, Univ. of Göttingen, Göttingen, Germany; [4]Campus Inst. for Dynamics of Biol. Networks, Göttingen, Germany; [5]Dept. of Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Higher-order mental representations underlying working memory function, are believed to arise in primates, with the development and expansion of the granular prefrontal cortex (PFC). For example, in vivo extracellular recordings from behaving non-human primates (NHPs) have shown that persistent firing representing the contents of working memory can be found in lateral PFC, but is absent in early sensory areas such as primary visual (V1) cortex. However, the mechanistic basis of the potentially unique persistent activity in primate PFC circuitry remains speculative, partly due to the lack of data on the biophysical and morphological features of the diverse cell-types present in different cortical areas in NHPs. Furthermore, whether single-neuron properties in PFC differ among different NHP species (e.g. new-world marmosets and old-world macaques) remains unclear. The latter is an important question, as marmosets are increasingly being used as models for human cognition, yet their microcircuitry components are poorly documented. In our international consortium (NeuroNex), we aim to characterize the biophysical and microanatomical properties of cortical neurons from the dorsolateral (DL)PFC and V1 of marmoset and macaque monkeys (Macaca mulatta, Macaca fascicularis, Callithrix jacchus) using patch clamp electrophysiology in acute brain slices. We systematically characterize the membrane voltage responses to different current injection protocols to analyze intrinsic membrane properties as in the Allen Institute Cell Type Database. To date, we accumulated over 500 electrophysiological recordings. In addition, we examined the anatomical features including dendritic type and laminar location of the reconstructed neurons. Some of these data can be browsed on the website ‘PrimateDatabase.com’, a publicly available collection of intracellular recordings and digital reconstructions of the morphology of a proportion of the recorded neurons. The goal of our project is to provide data that will allow the

search for differences in intrinsic properties across cortical areas and across NHP species and enable modelling studies of working memory using realistic single neurons simulations.

Disclosures: **M. Feyerabend:** None. **S. Pommer:** None. **M. Jimenez-Sosa:** None. **J. Rachel:** None. **J. Sunstrum:** None. **F. Preuss:** None. **S. Mestern:** None. **S. Matovic:** None. **M. Wiederman:** None. **S. Everling:** None. **G. Gonzalez-Burgos:** None. **D. Lewis:** None. **A. Neef:** None. **J. Staiger:** None. **W. Inoue:** None. **J. Martinez-Trujillo:** None.

Poster

403. Prefrontal Mechanisms of Working Memory: Nonhuman Primate Studies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 403.03

Topic: H.05. Working Memory

Support: NSF Grant Neuronex 2015276

Title: Parvalbumin neurons from mouse visual and prefrontal cortex differ in synaptic input and input-output transformation properties.

Authors: ***T. MIYAMAE**[1], Y. NISHIHATA[1,2], D. A. LEWIS[1], G. GONZALEZ-BURGOS[1]; [1]Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA; [2]Psychiatry, Nara Med. Univ., Kashihara, Japan

Abstract: Parvalbumin-positive (PV) interneurons are present, with a highly conserved laminar distribution, across all areas of the mammalian neocortex. However, the percentage of all interneurons that PV neurons represent differs significantly between cortical areas. For instance, PV neurons are a majority (>40%) of all interneurons in the visual cortex (VC) but a minority (<20%) in the medial prefrontal cortex (PFC). Despite these hierarchical differences in PV neuron percentage, little is known vis-à-vis whether the role of PV neurons in sensory and association areas exhibits regional specializations. To begin addressing this question, we studied PV neurons in layers 2-5 of the mouse VC and PFC using patch clamp electrophysiology in acute slices. To focus on potential differences in the synaptic inputs and their transformation into spike output, we recorded spontaneous excitatory postsynaptic currents (sEPSCs) and assessed the membrane potential response to injection of rectangular current steps. We also assessed the probability of spiking in response to variable current that mimicked the natural pattern of synaptic input observed during UP-DOWN states. Analysis of a database of sEPSC recordings (VC, n=26; PFC, n=17 cells) showed a higher sEPSC frequency in PV neurons from VC, and suggested an absence of difference in the sEPSC amplitude between PV neurons from VC and PFC. Analysis of 16 intrinsic membrane properties assessed with rectangular current steps (VC, n=26; PFC, n=21 cells) revealed that all VC and PFC neurons exhibited Fast Spiking (FS) properties typical of PV cells, with some similarities and differences between areas. Moreover, two firing patterns, termed dFS and cFS, were observed and distinguished, respectively, by the presence or absence of a delay to fire the first spike at rheobase currents, as previously reported by others. Interestingly, the fractions of dFS and cFS cells differed between VC and PFC, since

21/26 and 9/21 of the PV cells had dFS phenotype in VC and PFC, respectively. Analysis of the spike probability during variable, UP state-like input, will expand our assessment of potential differences between VC and PFC in the input-output transformation properties of PV neurons. Our preliminary assessments suggest that the greater PV neuron percentage in VC correlates with higher levels of excitatory input and with different input-output transformation properties relative to those of PV neurons from PFC.

Disclosures: **T. Miyamae:** None. **Y. Nishihata:** None. **D.A. Lewis:** Other; Currently receives investigator-initiated research support from Merck.. **G. Gonzalez-Burgos:** None.

Poster

403. Prefrontal Mechanisms of Working Memory: Nonhuman Primate Studies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 403.04

Topic: H.05. Working Memory

Support: CAMH Discovery Fund

Title: Understanding the contributions of voltage gated potassium channels to neuronal excitability by integrating transcriptomics with detailed ion channel kinetics.

Authors: *A. NIGAM[1,2], S. TRIPATHY[2], S. L. HILL[2];
[1]Univ. of Toronto Collaborative Program In Neurosci., Toronto, ON, Canada; [2]Krembil Ctr. for Neuroinformatics, Ctr. for Addiction and Mental Health, Univ. of Toronto, Toronto, ON, Canada

Abstract: Neuronal excitability is a core phenomenon where large and rapid changes in membrane potential occur in response to a small stimulus; having implications virtually across all neurological and neuropsychiatric disorders. Ion channels are the major players involved in its regulation among which, voltage gated potassium channels (Kv) on their own are part of a diverse family. The dysfunction in Kv channels is involved in channelopathy, epilepsy, schizophrenia and multiple other neurological and neuropsychiatric disorders. Here, we want to focus on understanding the generic principles for Kv channels, in terms of synergy, degeneracy, pleiotropy, and the functional overlap as hypothesized. By diving into the gene expression and electrophysiological properties of both current clamp and voltage clamp features of voltage gated potassium ion channels, we aim to identify key insights of potassium channels, including how they work together to contribute to neuronal excitability.
We use the deeply characterized homomeric ionic channels data from the Channelpedia database for kinetic characteristics and the mouse visual cortex patch-seq dataset for transcriptomics and cellular electrophysiology to study Kv genes. We can study at the single cell and the population level resolution of how the Kv channels work from gene expression to protein function. Hierarchical clustering is performed to integrate these three modalities. The clusters obtained from the correlation matrix of Kv gene expression and electrophysiology features revealed that channels can be categorized broadly into multiple clusters when looking at gene co-expression

(six), gene expression-electrophysiological feature associations (six), and genes with their kinetic characterization (three). These clusters are further analyzed to observe the similarities and the differences between these Kv ion channels. The clusters obtained from the correlation matrices of gene co-expression and gene expression-electrophysiology properties indicate high correlation of gene expression of certain Kv channels with fast neuronal spiking properties. Further, observing the voltage ranges of activation and inactivation and their association with electrophysiology features and gene expression has resulted in clusters of Kv genes corresponding to timescales (slow, medium, and fast).
Integrating these multiple modalities has highlighted principles of how Kv channels work together to regulate neuronal excitability. Eventually, we aim to understand these relationships between genes, transcripts, and alterations in neuronal excitability in brain disorders and neuropsychiatric illnesses.

Disclosures: **A. Nigam:** None. **S. Tripathy:** None. **S.L. Hill:** None.

Poster

403. Prefrontal Mechanisms of Working Memory: Nonhuman Primate Studies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 403.05

Topic: H.05. Working Memory

Support: CIHR
NeuroNex, NSF

Title: Contribution of synaptic activity to the activation profiles of primate cortical pyramidal neurons induced by electrical stimulation measured by Ca^{++} imaging

Authors: *S. VIJAYRAGHAVAN[1], H. IGARASHI[3], V. SUKUMAR[4], W. INOUE[5], A. PRUSZYNSKI[2], J. C. MARTINEZ-TRUJILLO[6];
[1]Western Univ., LONDON, ON, Canada; [2]Physiol. and Pharmacol., Western Univ., London, ON, Canada; [3]Dept. of Physiol. and Pharmacol., Robarts Res. Inst., London, ON, Canada; [4]UNIVERSITY OF WESTERN ONTARIO, LONDON, ON, Canada; [5]Univ. of Western Ontario, London, ON, Canada; [6]Dept. of Physiol. and Pharmacol. and Psychiatry, Schulich Sch. of Med. and Dentistry, Robarts Institute, Western Univ., London, ON, Canada

Abstract: A hierarchy of intrinsic timescales of neuronal activation across the different cortical sensory and association cortical areas has been identified as a potential mechanism by which association cortical areas encode and maintain information in working memory for longer periods of time (Murray et al., Nat. Neurosci. (2014), 17 (12), 1661-1663). The timescales of activation of neurons as measured by their autocorrelation function during the rest period in cognitive tasks shows a gradient along the cortical hierarchy from sensory to prefrontal association areas. Several mechanisms could contribute to the longer timescales in the neuronal autocorrelation functions in association cortical areas, including strength of recurrent synaptic

activity, differences in intrinsic physiological and anatomical properties of neurons, and differences in neuromodulatory tone between different areas in the cortical hierarchy. Ca^{++} imaging with genetically encoded indicators such as GCaMP in cortical slices combined with electrical microstimulation offers an attractive methodology to examine the response profiles of many neurons simultaneously to further address the mechanisms by which activation timescales vary between different areas. Here we performed two photon microscopy while electrically stimulating cortical slices from the common marmoset, Callithrix jacchus. We used a robot-guided injection system to transfect neurons in marmoset PFC, somatosensory cortex and area PE with AAV viral vectors wherein GCaMP6f was expressed under the control of the CAMKII promoter. We examined the time-course, latency of onset and amplitude and other parameters of Ca^{++} responses of pyramidal neurons to electrical stimulation of prefrontal and sensory cortical slices with and without blockade of ongoing synaptic activity with glutamate and GABA receptor blockers. We found that blockade of ionotropic glutamate and GABA receptors had mixed effects on parameters describing the activation profiles of pyramidal neurons. The activation of many neurons upon electrical stimulation was reduced after blockade of synaptic transmission, while it remained unchanged or occasionally augmented for other pyramidal neurons. We will discuss our findings in the context of potential cellular mechanisms modulating the intrinsic network timescales along the cortical hierarchy.

Disclosures: S. Vijayraghavan: None. **H. Igarashi:** None. **V. Sukumar:** None. **W. Inoue:** None. **A. Pruszynski:** None. **J.C. Martinez-Trujillo:** None.

Poster

403. Prefrontal Mechanisms of Working Memory: Nonhuman Primate Studies

Location: SDCC Halls B-H

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Program #/Poster #: 403.06

Topic: H.05. Working Memory

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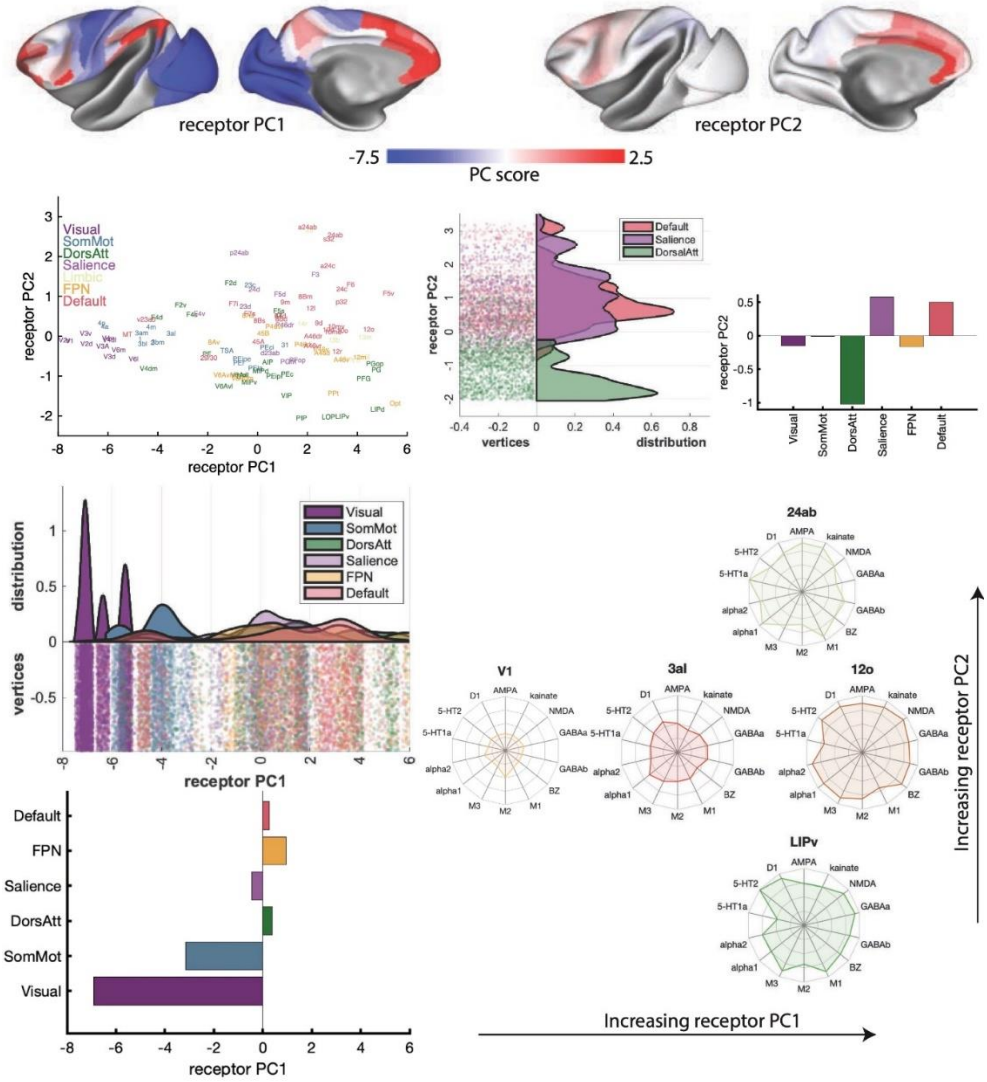
EU HBP SGA3 945539

Title: Gradients of receptor expression in the macaque cortex

Authors: *S. FROUDIST-WALSH[1], T. XU[2], M. NIU[3], L. RAPAN[3], L. ZHAO[3], D. S. MARGULIES[4], K. ZILLES[3], X.-J. WANG[1], N. PALOMERO-GALLAGHER[3];
[1]New York Univ., New York, NY; [2]Child Mind Inst., New York, NY; [3]Res. Ctr. Julich, Julich, Germany; [4]Univ. of Paris Cité, Paris, France

Abstract: Dynamics and functions of neural circuits depend on interactions mediated by receptors. Therefore, a comprehensive map of receptor organization across cortical regions is needed. Here we use *in-vitro* receptor autoradiography to measure the density of 14 neurotransmitter receptor types in 109 areas of macaque cortex. We integrate the receptor data with anatomical, genetic and functional-connectivity data into a common cortical space. We uncovered a principal gradient of receptor expression per neuron aligned with cortical hierarchy from early sensory cortex to higher cognitive areas. A second gradient, driven by serotonin 5-HT_{1A} receptors, peaks in the anterior cingulate, default-mode and salience networks, and is highly related to human gene expression, highlighting the potential for the macaque as a translational model of serotonergic processing and disorders. The receptor gradients may enable rapid, reliable information processing in sensory cortical areas and slow, flexible integration of information in higher cognitive areas.



Disclosures: **S. Froudist-Walsh:** None. **T. Xu:** None. **M. Niu:** None. **L. Rapan:** None. **L. Zhao:** None. **D.S. Margulies:** None. **K. Zilles:** None. **X. Wang:** None. **N. Palomero-Gallagher:** None.

Poster

403. Prefrontal Mechanisms of Working Memory: Nonhuman Primate Studies

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Topic: H.05. Working Memory

Support: Neuronex NSF2015276

Title: A comparative study of D1R expression in parvalbumin inhibitory neurons in dlPFC of macaque and marmoset

Authors: *M. P. JOYCE[1], F. M. KRIENEN[2], W. INOUE[4], J. C. MARTINEZ-TRUJILLO[5], S. MCCARROLL[3], A. F. ARNSTEN[6];
[1]Neurosci., Yale Univ., New Haven, CT; [2]Genet., [3]Dept. of Genet., Harvard Med. Sch., Boston, MA; [4]Univ. of Western Ontario, London, ON, Canada; [5]Dept. of Physiol. and Pharmacol. and Psychiatry, Schulich Sch. of Med. and Dentistry, Robarts Institute, Western Univ., London, ON, Canada; [6]Dept. Neurosci., Yale Med. Sch., New Haven, CT

Abstract: Dopamine (DA) significantly modulates network connectivity in the layer IIIb microcircuits of dorsolateral prefrontal cortex (dlPFC) that generate working memory. The dlPFC has expanded in primates and is most elaborate in humans, where it is thought to be a substrate for abstract thought and cognition. In macaque dlPFC under optimal non-stress conditions, DA promotes strong but flexible connectivity in layer IIIb recurrent microcircuits, enhancing spatial tuning and suppression of distractors in spatial working memory tasks. Under high levels of DA release such as in uncontrollable stress, D1 receptor (D1R) stimulation suppresses firing in layer IIIb microcircuits, likely through cAMP-mediated opening of potassium channels on spines of pyramidal neurons. DA may also reduce the output of dlPFC layer IIIb circuits by activating parvalbumin-containing (PV) inhibitory interneurons, which are excited by D1R. While marmosets exhibit the delay-related firing that is a signature of cognition-associated layer IIIb microcircuits of dlPFC, they appear more susceptible to distraction and stress-related decrements in spatial working memory function. This species discrepancy may be mediated by differences in D1R expression on PV interneurons in layer IIIb microcircuits. Here, we show phylogenetic differences in D1R transcripts and in situ protein expression on PV inhibitory neurons in layer IIIb microcircuits of the marmoset compared to the macaque. Inhibitory neurons, including PV neurons, contained higher D1R mRNA transcripts in marmosets than macaque, suggesting that the effect of DA release in marmoset dlPFC may be more strongly mediated by expression on inhibitory neurons than in macaques. Double immunolabeling and confocal microscopy to examine the in situ protein expression of D1R on PV inhibitory neurons in marmosets vs. macaques is consistent with species differences. Our results suggest that stress-related D1R signaling may more rapidly silence dlPFC recurrent pyramidal circuits in marmosets than in macaques via PV inhibitory neuron assemblies. Under lower DA release conditions, greater D1R expression on PV interneurons might enhance tuning

of mental representations. These results may reflect differences in the ability of marmosets and macaques to handle stress while keeping their PFC function intact.

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Poster

403. Prefrontal Mechanisms of Working Memory: Nonhuman Primate Studies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 403.08

Topic: H.05. Working Memory

Support: CIHR
BrainsCAN
CREF
NeuroNex
NSERC

Title: Large-scale mapping of the prefrontal cortex in the common marmoset

Authors: *R. K. WONG, J. SELVANAYAGAM, K. D. JOHNSTON, S. EVERLING; Physiol. and Pharmacol., Western Univ., London, ON, Canada

Abstract: The common marmoset (*Callithrix jacchus*) has rapidly gained attention in neuroscience research. This species' small brain size and relatively lissencephalic cortex offer practical advantages for laminar electrophysiology, optical imaging, and large-scale mapping of cortical areas. Electrophysiological investigations in macaque PFC have provided much insight into the functional specialization of PFC subregions. This understanding however, is based largely on area-specific recordings compiled across multiple animals, and not via simultaneous recordings spanning the entire PFC. Here, we exploited the small size and lissencephalic cortex of the marmoset to characterize the functions of PFC subregions using high-density array recordings covering a large portion of the PFC. Untethered extracellular recordings were carried out in two adult marmosets with 96-channel Utah arrays (4x4 mm, 1.5 mm electrode length, 400μm pitch) implanted in the left PFC, with coverage of areas 8, 9, 10, 46 and 47. To characterize these PFC subregions, marmosets performed a cognitive task and were also presented with sets of visual and auditory stimuli. These were comprised of a delayed-match-to-location (DMTL) task on a touchscreen attached to the home cage, a visual field mapping task, presentations of images belonging to different categories (human faces, marmoset faces, marmoset body parts and objects), and a set of auditory stimuli consisting of conspecific vocalizations. Well-isolated single units were characterized across a broad swath of prefrontal subregions including areas 46D, 46V, 47, 8aD, 8aV, 9 and 10. In the DMTL task, we observed modulations of activity in response to all task events in all subregions, but noted that the proportion of units with delay activity was relatively lower in areas 9 and 10. Units with visual

activity were found in all prefrontal subregions, with spatially tuned units preferentially present in area 8aV, which corresponds to the marmoset frontal eye fields (FEF). When marmosets were shown images belonging to different categories, units with visual responses were found in all prefrontal subregions. Units with auditory responses were found across all prefrontal subregions, but most prominently in areas 8aD, 9, 46D and the medial portion of area 10. Taken together, these findings suggest that subregions of marmoset PFC are broadly responsive during cognitive performance and in response to stimuli in multiple modalities and contexts, but that regional specialization is present. They additionally support the marmoset as a model animal for large-scale functional mapping of cortical areas.

Disclosures: R.K. Wong: None. **J. Selvanayagam:** None. **K.D. Johnston:** None. **S. Everling:** None.

Poster

403. Prefrontal Mechanisms of Working Memory: Nonhuman Primate Studies

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Program #/Poster #: 403.09

Topic: H.05. Working Memory

Support: CONP Scholars Program

Title: Optimal Transport-Based Domain Adaptation for Alignment of Intracellular Electrophysiology Datasets

Authors: *S. MESTERN[1], J. C. MARTINEZ-TRUJILLO[3], W. INOUE[2];
[1]Univ. of Western Ontario, Stratford, ON, Canada; [2]Univ. of Western Ontario, London, ON, Canada; [3]Dept. of Physiol. and Pharmacol. and Psychiatry, Schulich Sch. of Med. and Dentistry, Robarts Institute, Western Univ., London, ON, Canada

Abstract: The advent of open datasets cataloguing diverse cell types in the mammalian brain offers unique opportunities for further discoveries. However, intracellular electrophysiology datasets can be skewed by technical variables and species-dependent differences. Here, we propose optimal transport-based domain adaptation (OTDA) for transforming and aligning datasets across labs & species. We then use aligned datasets to allow grouping of similar cell types, and subsequently, comparing homologous cell types across datasets and species. Electrophysiological features have been extracted from several open datasets of cortical patch-clamp electrophysiology. First, we demonstrated the technique's robustness in aligning data. Using mouse datasets from a single source, a random sample of the data was skewed stochastically. Then, using OTDA, the skewed data was shifted back onto the remaining dataset. The OTDA method showed better reconstruction of original data compared to z-score or min-max normalization. Next, we demonstrated the usefulness of OTDA in understanding similar cell types across datasets. Data from several species (mouse and human) & labs were aligned using OTDA. Visual inspection confirmed the robust alignment of similar cell-types. Finally, we

performed unsupervised clustering on the aligned data and identified cells that belong to the same clusters (cell types). Thereafter, we used the features of the original data and inspected within-cluster differences between species. Overall, OTDA shows a robust methodology for cross-species and lab comparison of electrophysiological data while acknowledging the intrinsic differences.

Disclosures: S. Mestern: None. **J.C. Martinez-Trujillo:** None. **W. Inoue:** None.

Poster

403. Prefrontal Mechanisms of Working Memory: Nonhuman Primate Studies

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Topic: H.05. Working Memory

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Title: Activation of L-type Voltage-gated Calcium Channels (LVCC) by β1-AR Reduces Working Memory-Related Neuronal Firing in the Dorsolateral Prefrontal Cortex

Authors: *M. WANG, S. YANG, A. F. T. ARNSTEN;
Yale Univ. Sch. of Med., New Haven, CT

Abstract: The prefrontal cortex (PFC) in human and nonhuman primates is essential for working memory and higher cognitive function. The dlPFC has extensive, recurrent excitatory circuits to generate and sustain the mental representations in the absence of sensory stimulation to guide working memory and abstract thought. Our research has focused on the cellular basis of visuospatial working memory, where dlPFC "Delay cells" maintain spatially-tuned, persistent firing across the delay period. Our previous studies showed the persistent firing of Delays cells relies on NMDAR, including those with GluN2B subunits, receptors that flux high levels of calcium and are expressed within the synapse in layer III dlPFC. Spine synapses in layer III dlPFC also express the molecular machinery to magnify calcium signaling to support persistent firing. However, higher levels of calcium-cAMP signaling open nearby potassium (K^+) channels on spines, weakening connectivity and reducing neuronal firing. The L-type calcium channel (LTCC) encoded by *CACNA1C* are associated with cognitive impairment and increased risk of mental disorders, but it's not known why increased LTCC actions would be harmful to cognition. In heart, LTCC are activated by noradrenergic β1-adrenoceptor (β1-AR) stimulation during stress, increasing internal calcium release. The current study examined the physiological contributions of LTCC and β1-AR signaling to dlPFC working memory-related firing. The effects of β1-AR-$Cav_{1.2}$ signaling on dlPFC "Delay cell" firing were tested using single unit recordings coupled with iontophoresis in monkeys performing an oculomotor spatial working memory task. Our results showed that iontophoresis of a β1-AR agonist or an LTCC agonist reduced Delay cell firing and spatial tuning, while a β1-AR antagonist or low dose of an LTCC antagonist increased delay-related firing and spatial tuning. Furthermore, our data supported an

interaction between β1-AR and LTCCs, as the reduction in Delay cell firing induced by the β1-AR agonist was blocked by co-iontophoresis with the LTCC blocker. Further experiments examined the mechanisms by which increased LTCC opening reduces dlPFC Delay cell firing. Our results showed that SK and HCN channels contribute to the loss of firing produced by increasing the open state of LTCCs. Taken together, our data show that LTCC opening is needed to support persistent neuronal firing, but that excessive levels driven by β1-AR stimulation reduce firing through the opening of SK and HCN channels. Sustained reductions in network connectivity and firing would likely spur synapse loss and cortical thinning, as has been seen in many *CACNA1C*-linked disorders.

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Poster

403. Prefrontal Mechanisms of Working Memory: Nonhuman Primate Studies

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Topic: H.05. Working Memory

Support: Simons Foundation grant 543057SPI
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Title: Large scale modelling of distributed working memory: a comparative study between macaque and marmoset

Authors: *L. MAGROU[1], P. THEODONI[2], K. KNOBLAUCH[3], H. KENNEDY[3], M. G. P. ROSA[4], A. F. T. ARNSTEN[5], X.-J. WANG[1];
[1]Ctr. for Neural Science, New York Univ., New York, NY; [2]Ctr. for Mind, Brain and Consciousness, New York Univ., New York, NY; [3]Stem Cell and Brain Res. Institute, Univ. Lyon, Univ. Claude Bernard Lyon 1, INSERM, Bron, France; [4]Neurosci. Program, Biomedicine Discovery Inst. and Dept. of Physiology, Monash Univ., Clayton, VIC, Australia; [5]Dept. of Neuroscience, Yale Univ. Sch. of Med., New Haven, CT

Abstract: Recent large-scale models of Working Memory (WM) show that a distributed WM is significantly more resilient to distractions, perturbations, or even silencing than a more localised WM system. Such models provide insights regarding viable and biologically compatible mechanisms that explain the inherently distributed nature of WM. Building on this, we area now investigating the differences between two primates (i.e. macaque and marmoset) in their respective WM capabilities. Indeed, macaques are well known for their talents in this matter, which have been extensively studied. Conversely, emerging data indicate that the marmoset monkey has a shorter WM time span and is highly susceptible to distractions and perturbations. Using to the unique large-scale anatomical tract-tracing connectivity datasets (i.e. connectomes) from both the Kennedy/Knoblauch and the Rosa labs for macaque and marmoset respectively, we explored the effect of connectivity on large scale modelling of WM. Although the two

species have different parcellation schemes, they can be made to converge on a common 75-area consensus map that we developed, thus establishing reasonable area-to-area equivalences between these species' connectivities.
Major differences in cortical connectivity are found in the two species. Firstly, connexions from prefrontal to parietal areas, normally present in the macaque, were found to be absent in the marmoset, notably connexions from areas A46D and A8b to area LIP, suggesting that feedback from higher hierarchical levels to parietal cortex is essentially lacking in the marmoset, even though feedforward projections from parietal to prefrontal cortex are similar between species. Secondly, V1 projects more extensively to the prefrontal cortex in the marmoset than in the macaque, with direct projections to area A46D and A8b, meaning that early visual area feedforward connexions may have a stronger impact than in the macaque on high level areas. Taken together, such structural differences and others could form the basis of the increased distractibility of the marmoset species. Large scale modelling of distributed WM is uniquely suited to tackle this question. We therefore applied an identical to both species, the only difference being that of inter-areal connectivity and the related hierarchy. This tests to what extent these connectome differences are sufficient to explain the observed behaviour of these animals and, if not, will offer testable insights to explain such differences.

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Poster

403. Prefrontal Mechanisms of Working Memory: Nonhuman Primate Studies

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Topic: H.05. Working Memory

Support: CIHR
NSERC
NeuroNex
OGS

Title: Intrinsic properties and adaptation in macaque prefrontal cortex: comparing in vitro and in vivo

Authors: *B. CORRIGAN[1], M. FEYERABEND[1], R. A. GULLI[3], M. S. JIMENEZ-SOSA[2], J. K. SUNSTRUM[4], S. MATOVIC[8], M. ROUSSY[1], R. LUNA ALMEIDA[10], S. A. MESTERN[1], B. MAHMOUDIAN[11], S. VIJAYRAGHAVAN[5], H. IGARASHI[9], E. S. KUEBLER[6], K. PRADEEPAN[5], W. J. ASSIS[5], A. PRUSZYNSKI[7], S. TRIPATHY[12], S. TREUE[13], S. EVERLING[14], W. INOUE[1], J. C. MARTINEZ-TRUJILLO[15];
[2]Dr. Julio Martinez-Trujillo Lab., [1]Univ. of Western Ontario, London, ON, Canada; [3]Columbia Univ., New York, NY; [4]Neurosci., [6]Physiol. and Pharmacol. - Robarts Res. Inst., [7]Physiol. and Pharmacol., [5]Western Univ., London, ON, Canada; [8]Neurosciences, [9]Dept. of Physiol. and

Pharmacol., Robarts Res. Inst., London, ON, Canada; [10]Univ. Autonoma de Chihuahua, Chihuahua, Mexico; [11]Univ. of Western, London, ON, Canada; [12]Psychiatry, Univ. of Toronto, York, ON, Canada; [13]Cognitive Neurosci. Lab., German Primate Ctr., Goettingen, Germany; [14]Physiol., Univ. Western Ontario, London, ON, Canada; [15]Dept. of Physiol. and Pharmacol. and Psychiatry, Schulich Sch. of Med. and Dentistry, Robarts Institute, Western Univ., London, ON, Canada

Abstract: While the intrinsic properties of neurons have long been studied using patch clamp recordings, the effects of these properties within neuronal circuits in a behaving animal are not well known. Patch-clamp recordings *in vivo* are heroic endeavours rarely done in primates (Chen & Fetz, 2005; Gao, Kostlan, Wang, & Wang, 2016). In an effort to ascertain whether intrinsic adaptation could be affecting cells in the primate lateral prefrontal cortex, we recorded from 212 cells from macaque LPFC (2M *Macaca mulatta* and 4F and 3M *Macaca fascicularis*) using *in vitro* patch clamp methods, as well as recording from two male macaques with two 96-channel Utah arrays in the same region from which we recorded 325 well isolated cells. In vivo recordings were aligned to target appearance in a guided saccade task and a learning task in virtual reality. We separated cells based on morphology and/or spike width, using 50 spiny cells with mostly broad waveforms, and defining 51 putative parvalbumin interneurons as cells with narrow waveforms. For *in vivo* we only used waveform to separate cells with 24 responsive narrow cells and 206 responsive broad cells. We calculated the adaptation slope using an exponential curve $y = ae^{bx}$ for the *in vitro* sweep with the most spikes, and for the 25 trials with the most spikes in the 500ms following target onset for *in vivo*. We found that there was little overlap between adaptation slopes for spiny and PV neurons *in vitro,* and more overlap between broad cells *in vivo* and spiny *in vitro*. *In vivo* narrow cells had slopes closer to the broad cells than the *in vitro* PV cells. We believe the overlap for putative pyramidal cells indicates that intrinsic adaptation could be contributing to firing patterns of pyramidal cells *in vivo* in the primate LPFC. This could be relevant to modelers, some of whom have already tried implementing intrinsic adaptation in deep learning models (Vinken, Boix, & Kreiman, 2020).

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Poster

403. Prefrontal Mechanisms of Working Memory: Nonhuman Primate Studies

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Topic: H.05. Working Memory

Support: DFG 436260547
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Title: The dynamic gain function: A powerful tool to study in vivo-like neuronal functions across species.

Authors: *S. POMMER[1], R. M. MERINO[2], M. MIETSCH[3,4], R. HINKEL[3,4,5], J. F. STAIGER[1], F. WOLF[2,6,7,8], A. NEEF[2,6,7,8];
[1]Inst. for Neuroanatomy, Univ. Med. Center, Univ. of Göttingen, Göttingen, Germany; [2]MPI for Dynamics and Self-Organization, Göttingen, Germany; [3]German Primate Ctr. (DPZ), Leibniz Inst. for Primate Res., Göttingen, Germany; [4]DZHK (German Ctr. for Cardiovasc. Research), Partner Site Göttingen, Göttingen, Germany; [5]Inst. for Animal Hygiene, Animal Welfare and Farm Animal Behavior, Univ. of Vet. Med., Hannover, Germany; [6]Göttingen Campus Inst. for Dynamics of Biol. Networks, Göttingen, Germany; [7]MPI for Multidisciplinary Sci., Göttingen, Germany; [8]Bernstein Ctr. for Computat. Neurosci., Göttingen, Germany

Abstract: Meaningful physiological neuron characterizations need to be designed with the network function in mind. Conventional in vitro neuron characterizations use regular stimuli, i.e. steps or ramps. The resulting responses are typically used to create neuron models of varying complexity, which are linked up to simulate network activity. This long-established method allows us to link response features (voltage sags, after-polarizations, rebound spikes, etc.) to ion channel types and signaling mechanisms. Although these models optimally respond to constant, artificial stimuli, their utility for network simulations is unclear, because in network models and in vivo, neurons operate in a fluctuation-driven regime. The respective characteristics are best measured using a statistical quantification of responses to in vivo-like stochastic inputs with fluctuating amplitudes and correlation times. Here we used the dynamic gain function (DGF) to characterize neurons in prefrontal cortex slices of mouse and marmoset (*Callithrix jacchus*). The DGF is the linear response function of a neuronal population. It captures the frequency preference of neurons and their ability to tune-in to rhythmic activity in recurrent networks. We combined DGF measurements with conventional characterization. Mouse and marmoset neurons showed a large diversity of neuronal characteristics. However, we found qualitatively and quantitatively similar features across species: 1) The range of frequency that could be faithfully encoded was similar, with cut-off frequencies around 400 Hz. High-bandwidth, ultrafast encoding seems to be a general feature and not exclusive to primates. 2) Adapting neurons shifted their frequency preference when we changed the input statistics, mimicking shifts in brain state. 3) Decomposing DFG into sub- and supra-threshold components we found that the drop in low-frequency sensitivity is likely caused by sub-threshold activation of potassium channels. In contrast, the increase in high-frequency sensitivity stems from peri- and supra-threshold activations. Therefore, our recent finding (Merino et al. 2021) holds for marmoset: spike-rate adapting interneurons are tunable to gamma and ripple frequencies, when the population dynamics slows down. This demonstrates that DGF measurements, can greatly contribute to identify the mechanisms of prefrontal cortex information routing in marmosets. In conclusion, DGF measurements, offer a neuronal characterization with fluctuating, in vivo-like input. Furthermore, our novel DGF decomposition identifies the biophysical mechanisms that shape the DGF properties.

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Poster

403. Prefrontal Mechanisms of Working Memory: Nonhuman Primate Studies

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Topic: H.05. Working Memory

Support: CIHR
NSERC
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NSF
CONACYT

Title: Electrophysiological and morphological characterization of non-human primate fast spiking neurons across cortical areas

Authors: *M. S. JIMENEZ-SOSA[1], M. FEYERABEND[1], E. S. KUEBLER[1], J. K. SUNSTRUM[1], S. A. MESTERN[1], S. MATOVIC[1], M. WIEDERMAN[1], P. TRUSCHOW[2], B. W. CORRIGAN[1], M. ROUSSY[1], B. MAHMOUDIAN[1], D. F. BUITRAGO-PIZA[1], S. TREUE[4], J. F. STAIGER[3], S. EVERLING[1], W. INOUE[1], J. C. MARTINEZ-TRUJILLO[1];
[1]Dept. of Physiol. and Pharmacology, Schulich Sch. of Med., Univ. of Western Ontario, London, ON, Canada; [2]Dept. of Neuroanatomy, Ctr. of Anat., Georg-August-Universität Göttingen, Goettingen, Germany; [3]Dept. of Neuroanatomy, Ctr. of Anat., Georg-August-Universität Göttingen, Göttingen, Germany; [4]Cognitive Neurosci. Lab., German Primate Ctr., Goettingen, Germany

Abstract: Parvalbumin (PV) fast spiking (FS) cells in the primate dorsolateral prefrontal cortex (DLPFC) have been implicated in higher cognitive functions and shown to play a role in many psychiatric disorders. For one, a decrease in PV cell numbers or activity has been associated with schizophrenia. On the other hand, PV cell activity is also associated with gamma activity which correlates with cognitive phenomena such as working memory. Compared to rodents, the primate cortex shows a much stronger differentiation across cortical areas with the DLPFC being a prominent example of innovation in cytoarchitecture. Consequently, the question arises if FS cells of the primate DLPFC have been subjected to new evolutionary pressure leading to changes in their intrinsic properties outside of their hallmarked waveform. In this study, we have developed an experimental pipeline combining whole cell patch-clamp recordings with biocytin labeling and three-dimensional reconstruction to compare subthreshold properties and morphology of putative cortical FS (pFS) cells across species as well as anatomical areas PFC and V1. Understanding how biophysical features of FS neurons varied across anatomical areas and species will help us to gain insight into how demands of different cortical circuits shape basic integrative properties like excitability. We found significant differences in rheobase, time constant and sag ratio across all species. Input resistance and resting membrane potential was significantly higher in macaque (especially in PFC vs V1). Interestingly, most parameters did not follow the expected gradient of mouse-macaque-human. NHP pFS cells showed on average

highest values. However, macaque and human neurons showed similar tendencies compared to mouse. To further clarify these differences we performed a 2-way ANOVA with species and cell types (FS/Spiny-Exc) as factors. All measurments showed a signifficant interaction effect, and surprisingly, classic features of the FS cell known from rodents (low input resistance and time constant, high rheobase) were not present in primates. Our results suggest that the expansion of the cortical surface in macaques and humans relative to mouse is accompanied by changes in the intrinsic electrophysiological and somatodendritic configuration of FS interneurons increasing the input-output gain to accommodate the increased need of the network.

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Poster

403. Prefrontal Mechanisms of Working Memory: Nonhuman Primate Studies

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Topic: H.05. Working Memory

Support: American Federation for Aging Research (AFAR)
R01 AG061190-01

Title: Signature of vulnerability for Alzheimer's disease in rhesus macaque entorhinal cortex layer II

Authors: *I. J. PERONE[1,2], J. I. ARELLANO[1], Y. M. MOROZOV[3], A. F. T. ARNSTEN[4,1], D. DATTA[1];
[1]Neurosci., [2]Interdepartmental Neurosci. Program, Yale Univ., New Haven, CT; [3]Dept Neurobiol, Yale Univ. Sch. Med., New Haven, CT; [4]Dept. Neurosci., Yale Med. Sch., New Haven, CT

Abstract: Tau pathology emerges in a distinct spatial and temporal pattern in Alzheimer's Disease (AD). Anatomical studies in AD subjects and rhesus macaques show earliest signs of tau pathology in the stellate cell islands in entorhinal cortex (ERC) layer II. However, the molecular mechanisms that confer vulnerability to ERC layer II cells early in AD is unknown. Our previous research in aging monkeys has shown that neurons vulnerable to tau pathology have the molecular machinery to magnify calcium signaling near the synapse, with calcium dysregulation associated with the emergence of tau pathology. In macaque dlPFC, these vulnerable synapses express nearby phosphodiesterases (e.g. PDE4D) and HCN channels, allowing dynamic changes in synaptic strength needed for higher cognition, but conferring vulnerability when regulation is lost with age (Arnsten et al. *Mol Psychiatry* 26: 3684-3700, 2021). Although ERC grid cells in

rodent are known to show similar dynamic changes with altered cAMP-HCN channel signaling (Giocomo et al. *Cell* 147: 1159-70, 2011), it is not known if layer II ERC in primate also shows this molecular "signature of flexibility" that may become a "signature of vulnerability" with advancing age. The current study examined these molecular mechanisms that confer risk of AD pathology in layer II ERC of macaque, where perfusion fixation allows preservation of membranes and phosphorylation state. High-spatial resolution immunoEM found that PDE4D and HCN1 were primarily observed in postsynaptic compartments in macaque ERC layer II, similar to their subcellular locations in dlPFC. In dendritic spines, PDE4D was concentrated on the SER spine apparatus and in postsynaptic density, and HCN1 expressed in the membrane near excitatory synapses. Within dendritic shafts, PDE4D labeling was observed along microtubules and near mitochondria, whereas HCN1 was organized in discrete clusters along the plasma membrane. These data suggest that PDE4D is optimally positioned to modulate cAMP microdomains and control calcium extrusion from the SER. HCN1 channels are localized in subcompartments to facilitate dynamic physiological representation of sensory experience and visual space governed by cAMP-PKA signaling. The anatomical patterns in ERC layer II corroborate our findings in vulnerable glutamatergic circuits in prefrontal cortex, suggesting conserved molecular features in association cortices most susceptible in AD. Ongoing multi-label immunofluorescence experiments are evaluating the emergence of calpain-2 and phosphorylated and hyperphosphorylated tau residues in aging macaque ERC layer II.

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Poster

403. Prefrontal Mechanisms of Working Memory: Nonhuman Primate Studies

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NSF Grant Neuronex 2015276
NSF Grant DMS 1951099

Title: Mechanisms regulating the frequency of inhibition-based gamma oscillations in primate prefrontal and parietal cortices

Authors: *G. GONZALEZ-BURGOS[1], T. MIYAMAE[1], N. REDDY[1], S. DAWKINS[1], C. CHEN[2], A. HILL[3], J. F. ENWRIGHT[1], G. ERMENTROUT[1], D. A. LEWIS[1];
[1]Univ. of Pittsburgh, Pittsburgh, PA; [2]Carnegie Mellon Univ., Pittsburgh, PA; [3]Wheaton Col., Wheaton, IL

Abstract: In primates, the dorsolateral prefrontal (DLPFC) and posterior parietal (PPC) cortices are critical nodes in the network mediating cognitive functions including attention and working

memory. During working memory tasks, gamma oscillations, usually prominent in layer 3 (L3), are induced in both DLPFC and PPC but, notably, exhibit higher frequency in the DLPFC. These oscillation frequency differences might be crucial for working memory function, but the mechanisms producing different oscillation frequencies in monkey DLPFC and PPC remain poorly understood. To investigate the basis of the DLPFC-PPC differences in oscillation frequency, using patch-clamp recordings in acute slices we studied GABAAR-mediated inhibition, which plays a crucial role in gamma oscillation production, in L3 pyramidal neurons (L3 PNs) from the rhesus monkey DLPFC or PPC. Recordings of GABAAR-mediated synaptic currents from L3 PNs, while suggesting a contribution to network synchronization in both areas, revealed no DLPFC-PPC differences in the strength or kinetics of GABAAR-mediated inhibition. Likewise, the expression of GABAAR genes in L3 PNs did not differ between regions. In the absence of differences in inhibition, DLPFC L3 PNs showed greater dendritic spine density and higher expression of AMPAR and NMDAR subunit genes relative to PPC L3 PNs, suggesting that the excitatory synaptic drive onto L3 PNs could be stronger in the DLPFC. Simulations in computational models of the cortical microcircuit showed that, with constant synaptic inhibition, increasing the strength of recurrent excitatory synaptic drive increased the network oscillation frequency. Hence, the DLPFC-PPC differences in gamma oscillation frequency could depend on stronger recurrent excitation in the DLPFC relative to PPC. Our data suggest that the mechanisms of GABAAR-mediated inhibition are highly conserved between DLPFC and PPC but that stronger drive from local recurrent excitation may produce inhibition-based rhythms of higher frequency in the DLPFC network.

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Poster

404. Social Cognition and Behavior: Mechanisms and Models

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Program #/Poster #: 404.01

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH ES100221

Title: Hippocampal area CA2 activity supports resilience following acute social defeat stress

Authors: *D. RADZICKI[1], K. E. MCCANN[2], S. M. DUDEK[3];
[1]NIEHS/NIH, Durham, NC; [2]Human Genet., Emory Univ. Sch. of Med., Atlanta, GA;
[3]Neurobio. Lab., Natl. Inst. of Env. Hlth. Sci., NIH, Research Triangle Park, NC

Abstract: In mice, only a subset of individuals will go on to develop lasting avoidance behavior following an exposure to an environmental stressor. For example, exposure to an aggressive mouse results in about half of tested mice developing a socially avoidant phenotype while a more

resilient population exhibit no overt effects of the experience. The hippocampus is critical for memory acquisition and retrieval and influences an animal's response to stress. Moreover, neurons in area CA2 are required for social recognition memory and aggression. Given the high concentration of mineralocorticoid receptors in area CA2, this region is also positioned to play an important role in the greater hippocampal stress response. To further interrogate the effects of stress on CA2 dependent behaviors, we chemogenetically manipulated neuronal activity *in vivo* during an acute, socially derived stressor and tested whether memory for the defeat was influenced. Using the acute social defeat (ASD) paradigm, wildtype males underwent a five-minute bout of social defeat in the home cage of a large male CD1 aggressor. 24 hours after the defeat, we observed that defeated mice significantly less time investigating a novel CD1 mouse when compared to control mice that had not been exposed to the aggressor mice (50.3 +/-10.5 vs. 99.4 +/-5.1 s, n=22,18, p=0.0001). This avoidant phenotype persisted for up to one month following a single defeat: mice exhibited either a susceptible/avoidant or a resilient/investigative behavioral phenotype. When CA2 activity was inhibited using Gi-DREADD during the defeat, however, subject mice exhibited a significantly higher amount of social avoidance one day later when compared to defeated littermates not expressing DREADDs (241.6 +/-12.7 vs. 188.6 +/-16.4 s, n=18,18, p=0.02). This increased avoidance was driven by a loss of the 'resilient' population of defeated mice. CA2 inhibition during defeat also resulted in changes in neuronal activity following social investigation, as measured by staining for the immediate early gene (IEG) *cfos*, both within the hippocampal subfields and in downstream corticolimbic regions. Lastly, given CA2's known role in aggression, we asked whether inhibition of CA2 also modulated the behavior of subjects during the defeat itself. We observed a significant reduction in the amount of defensive submission during defeat when CA2 was inhibited (24.5 +/-5.1 vs. 44.1 +/-6.3 s, n=18,18, p=0.02). Taken together these results indicate that CA2 neuronal activity supports resilience following an acute social stressor and that submissive defensive behavior during the defeat (vs. fleeing) is a predictor of future resilience to social stress.

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Poster

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Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH ES100221

Title: Defining hippocampal area CA2 in the fox brain

Authors: A. N. PHOENIX[1], D. V. SHEPELEVA[2], Y. E. HERBECK[2], L. N. TRUT[2], S. FARRIS[3], A. V. KUKEKOVA[4], *S. M. DUDEK[1];
[1]Natl. Inst. of Env. Hlth. Sci., NIH, Research Triangle Park, NC; [2]Siberian Br. of the Russian Acad. of Sci., Inst. of Cytology and Genetics,, Novosibirsk, Russian Federation; [3]Fralin Biomed.

Res. Inst., Virginia Tech., Roanoke, VA; [4]Dept. of Animal Sci., The Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract: Since 1959, the Russian Farm-Fox study has bred foxes to be either tame or aggressive, and neuroscientists have studied them to gain insight into the brain structures associated with aggression (Hecht 2021). In mouse, hippocampal area CA2 has emerged as one of the essential regulators of social aggression (Pagani 2015; Leroy 2018). To determine whether we could identify differences in CA2 between tame and aggressive foxes, we first sought to identify CA2, as no clearly defined area of CA2 has been described in species such as cats, dogs, or pigs. In cats, for example, mossy fiber projections were found to extend right up to the area of small pyramidal cells (CA1), suggesting that felines have no separate CA2 (Laurberg 1980; Hirama 1997). In the case of dogs, one study was able to show a 'transition zone' between CA1 and CA3, but described it as a mixing of the two cell types (Amayasu 1999). Thus, it was not at all clear whether CA2 could be identified in foxes. In this study, we used temporal lobes from both male and female red foxes (*Vulpes vulpes*), cut perpendicular to the long axis of the hippocampus, and sectioned at dorsal, middle, and ventral levels. To determine if markers previously shown to label pyramidal cells in CA2 of rats and mice could also be used to identify CA2 in foxes, we used antibodies against Purkinje cell protein 4 (PCP4), regulator of G protein signaling 14 (RGS14), and striatal-enriched phosphatase (STEP). As perineuronal nets (PNNs) are also enriched in CA2 in mice, we also stained PNNs with Wisteria Floribunda Agglutinin (WFA). Antibodies against calbindin-1 were used to visualize mossy fiber axons. As in cats and dogs, we found mossy fibers to reach all the way around the curve of the pyramidal cell layer, well into the regio superior. Nevertheless, we observed PCP4 staining in the area spanning the end of the mossy fiber staining and the beginning of the pyramidal cells lacking mossy fibers, like in mice and rats. Although some isolated PCP4+ cells were seen in CA1 like in mice, this pattern of concentrated staining around the distal mossy fibers likely represents area CA2 in the fox. Anti-STEP antibodies displayed a similar staining, whereas anti-RGS14 antibodies failed to stain any cells. WFA clearly labeled some interneurons and stained neuropil that was excluded from the striatum lucidum, however staining in CA2 was inconsistent. Our findings suggest that foxes have a molecularly defined CA2, and suggest that other carnivores like dogs and cats might as well. With this being the case, foxes could be useful to study CA2 as it relates to aggression.

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Poster

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Title: Hardwired to attack: developmentally distinct cellular subpopulations for innate social behaviors in the medial amygdala.

Authors: J. E. LISCHINSKY[1], L. YIN[1], C. SHI[2], N. PRAKASH[3], J. BURKE[4], G. SHEKARAN[4], M. GRBA[4], J. G. CORBIN[3], D. LIN[1];
[1]Neurosci. Inst., New York Univ. Sch. of Med., New York, NY; [2]Hunter Col., New York, NY; [3]Ctr. for Neurosci. Res., Children's Natl. Hosp., Washington, DC; [4]New York Univ., New York, NY

Abstract: Innate social behaviors are crucial for survival, thus shared across animal species. In humans, psychiatric disorders with deficits in social interactions, e.g. autism spectrum disorders, can be observed during child development and have been associated with amygdala dysfunction. There is still a lack of understanding of the circuitry and developmental mechanisms for the generation of social behaviors. We have focused on the murine medial amygdala (MeA) as it is sufficient for the production of social behaviors including aggression and mating. Given that these diverse social behaviors differ in their sensory trigger and behavioral outcomes, can the neuronal substrates for these behaviors be distinct? Taking a developmental approach, we have previously characterized two MeA GABAergic neuronal subpopulations, marked by the expression of the transcription factors Foxp2 and Dbx1 which both originate from the embryonic preoptic area. The Foxp2+ and *Dbx1*-derived subpopulations are spatially, molecularly and physiologically distinct. Interestingly, we have now observed that these two subpopulations differ in their *in vivo* processing of social conspecific information. The MeA Foxp2+ cells are uniquely processing male sensory cues and are functionally relevant for aggression, while *Dbx1*-derived cells respond broadly to social cues. To determine the extent to which these neuronal responses are hard-wired, we investigated the social tuning of Foxp2+ cells across development by recording the neuronal activity to social cues in freely-moving juvenile mice at multiple postnatal days, providing a developmental understanding on how neuronal responses are established. Finally, we uncovered through channelrhodopsin-assisted circuit mapping that these subpopulations possess distinct functional inputs from the olfactory bulb, which may be leading to the differences seen in sensory processing. In conclusion, developmentally distinct MeA neuronal subpopulations differ in their circuitry, are differentially relevant for processing conspecific sensory cues and mediating social behaviors.

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Poster

404. Social Cognition and Behavior: Mechanisms and Models

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Topic: F.02. Neuroendrocrine Processes and Behavior

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Title: Expanding machine-learning classifiers of agonistic behaviors in mice

Authors: *A. RODRIGUES, Z. C. NORVILLE, A. OSTERMAN, A. SHANK, G. TOUPONSE, R. C. MALENKA, N. ESHEL;
Stanford Univ., Stanford, CA

Abstract: Most aggression research in animals relies on the resident-intruder paradigm, in which a strong resident mouse is pit against a weaker intruder, giving the resident both physical and environmental advantages. Such pairings provide a limited view of agonistic interactions, as recorded behavior is often unidirectional, with only the resident initiating. Here we aimed to expand the repertoire of animal aggression models by pitting the resident mouse against a stronger intruder, in this way splitting the physical and environmental advantages: the intruder has the physical advantage while the resident has the environmental advantage. We used Simple Behavioral Analysis (SimBA), a recently-developed machine-learning algorithm, to build a model that classifies, for the first time, behaviors occurring within strong intruder/weak resident footage. The strong intruders were sexually experienced CD-1 males; weak residents were smaller, sexually naive adult CD-1/C57 hybrid males. Mice encountered each other for 5-10 minutes while an overhead camera captured their behavior at 20 frames per second. The model's training data contained 92,750 video frames, which we manually annotated as having up to 13 behaviors of interest, including 6 resident-initiated and 7 intruder-initiated behaviors. Data were derived from 10 videos, showcasing 18 mice. We then tested the model against 3 naive videos (37,260 frames total) and calculated the sensitivity and specificity of classification. The model achieved a sensitivity range of 0.65-0.8 for different behaviors and a specificity of greater than 0.95. In typical sessions, >50% of frames contained classified behavior, including 3.6% IntruderAggression (range 0-10%), 24% IntruderInteraction (range 15-55%), 4.7% IntruderDefense (range 0-10%), 3.8% ResidentAggression (range 0-10%), 21.6% ResidentInteraction (range 10-40%), and 6.7% ResidentDefense (range 0-10%). The model's focus on both resident- and intruder-initiated behaviors also enabled the classification of simultaneous behaviors. Identified concurrence patterns include: IntruderAggression with ResidentDefense, IntruderDefense with ResidentAggression, IntruderInteraction with ResidentInteraction, and less commonly, IntruderAggression and ResidentAggression. We succeeded in expanding machine learning algorithms to classify behavior during strong

intruder/weak resident interactions with high specificity. Future studies involve pairing the behavior classifications with simultaneous neural recordings to discern the circuit underpinnings of a greater array of agonistic behaviors.

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Poster

404. Social Cognition and Behavior: Mechanisms and Models

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Topic: F.02. Neuroendrocrine Processes and Behavior

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Title: Dorsal raphe serotonin neurons inhibit reactive aggression in mice.

Authors: *Z. C. NORVILLE, A. SHANK, A. OSTERMAN, B. S. BENTZLEY, R. C. MALENKA, N. ESHEL;
Stanford Univ., Stanford, CA

Abstract: Serotonin (5-HT) has long been thought to modulate aggression, though few studies have recorded from serotonin neurons or manipulated their activity during aggressive behavior. In this work, we leveraged genetically targeted optical tools and unbiased behavioral decoders to characterize the function of dorsal raphe (DR) serotonin neuron activity in aggression. To test serotonin's causal role in aggression, we injected 13 male SERT-Cre x CD-1 hybrid mice with either *DIO*-ChRmine (an excitatory opsin; n = 7) or *DIO*-mScarlet (an inert fluorescent protein; n = 6) and *DIO*-GCaMP6f in DR serotonin neurons. After recovery from surgery, we introduced mice to weak male intruders in 10-minute daily sessions over 10 days. During each resident-intruder session, we optogenetically excited serotonin neurons in alternating 30-second epochs. Behavior was extracted from videos using supervised algorithmic decoding. Stimulation of DR serotonin neurons reduced algorithm-scored aggression in ChRmine-injected mice compared to mScarlet controls (2-way ANOVA, interaction of experimental group vs. stimulation period, $p < 0.05$), with no significant impact on other social behaviors. To record serotonin dynamics during

aggression, we injected 5 additional male mice with $GRAB_{5\text{-}HT1.0}$ (a fluorescent serotonin sensor) and used fiber photometry to measure serotonin release in the DR during aggressive bouts. When animals were recorded in the absence of photo-stimulation, serotonergic cell body activity increased ($p < 0.001$) and local DR serotonin release decreased ($p < 0.001$) immediately prior to aggression onset. Signal displacements persisted throughout each aggressive bout and returned to baselines upon the bout's completion. The mechanisms underlying the surprising discrepancy between calcium and serotonin recordings are currently being explored. Together, these findings inform an updated model of serotonin-aggression dynamics, implicate candidate target areas for further investigation, and support prior work showing that DR serotonin release enhances prosocial behavior.

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Poster

404. Social Cognition and Behavior: Mechanisms and Models

Location: SDCC Halls B-H

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Program #/Poster #: 404.06

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: Subcutaneous Mycobacterium vaccae modulates anxiety-like behavior and hippocampal gene expression but not gut microbiome in a high-fat diet-induced obesity rat model

Authors: *S. I. S. R. NORONHA[1], L. A. G. MORAES[2], J. E. HASSELL JR[3], C. E. STAMPER[3], J. D. HEINZE[3], A. I. ELSAYED[3], M. FRANK[4], F. C. S. SILVA[1], D. A. CHIANCA JR[1], R. C. A. DE MENEZES[1], C. A. LOWRY[3];
[1]Dept. of Biol. Sci., [2]Dept. of Computing Sci., Federal Univ. of Ouro Preto, Ouro Preto, Brazil; [3]Integrative Physiol. and Ctr. for Neurosci., [4]Psychology and Neurosci., Univ. of Colorado at Boulder, Boulder, CO

Abstract: High-fat diet (HFD) induced-obesity condition has been connected to the development of several chronic health conditions, including gut dysbiosis, chronic low-grade inflammation, and stress-related psychiatric disorders such as anxiety disorders. Immunization using heat-killed bacterium *Mycobacterium vaccae*, a bacterium with anti-inflammatory, immunoregulatory, and stress resilience properties, has a protective action against stress-induced intestinal disease and anxiety. We hypothesized that treatment with *M. vaccae* would prevent HFD-induced hippocampal neuroinflammation and anxiety-like defensive behavioral responses. To test the hypothesis, we exposed rats to a 9-week HFD-induced obesity protocol (45% fat; kcal/g) or a control diet (CD; 11% fat; kcal/g). During the diet protocol, we injected *Mycobacterium vaccae*

(100 µg/0.1 ml s.c.) or vehicle once a week resulting in HFD, HFD treated with *M. vaccae* (HFDmv), CD and CD treated with *M. vaccae* (CDmv) groups. Effects on the gut microbiome were assessed using 16S rRNA gene sequencing while effects on hippocampal neuroinflammation were assessed using RT-qPCR and anxiety-related defensive behavioral responses were assessed using the elevated T-maze (ETM). We found that *M. vaccae* treatment did not impact overall alpha diversity of the gut microbiome. In terms of beta diversity, we found diet had an effect ($p < 0.0001$), but *M. vaccae* did not affect the diet-induced changes. Despite the absence of *M. vaccae* effects on the gut microbiome, we found that this bacterium restored/modulated the relative hippocampal expression of genes encoding the IL-4 receptor (HFD mean ± SD: 2.20 ± 0.57; HFDmv mean:1.54 ± 0.42; $p = 0.0021$); Nlrp3 (HFD mean: 2.11 ± 0.42; HFDmv mean: 1.57 ± 0.28; $p = 0.0013$); Gfap (HFD mean: 1.97 ± 0.67; HFDmv mean: 1.49 ± 0.25; $p = 0.0115$), Hmgb1 (HFD mean: 1.93 ± 0.70; HFDmv mean: 1.42 ± 0.26; $p = 0.0063$), and Ahr (CDmv mean: 2.07 ± 0.58; HFDmv 1.68 ± 0.40; $p = 0.0191$). Finally, HFD-fed rats presented with increased anxiety-related defensive behavioral responses when tested in the ETM, taking longer to leave the enclosed arms on avoidance 1 compared to CD (CD mean: 56.8 ± 26.5; HFD mean: 152.2 ± 70.2; $p = 0.0010$). The *M. vaccae* treatment decreased the latency in avoidance 2 trial in the HFD group (HFD mean 215.8 ± 87.3; HFDmv mean: 127.1 ± 90.3; $p = 0.0298$), suggesting an anxiolytic effect. These data show that a HFD altered the gut microbiome, increased biological signatures of neuroinflammation, and increased anxiety-like defensive behavioral responses. These changes were partially reversed by the treatment with *M. vaccae.* Our results give new insights over the immunomodulatory action of *M. vaccae*.

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Poster

404. Social Cognition and Behavior: Mechanisms and Models

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Title: Behavioral and neural correlates of flexible social rank state

Authors: *D. BLACKMAN[1], O. TIMMERMANS[1], S. OLINE[1], A. P. FINK[2], C. E. SCHOONOVER[2], A. L. FALKNER[1];
[1]Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ; [2]Neurosci., Columbia Univ., New York, NY

Abstract: Across social species, groups of individuals frequently exhibit hierarchical social organization. High rank animals use a well-characterized suite of social behaviors and chemosensory cues to assert their status, thus gaining preferential access to limited resources. Though hierarchies are typically stable, social rank is not fixed: an individual can rise or fall in the hierarchy depending on changing social and environmental circumstances. Therefore, we define rank as a flexible state that patterns broad sets of actions in response to social stimuli, and hypothesize that changes in rank can re-pattern these behavioral responses. However, it is unclear where in the brain maintains a flexible representation of rank, or how this representation is updated following a change in rank. Here, we use a novel assay that allows precise millisecond-level monitoring of behavioral engagement, aversion, and arousal to chemosensory social stimuli to show that social rank predicts behavior: low-ranking males avoid these cues significantly more than high-ranking males. Notably, the magnitude of the behavioral difference between ranks is highest in response to the chemosensory (urine) cues of highly-aggressive males. Further, we show that experience flexibly changes behavior in this assay: we manipulate individuals' positions in their hierarchies and show that they update their behavioral responses after this rank change. Administration of a testosterone "pulse" similarly induces changes in rank-related behaviors. The ventral premammillary nucleus (PMv), a hypothalamic node in the social behavior network that richly expresses androgen receptors (ARs), has previously been implicated in both male conspecific aggression and sensory processing, making it well-poised to integrate incoming sensory information and influence rank-specific behaviors (Stagourakis et al. 2018; Chen et al. 2020). To test whether the PMv encodes a flexible representation of social rank, we performed chronic in vivo electrophysiology using multi-shank silicon probes to track single units in the PMv across time, as animals underwent either a change in social rank or hormone state. We find a heterogenous population of PMv neurons whose response properties vary both as a function of chemosensory cues and hormone state. In addition, we find that genetic deletion of AR in the PMv during adulthood or chemogenetic suppression of a subpopulation of PMv neurons promotes a range of "subordinate-like" behaviors. Taken together, these findings suggest a role for an androgenic mechanism in the PMv for mediating flexibility and stability of social rank.

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Poster

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Topic: F.02. Neuroendrocrine Processes and Behavior

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Title: An essential role of oxytocin receptors in the ventromedial hypothalamus in defeat-induced social avoidance in female mice

Authors: *Y. JIANG, T. OSAKADA, R. YAN, R. TABUCHI, D. LIN;
New York Univ. Neurosci. & Physiol., New York, NY

Abstract: It is crucial for animals to stay away from disadvantageous conflicts with superior opponents due to the potential severe consequence of defeat. Although naïve animals are naturally attracted to conspecifics, they learn to avoid the winner mouse after a single defeat. We established a behavior paradigm using lactating mothers as aggressors to investigate the neural mechanisms underlying defeat-induced social avoidance in female mice. Similar to males, female mice learn to avoid the aggressor after a single defeat. We then examined defeat-induced c-Fos expression pattern and found increased c-Fos in the anterior ventrolateral part of the ventromedial hypothalamus (aVMHvl), a region essential for conspecific defense. Furthermore, we found that the defeat-induced c-Fos in the aVMHvl overlaps well with the oxytocin receptor (OXTR). Interestingly, aVMHvl OXTR expressing cells show a clear change in response to the aggressor during social interaction post-defeat based on in vivo Ca^{2+} recordings. Specifically, the aVMHvl OXTR expressing cells increased activity during aggressor investigation after defeat while behaviorally, animals showed robust social avoidance. Knockout of OXTRs in aVMHvl reduced social avoidance after defeat. These results suggest an important role of oxytocin signaling in the aVMHvl in defeat-induced social avoidance in female mice.

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Poster

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Title: Seasonality in the estrogenic modulation of agonistic behavior in teleost wild-caught females

Authors: *G. VALIÑO[1], C. JALABERT[3,4], L. ZUBIZARRETA[1,6], J. FARIAS[2], J. SOTELO-SILVEIRA[2,7], K. K. SOMA[5,3,4], L. QUINTANA[1];
[1]Lab. de Neurofisiología Celular y Mol., [2]Dept. de Genómica, Inst. de Investigaciones Biológicas

Clemente Estable, Montevideo, Uruguay; [3]Zoology, [4]Djavad Mowafaghian Ctr. for Brain Hlth., [5]Psychology, Univ. of British Columbia, Vancouver, BC, Canada; [6]Dept. de Fisiología, Facultad de Medicina, Montevideo, Uruguay; [7]Dept. de Biología Celular y Mol., Facultad de Ciencias, Univ. de la República, Montevideo, Uruguay

Abstract: Agonistic behavior is an adaptive social behavior that emerges from the competition among conspecifics for limited resources. It has been shown in many species that agonistic behavior is strongly modulated by estrogens. Nevertheless these experiments were conducted mainly in breeding males. To fully understand the hormonal modulation of the agonistic behavior is necessary to study animal models which express this behavior in other conditions. In this sense, *Gymnotus omarorum*, a weakly electric fish from South America, emerges as an excellent model. *G. omarorum* expresses year-round agonist encounters in both males and females. The expression of its non-breeding agonistic behavior is independent of gonadal hormones, as castrated individuals express this behavior. Nevertheless it depends on rapid estrogen synthesis as the inhibition of the aromatase (enzyme that synthesis estrogens) abolish this behavior during the non-breeding season. Our aim was to fully characterize this female year-round aggression and analyze potential seasonality in its underlying estrogenic mechanisms. The rapid inhibition of aromatase (Fadrozole 20ug/g) in intrasexual female dyads (n=12 and n=11 controls) had no effect in any locomotors nor electric variables during the breeding season, in contrast to the reported effect during the non-breeding season. Circulating hormones were quantified from wild-caught fish (n=11 non-breeding and n=15 breeding females) by LC-MS/MS. Sexual steroids showed a seasonal difference in both androgens and estrogens. Estrogens were only present during the breeding season, while androgens were higher during the non-breeding season. From the same individuals that hormones where measured, gene expression of aromatase, estrogen receptor alpha and estrogen receptor beta was quantified by qRT-PCR in 2 areas of the social brain network (the pre-optic area and the lateral septum). There were no seasonal differences in the expression of these genes in neither area. In sum, our results show that *G. omarorum* year-round agonistic behavior depends on different seasonal mechanisms in females. During the non-breeding seasons, it depends on rapid modulatory effects of neuroestrogens possible synthesized from circulating androgens. While during the breeding season it might depend on long-term effects from gonadal estrogens. This seems to be a shared strategy with birds and mammals.

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Poster

404. Social Cognition and Behavior: Mechanisms and Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 404.10

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: Postpartum resource scarcity alters the nature of maternal defensive behavior in rats

Authors: *S. KU[1], A. CUARENTA[1], M. DUPUIS[1], J. FLOWERS[2], R. KARBALAEI[3], C. ARDEKANI[1], M. E. WIMMER[1], D. A. BANGASSER[4];
[1]Psychology, Temple Univ., Philadelphia, PA; [2]Temple Univ., philadelphia, PA; [4]Dept. of Psychology and Neurosci., [3]Temple Univ., Philadelphia, PA

Abstract: Postpartum affective disorders, such as postpartum depression and anxiety disorders, are debilitating diseases with limited treatments. Two extremely high risk factors for postpartum affective disorders are postnatal stress and low socioeconomic status (SES). A low SES environment can be mimicked using a limited bedding and nesting (LBN) manipulation, in which the dam's access to nesting materials is restricted during the beginning of the postpartum period. Furthermore, the dam and pups reside on a metal grate, which induces additional postnatal, environmental stress. Previously, we found that the LBN manipulation increases dams' pup-directed behavior (passive nursing, blanket nursing, arched back nursing, licking, and grooming of pups) and decreases self-care (self-grooming, eating, and drinking), a phenotype which likely reflects hyperarouasal. Conversely, dams exposed to extremely enriched postnatal environments display very little time on the nest and overall decreased frequency of nursing. Given this clear, demonstrable effect of resource availability on maternal care and postpartum affective behavior, we sought to leverage our LBN model to understand how both postnatal chronic stress and resource scarcity affect postpartum affective behavior. On postnatal day (PND) 2, Long Evans dams (60-100 days old) were placed in either standard housing (able bedding, 2 nestlets, and 1 enrichment, n = 11) or LBN (n = 9) housing conditions. On PND10, we ran dams through a resident/intruder task to elicit aggression, where a late adolescent (50-75 days old) male intruder rat was placed in the dams' home cage for 15 minutes. Each resident/intruder interaction was recorded with a GoPro, then hand scored using SolomanCoder for various forms of aggression. Though data analysis is still ongoing, our preliminary findings indicate that LBN does not alter the frequency or likelihood of aggressing. Rather, LBN appears to affect the nature of aggression: LBN dams pin the intruder significantly less than control dams and, if at all, for significantly shorter durations. This pattern of aggression in LBN dams is consistent with a more defensive type of aggression versus a more offensive type displayed by control dams. We are currently conducting RNA sequencing on the medial amygdala (MeA) in control and LBN dams to ascertain how resource scarcity alters transcription in this key region for aggression. Together, these studies will reveal mechanisms by which resource scarcity alters maternal defensive behavior. Clinically, this work may reveal novel targets for treating postpartum anxiety disorders, particularly symptoms regarding overprotective or anxious parenting.

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Poster

404. Social Cognition and Behavior: Mechanisms and Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 404.11

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: R01MH113007

Title: Role of BNST-CRF neurons in defensive behaviors: effect of stress, sex, and estrous cycle

Authors: *R. CHUDOBA[1], V. OLIVERA[3], S. OLSON[4], W. FRANCESCONI[2], F. BERTON[2], J. A. DABROWSKA[5];
[2]Dept. of Cell. and Mol. Pharmacol., [1]Rosalind Franklin Univ. of Med. and Scien, North Chicago, IL; [3]Cell. and Mol. Pharmacol., Rosalind Franklin Univ. Sch. of Grad. and Postdoctoral Studies, North Chicago, IL; [4]Cell. and Mol. Pharmacol., Rosalind Franklin Univ., North Chicago, IL; [5]Chicago Med. Sch. RFUMS, North Chicago, IL

Abstract: Corticotropin-releasing factor (CRF) is a stress-related neuropeptide that is produced in the dorsolateral bed nucleus of the stria terminalis ($BNST_{dl}$), a brain region that mediates behavioral responses to stressors. $BNST_{dl}$ neurons can be classified into three types (Type I-III) based on their electrophysiological properties. To investigate the properties of $BNST_{dl}$-CRF neurons, we used a CRF-Cre transgenic rat model, in which Cre-recombinase is expressed from the CRF promoter, to specifically tag these neurons for electrophysiological recordings. We found that, in response to hyperpolarizing and depolarizing current pulses, all CRF neurons in males (n=32) and females (n=5) have a hyperpolarized resting membrane potential relative to Type I and II neurons, delayed first spike, and prominent inward rectification, classifying them as Type III neurons of the $BNST_{dl}$. To understand how this neuron population is involved in modulating anxiety-like behaviors, we used Cre-dependent designer receptors exclusively activated by designer drugs (DREADDs) to silence $BNST_{dl}$-CRF neurons prior to measuring elevated plus maze (EPM) exploration, predator odor-induced freezing, and shock-induced acoustic startle sensitization (SS) in male and female rats. Inhibition of $BNST_{dl}$-CRF neurons did not affect time in EPM open arms or time spent freezing to predator odor for males or females. However, control males spent less time in the open arms ($p=0.04$, unpaired t-test) and more time freezing to the predator odor ($p=0.006$) compared to control females. For SS, we measured acoustic startle responses before and after 10-footshocks and found a significant effect of shock on startle in males ($F_{1,45}=8.88$, $p=0.005$, two-way ANOVA) and females ($F_{1,17}=10.21$, $p=0.005$). In control rats with no neuronal silencing, metestrous and diestrous females show significantly higher shock-induced startle sensitization compared to proestrous females ($p=0.0029$, unpaired t-test) as well as males ($p=0.007$). Meanwhile, $BNST_{dl}$-CRF neuron silencing attenuated shock-induced startle sensitization in males ($p=0.64$) yet increased sensitization in proestrous females ($p=0.0306$, n=8, unpaired t-test). Thus, our results show that both estrous phase and sex influence shock-induced startle sensitization and Type III, $BNST_{dl}$-CRF neurons contribute to this behavior.

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Poster

404. Social Cognition and Behavior: Mechanisms and Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 404.12

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: Neuroscience Student Research Award

Title: Contributions of taurine and baclofen to dominant female crayfish Procambarus clarkii

Authors: S. A. LISKOWICZ, O. R. SANDER, K. A. TILL, S. N. BRISTOL, C. B. WEBBER, *R. F. WALDECK;
Biology/Neuroscience, Univ. of Scranton, Scranton, PA

Abstract: Taurine is a nonessential amino acid commonly found in energy drinks and acts as a $GABA_A$ and $GABA_B$ receptor agonist. Invertebrates like crayfish, *Procambarus clarkii*, possess GABAergic systems that can be used to study this inhibitory neurotransmitter. Crayfish are also excellent aggression models, readily engaging in agonistic encounters, and form social hierarchies through these fights. Thus, crayfish may be used to investigate the role of GABA on aggression, accompanying behaviors such as the tail flip, and winner and loser effects. In a previous poster presented at SFN in 2019, administration of taurine (25mg/L) to subordinate female crayfish resulted in a significant decrease in aggression and a significant increase in frequency of non-tail flip retreats. Subordinate female crayfish given $GABA_B$ receptor agonist baclofen (17.5mg/L) displayed a significant decrease in aggression and significant increases in both non-tail flip retreats and tail flip frequency. Electrophysiological studies confirmed that both taurine and baclofen induce changes at the cellular level when recording from the crayfish ventral nerve cord. To investigate the role of the two agonists on not only the loser effect but also the winner effect, dominant female crayfish were administered taurine or baclofen. Dominant female crayfish receiving taurine exhibited a significant decrease in aggression but no significant changes in the frequency of tail flips or non-tail flip retreat. Dominant female crayfish given baclofen exhibited no significant changes in aggression, tail flip frequency, or non-tail flip retreat frequency. This data supports the existence of different roles for GABA in modulating the winner and loser effects in dominant and subordinate crayfish, respectively, and may suggest alterations to the crayfish GABAergic system occur with hierarchy formation. Furthermore, another experiment in which subordinate female crayfish were administered serotonin-1 (5-HT1) receptor antagonist propranolol (10uM) was performed to determine if the action of the GABA agonists may be mediated through the disinhibition of the serotonergic system in crayfish. In this experiment, there were no significant changes in aggression, tail flip frequency, or non-tail flip retreat frequency. This data does not support that GABA is acting through the disinhibition of the serotonergic system via 5-HT1 receptors.

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Poster

404. Social Cognition and Behavior: Mechanisms and Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 404.13

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH NIGMS R35GM119844
Kavli Institute for Brain and Mind Postdoctoral Scholarship
Salk Pioneer Fund Postdoctoral Scholar Award

Title: Dissecting the neural circuits regulating social conflict in drosophila melanogaster

Authors: *D. VENTIMIGLIA[1], E. CHEMIAC-CASE[2], S. POTDAR[1], K. ASAHINA[1];
[1]Salk Inst. for Biol. Studies, La Jolla, CA; [2]Neurosci., UCSD, La Jolla, CA

Abstract: To fight or flee? This is a critical decision faced throughout the animal kingdom and a key process in the formation and maintenance of social hierarchies. Losing to a dominant opponent results in social defeat, an internal state that halts aggression and triggers fleeing. Using an optogenetically induced fighting assay in Drosophila melanogaster, we have established a tractable paradigm to study social defeat in the fly. Upon experiencing social defeat, we found that specific classes of aggression-promoting neurons become unable to induce aggression. This effect can last many hours and is accompanied by enhanced fleeing behavior in response to subsequent attacks. We find that social defeat onset is modulated by hunger-state and resource availability and exhibits temporal dynamics that closely match theoretical predictions from evolutionary game theory, suggesting that flies follow an optimal strategy to resolve social conflict. We conducted a neuronal silencing screen and identified subpopulations of dopaminergic and mushroom body output neurons required for social defeat: blocking synaptic transmission of these neurons prevents the formation of losers in aggressive contests. Although social hierarchies have been noted since the earliest descriptions of fly aggression, specific neurons mediating these effects have not yet been identified. Our work suggests that circuits important for aversive learning and memory are used to regulate social conflict and generate context-dependent aggression in the fly.

Disclosures: D. ventimiglia: None. **E. Chemiac-Case:** None. **S. Potdar:** None. **K. Asahina:** None.

Poster

404. Social Cognition and Behavior: Mechanisms and Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 404.14

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH NIGMS R35GM119844
L.I.F.E. Foundation Research Grant

Title: Genetic and functional dissection of Drosophila octopaminergic neurons

Authors: K. ISHII, A. KOGER, M. CORTESE, A. MUKIM, M. SHOKHIREV, L. HUANG, ***K. ASAHINA**;
Salk Inst. for Biol. Studies, La Jolla, CA

Abstract: Octopamine (OA), an invertebrate neuromodulator that is both chemically and functionally analogous to mammalian norepinephrine, regulates a variety of behaviors in the fruit fly *Drosophila melanogaster*, including locomotion, sleep/wake states, egg-laying, taste modulatory, olfactory conditioning, and social behaviors such as courtship and aggression. OA neurons consist of more than two dozens of anatomically distinct subtypes (Busch et al., 2009), raising a possibility that each subtype has different behavioral functions. Through a genetic screening of 1,408 genes, we found that a knock-down of the transcriptional regulator *nervy* (*nvy*) specifically in OA neurons significantly elevated aggressive behavior of both male and female flies that were reared in groups. Only a subset of OA neurons express *nvy*. Genetic silencing of this population elevated aggression in group-reared flies, while silencing of a complementary, *nvy*-negative OA neurons suppressed aggression in socially isolated flies. This data suggests that OA neurons contain at least 2 subpopulations that oppositely regulate aggressive behavior. To further characterize both the genetic and circuitry mechanisms by which OA modulates aggression, we are characterizing split GAL4 lines (Dionne et al., 2018) that label specific OA subtypes. We have so far analyzed expression patterns of 379 split GAL4 lines, and identified several combinations that label specific subtypes in both *nvy*-positive and *nvy*-negative groups. In parallel, we also employ single-cell RNAseq (scRNAseq) to molecularly characterize aminergic neurons including OA subtypes. Aminergic neurons labeled by *Vmat-GAL4* (Diao et al., 2015) were dissociated and collected by the fluorescent activated cell sorter, and single cell cDNA libraries were synthesized using 10X Chromium platform. Analysis of 3,959 single cell transcriptomes obtained from Illumina NovaSeq revealed that major classes of aminergic neurons (dopamine, serotonin, and octopamine) were hierarchically segregated. Each class was further divided into distinct subtypes. OA neurons were classified into 4 subtypes, which is consistent with the number of anatomically defined major groups. These results help generate genetic infrastructure to investigate subtype-specific functions of OA neurons.
References: Busch S, et al. (2009) J Comp Neurol 513:643-667; Diao F, et al. (2015) Cell Rep 10:1410-1421; Dionne H, et al. (2018) Genetics 209:31-35.

Disclosures: K. Ishii: None. **A. Koger:** None. **M. Cortese:** None. **A. Mukim:** None. **M. Shokhirev:** None. **L. Huang:** None. **K. Asahina:** None.

Poster

404. Social Cognition and Behavior: Mechanisms and Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 404.15

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH NIDCD R01DC015577

Title: Neuromodulation of protein-hunger induced aggressive behavior in Drosophila melanogaster

Authors: *S. POTDAR, K. ASAHINA;
Mol. Neurobio. Lab., Salk Inst. for Biol. Studies, San Diego, CA

Abstract: Animal conflicts over resource are often resolved by aggressive behaviors. How aggression is regulated by internal states such as hunger – which represents a physiological demand for food, is less understood. Hunger promotes food-seeking and feeding-related behaviors and enhances sensitivity to food-related chemosensory stimuli. On the other hand, hunger inhibits cost-inducing competing behaviors such as courtship and mating. It is tempting to speculate that aggression is promoted by hunger state, especially in the presence of food-specific chemosensory cues. However, excessive aggression in the absence of food while hungry can deplete precious energy. Thus, hunger circuits can modulate aggressive behavior depending upon context. Clarifying physiological or behavioral conditions that are relevant for aggressive interactions in a systematic manner is key to uncovering neuronal circuits which enable animals to perform a cost-benefit analysis while considering need and availability.To address this question, we have developed a new behavioral paradigm in *Drosophila*, which display stereotyped and complex aggressive behaviors. Flies were raised on diets lacking essential nutrients, then tested for aggressive behaviors in the presence of various food materials. Dietary restriction of yeast or only amino acids in adult flies results in decreased whole-body protein level and increased aggression only when flies encounter yeast in the assay chamber. Absence of yeast in assay chamber, or presence of other nutrients such as sugar or cholesterol (fat), does not increase aggression in protein-deprived flies. To discover the neuronal circuits important for modulating protein-hunger induced aggression, we functionally screened for neurons whose activity is important for aggression induced only in the context of protein deprivation. Our results point toward several promising candidates including several types of neuropeptidergic cells that have been previously linked to feeding behaviors. Interestingly, neurons that release neuropeptide F (a *Drosophila* homolog of neuropeptide Y) were not required for elevating aggression in protein-deprived flies. Our ongoing effort to characterize genetic and neural mechanisms that promote aggression in nutrition- and food availability-dependent manner can reveal a neural logic of cost-benefit calculation.

Disclosures: S. Potdar: None. **K. Asahina:** None.

Poster

404. Social Cognition and Behavior: Mechanisms and Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 404.16

Topic: G.08. Other Psychiatric Disorders

Support: JSPS KAKENHI Grant Number JP19K17088
JSPS KAKENHI Grant Number JP22K15786

Title: Decreased behavioral alteration in the social environment among subjects with Anorexia Nervosa

Authors: *M. ISOBE[1], M. SUNADA[2], T. NODA[2], K. TOSE[2], R. MISHIMA[2], M. KAWABATA[2], H. KOZUKI[1], T. MURAI[2];
[1]Kyoto Univ. Hosp., Kyoto, Japan; [2]Kyoto Univ., Kyoto, Japan

Abstract: [Aims] It is often reported that shoplifting is observed in patients with anorexia nervosa (AN). In addition to the difficulty in behavioral change possibly caused by impaired cognitive flexibility, the low sensitivity to punishment stimuli may lead to the continuation of socially inappropriate behavior. In the present study, we modified the Inspection Game (Hampton et al., 2008), which is an economic game, and created a psychological experimental task that mimics the situation of shoplifting, and used the task to compare the degree of behavioral change induced by punishing stimuli between AN patients and healthy subjects (HC). The purpose of this study was to compare the degree of behavioral change caused by punishment stimuli between patients with AN and HC, and to deepen the understanding of the mechanism that makes shoplifting frequently observed in AN compared with other psychiatric disorders. [Methods] The task used in the current study is a competitive game between an employer and an employee. The employee chooses whether to work or shirk, and the employer chooses whether to inspect or not inspect. Subjects participate as employees and are rewarded if they are either "inspected by the employer when they are working" or "not inspected by the employer when they are slacking off. However, if they are inspected by their employer when they are slacking off, they will receive a fine. Subjects need to change their behavior depending on the frequency of inspections by their employers and the amount of the fine when they are inspected for skipping work. [Results] The subjects who participated in this study were 26 HCs and 28 ANs, all of whom were female. ANs showed significant lower body mass index (BMI) than HCs. There was no significant difference in the level of understanding of the tasks asked in the post interview, and both groups understood the tasks well. Subjects in both groups tended to shirk less often when the amount of fines increased when they skipped work. In trials after experiencing negative stimuli during the task, such as “not being rewarded” or “being fined”, the frequency of behavior alteration was lower in AN than in HC. [Discussion] The results suggest that AN patients may be less sensitive to punishment stimuli and less likely to change their behavior.

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Poster

405. Prefrontal Cortex Networks and Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 405.01

Topic: F.03. Stress and the Brain

Support: NIH 1R01HL137103-01A1

Title: Evaluation of prefrontal cortex expressing angiotensin type 2 receptors in fear-related behavior

Authors: *H. C. SMITH, Z. YU, L. IYER, A. KISNER, A. M. POLTER, P. J. MARVAR; George Washington Univ., Washington, DC

Abstract: Growing evidence suggests that the renin-angiotensin system (RAS), a regulator of blood pressure and fluid homeostasis, is a potential therapeutic target for PTSD. Brain angiotensin II receptors are expressed across corticolimbic circuits of the brain, such as the medial prefrontal cortex (mPFC), however, their role and mechanism are largely unknown. Given the importance of the mPFC in fear and anxiety-related behavior, our current studies sought to examine the role of mPFC expressing angiotensin type 2 receptors (AT_2R) neurons in fear-related behavior. To characterize mPFC AT_2R-$eGFP^+$ cells and function in fear-related behavior, auditory-cue-dependent Pavlovian fear conditioning and immunohistochemistry plus retrograde tracing and whole-cell patch-clamp recording in an AT_2R-eGFP BAC reporter mouse were used. We determined that the majority of AT_2R-$eGFP^+$ cells were glutamatergic and highly expressed in the prelimbic (120.6±13.25 cells/mm^2) and infralimbic cortex (163.7±17.30 cells/mm^2) of the mPFC. Retrograde labeling further demonstrated that AT_2R-$eGFP^+$ neurons project to the basolateral amygdala (BLA) while ex-vivo activation using slice electrophysiology of mPFC-AT_2R-$eGFP^+$ neurons decreased the frequency of spontaneous excitatory postsynaptic currents (sEPSC). Following auditory cue-dependent retrieval of fear memory, mPFC-AT_2R mRNA expression was significantly increased (1.1±0.4 Control vs 3.0±0.63 retrieval, $t(14)=3.38$, $p<0.01$) suggesting activation of this receptor during fear recall. These findings provide neuroanatomical, electrophysiological and behavioral evidence that AT_2R-mPFC expressing neurons may participate in the modulation of a conditioned fear memory, possibly by decreasing excitatory glutamatergic output from the mPFC onto key regions involved in fear learning such as the BLA. Current studies are underway, using Cre/Lox and chemogenetic approaches to further test this hypothesis.

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Poster

405. Prefrontal Cortex Networks and Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 405.02

Topic: H.08. Learning and Memory

Support: Brain/MINDS
KAKENHI JP22H00432
KAKENHI JP17H06312

Title: Neuronal activity dynamics in the prelimbic cortex underlying fear memory processing

Authors: *H. KONDO[1], R. KIM[1,2], H. SONG[1], J. KIM[1], T. SHAKUNO[1], R. SHIMODA[1], R. YAMASHITA[1], L. S. BREBNER[1], K. INOKUCHI[1], Y. KONDO[1], M. OKAMURA[1], H. OKUNO[3], K. OTA[1], H. FUJII[1], H. BITO[1];
[1]Dept. of Neurochemistry, Grad. Sch. of Med., The Univ. of Tokyo, Bunkyo-ku, Japan; [2]Dept of Psychiatry, U Texas Southwest Med. Cntr, Dallas, TX; [3]Dept of Biochem & Mol Biol, Grad. Sch. of Med. and Dent. Sci., Kagoshima Univ., Kagoshima, Japan

Abstract: The medial prefrontal cortex is one of the key structures in the mammalian brain, being critically involved with encoding, retrieval, and extinction of an emotional memory. Due to the low spatio-temporal resolution of previous studies, little is known, however, about the neuronal activity and its dynamics that directly determine the memory processing at the single-cell and ensemble levels. To investigate the specific time course and significance of neuronal activity dynamics in one prefrontal area, prelimbic cortex (PrL) of mice, we established an in vivo 2-photon calcium imaging protocol to simultaneously monitor the ongoing neural activity and behavioral representation during and following an auditory fear conditioning paradigm. To approach the PrL neurons despite the presence of a major sinus directly above it, we inserted a relatively thin GRIN lens (φ0.5 - 0.6 mm, NA 0.5) with about a 10-degree tilt. Around 30-50 somatic and dendritic activities were recorded in a single optical plane at up to 30 Hz using virally expressed XCaMP-G. Our preliminary data here suggests the presence of fear memory encoding neurons in PrL. We found that about 20% of neurons responded to the unconditioned stimuli (electrical shock) in a significant manner. We also found that about 30% of neurons showed significant responses to the conditioned stimuli (tone) after the associative learning of fear ($p < 0.001$, Wilcoxon rank sum test). To explore the role of reactivation of the putative fear memory neurons, we then performed a dual IEG activation mapping and compared, within the same individual mice, the neural activity during fear memory encoding and extinction, using a contextual fear conditioning paradigm. Combining newly generated Arc-hybrid-promoter-based reporter Tg mice, capable of activity-dependent Venus expression, with c-Fos immunohistochemistry, we computed the reactivation rate in multiple brain regions. Among five candidate areas that are previously implicated in contextual fear memory processing, namely, PrL, infralimbic cortex (IL), hippocampal CA3, dentate gyrus, and BA, we found that the reactivation rate was significantly augmented in PrL, in the extinction-induced group (7.5% ± 1.4, n=7) compared to the retrieval-only group (0.8 ± 0.4, n=8) ($p < 0.001$, two-tailed unpaired t test). By combining further analyses of the PrL and by recording from adjacent cortical structures, such as IL, which has been implicated in fear extinction, we will shed new light on the activity dynamics that underlie fear memory expression and extinction and better understand how memory consolidation and extinction are balanced via top-down control mediated by the prefrontal cortex.

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Poster

405. Prefrontal Cortex Networks and Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 405.03

Topic: H.08. Learning and Memory

Title: Switching between functional cortical-subcortical modules during associative learning

Authors: N. J. KILLIAN[1], E. N. ESKANDAR[2];
[2]Neurosurg., [1]Albert Einstein Col. of Med., BRONX, NY

Abstract: Here we provide evidence for changes in cortical-subcortical synchrony during associative learning progression using a novel behavioral metric. The metric is based on eye movement tracking during the image viewing phase of an associative learning task (ALT, as in *Martinez-Rubio et al., 2018*). In the ALT, the rhesus macaque subjects were tasked with associating two optically-presented items—a neutral image and a circular spot. First, the image is viewed, and then a saccade is made to one spot in an array, signaling a choice. Correct choices are rewarded, and subjects learn the paired associates by trial and error. Image viewing provided surprising insight into the learning process. Subjects made eye movements within the image borders, including small saccades and fixational eye movements. We used summary measures of gaze spread and fixational jitter as ingredients in a novel biomarker of learning state. Plotting the gaze trajectories in (spread, jitter) coordinates revealed two distinct viewing modes: *exploration mode* and *exploit mode*. Exploration mode viewing patterns corresponded to a 60% higher learning rate ($P < 0.0005$, rank sum test), whereas exploit trials had 24% more-advanced learning states ($P < 0.0005$, rank sum test). Exploration mode viewing uses dispersed sampling with tighter fixations after saccades, consistent with the ambient exploration mode observed early in novel scene viewing (*Ito et al., 2017*). Image viewing switches to the exploit mode as associations are learned, which may be energetically favorable (*Costa et al., 2019*). We converted the 2-D viewing values to a 1-D exploration index (EI) to facilitate the correlation between continuous brain activity and behavior. Our central hypothesis is that cortical-subcortical communication can be framed as cognitive modules that shift during learning from exploration to exploitation. Nicotinic cholinergic signaling from the nucleus basalis (NB) to the neocortex may support module selection, which may be coordinated with inter-areal muscarinic communication in the neocortex for module transition. We discovered a powerful low-beta (14 Hz peak) communication channel when examining interactions between the NB and the dorsolateral prefrontal cortex (DLPFC). High EI trials found early in learning were correlated with significantly-higher low-beta power transfer from NB to DLPFC, assessed with spectral Granger causality applied to the image viewing phase ($P = 0.005$, nonparametric cluster-based permutation test, 10 to 20 Hz, 250 to 925 msec). Future studies will be enhanced by simultaneously recording multiple cortical and subcortical areas of importance in the explore-exploit framework.

Disclosures: N.J. Killian: None. **E.N. Eskandar:** None.

Poster

405. Prefrontal Cortex Networks and Behavior

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

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Title: Novel value learning dynamics in the prefrontal cortex

Authors: *E. GUTIERREZ[1], S. VESELIC[1], J. L. BUTLER[1], T. H. MULLER[1], N. W. M. MALALASEKERA[1], L. T. HUNT[2], T. E. J. BEHRENS[2,3], S. W. KENNERLEY[3]; [1]UCL, London, United Kingdom; [2]Univ. of Oxford, Oxford, United Kingdom; [3]Univ. Col. London, London, United Kingdom

Abstract: Every day we are faced with choices between novel options whose value we must learn. While neurons encoding value of decision variables have been widely reported in the prefrontal cortex (PFC) of non-human primates, most evidence stems from tasks performed for thousands of trials. How encoding of novel values arises in prefrontal neurons thus remains largely unexplored. We therefore recorded neuronal activity from the anterior cingulate cortex (ACC, $N = 156$) and orbitofrontal cortex (OFC, $N = 160$) of two rhesus macaques while they performed an associative learning and decision-making task. During each session, subjects first learnt the value associated with novel reward-predicting cues by pairing each cue with an overlearned secondary reinforcer which indicated the novel cue's value, with corresponding reward outcome following a forced choice of the cue ("Conditioning phase"). Subjects experienced 10 repetitions of each of the 10 novel cues. Subsequently, binary choices between these recently learned ("novel") or well-learned ("overtrained") cues were presented ("Choice phase"). Subjects were able to quickly learn the value of these novel cues (Cavanagh *et al.*, 2019, PNAS). Here we show that neurons in ACC and OFC rapidly acquire novel value representations, albeit with divergent strategies. During the Conditioning phase, value signals were evident at the secondary reinforcer from the initial trials and remained constant throughout the entire Conditioning phase. In contrast, cue-related value representations gradually emerged within ten cue presentations. ACC neurons particularly exhibited more pronounced and rapidly-arising value selectivity, with some neurons exhibiting cue-related value signals within only five presentations of the novel stimulus. In both regions, value selectivity at the secondary reinforcer was correlated with the emergence of cue-related value coding. Additionally, we found that OFC selectivity was modulated by novelty. During the Choice phase, when a choice was offered

between the novel and overtrained cues, OFC strongly dissociated between which of the two sets the cues belonged to, independently of their value; this may reflect OFC's role in representing behaviourally-relevant variables to optimise choice based on task structure. Altogether, our findings imply a propagation of value from previously learnt secondary reinforcers onto novel cues, as predicted by reinforcement learning theory. They further indicate distinct prefrontal contributions to learning dynamics: ACC promptly integrates novel value representations into a common value code, whereas OFC precisely tracks the identity and novelty of each cue.

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Poster

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Title: Deep brain imaging of axon initial segment dynamics during associative fear learning.

Authors: *C. M. BENOIT[1,2], D. A. GANEA[1], C. THOME[3,4], A. SATTIN[5], S. M. INNOCENTI[1], T. FELLIN[5], M. ENGELHARDT[3], J. GRÜNDEMANN[1,2];
[1]Dept. of Biomedicine, Univ. Basel, Basel, Switzerland; [2]DZNE, Bonn, Germany; [3]Inst. of Anat. and Cell Biology, Med. Fac., Johannes Kepler Univ., Linz, Austria; [4]Inst. for Stem Cell Biol. and Regenerative Med., Standford Univ., Stanford, CA; [5]Dept. of Neurosci. and Brain Technologies, Inst. Italiano di Tecnologia, Genova, Italy

Abstract: The axon initial segment (AIS) is the site of action potential initiation and plays a crucial role in the generation of neuronal activity and the maintenance of network function during sensory processing and learning. Previous studies identified the AIS as a site of homeostatic plasticity. However, whether and how structural AIS plasticity occurs *in vivo* and its potential link to learning remains unknown. Here we established a gradient refractive index (GRIN) lens based two-photon imaging approach to monitor the structure and dynamics of the AIS in the medial prefrontal cortex (mPFC) network of mice expressing an intrinsic AIS live-stain. We visualised and tracked AIS structure in the infralimbic subdivision of mPFC across a four-day fear conditioning and extinction paradigm and identified population level changes in AIS length with increasing levels of fear extinction. Importantly, AIS length remained stable during consecutive baseline sessions outside of the learning paradigm. Our results demonstrate that AIS length is dynamic during associative learning *in vivo* and might mediate experience-dependent changes in neuronal excitability and output upon learning.

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Poster

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Support: IBS-R002-A1

Title: Role of VIP neurons and dopamine D1 receptors in working memory in the prefrontal cortex

Authors: ***J. BAE**[1], J. YI[2,3], S. CHOE[2], Y. YOON[1], M. JUNG[1];
[1]Dept. of Biol. Sci., Korea Advanced Inst. of Sci. and Technol. (KAIST), Inst. for Basic Sci. (IBS), Yuseong-gu, Daejeon, Korea, Republic of; [2]Ctr. for Synaptic Brain Dysfunctions (CSBD), Inst. for Basic Sci. (IBS), Yuseong-gu, Daejeon, Korea, Republic of; [3]These authors contributed equally, ., Korea, Republic of

Abstract: The prefrontal cortex (PFC) plays a critical role in working memory, and dopamine is known to modulate PFC neural processes including those supporting working memory. Of various types of neurons in the PFC, vasoactive intestinal polypeptide (VIP)-expressing neurons are thought to exert powerful influences on PFC circuit operations by modulating other inhibitory neuronal activity. Also, as the only inhibitory neurons expressing dopamine D1 receptors, they may also play a key role in mediating dopaminergic influences on the PFC. We investigated these issues in the medial PFC in mice performing a delayed match-to-sample task. The mice were trained to perform the task as the duration of delay was increased gradually from 0.5 to 4 s. Chemogenetic inactivation (n = 11) of VIP neurons or selective knockdown of VIP neuronal D1 receptors (KD = 7, CTR = 5) impaired behavioral performance when delay duration was increased to 3-4 s, but not before this phase. In well-trained mice, VIP neurons conveyed significant working-memory signals and their inactivation impaired trial-by-trial behavioral performance. In contrast, selective knockdown of VIP neuronal D1 receptors did not impair working-memory performance. These results indicate that PFC VIP neurons are critically involved in working memory, and that dopamine modulation of VIP neurons via D1 receptors is critical for learning to use, but not the maintenance of, working memory for guiding behavior.

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Poster

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Topic: H.08. Learning and Memory

Support: DFG SFB870
DFG RTG 2175
Max Planck Society

Title: Neural representations of learned categories in mouse prefrontal cortex

Authors: *S. REINERT, M. HÜBENER, T. BONHOEFFER, P. M. GOLTSTEIN;
Max Planck Inst. for Biol. Intelligence, Martinsried, Germany

Abstract: Grouping objects and experiences into categories is a fundamental skill for humans and many animals. Particularly, learning and recalling rules for categorization enables us to flexibly adapt to changes in context. Because the neuronal mechanisms underlying this process are not fully understood, we characterized representations of learned categories in medial prefrontal cortex (mPFC) by chronically recording neuronal activity of mice learning visual categorization.
In a head-fixed go/no-go task, animals were trained to categorize drifting gratings either based on orientation or spatial frequency. All mice successfully learned to group the stimuli into categories and generalized the learned rule to novel stimuli. Upon a rule-switch, mice quickly remapped the stimuli onto new categories. Strikingly, the animals also generalized the new rule to stimuli they had previously only experienced with the old rule. Thus, mice can learn rules for categorization.
Throughout this learning paradigm, we followed the activity of neurons in layer 2/3 of mPFC using two-photon calcium imaging. We found that a set of neurons acquired category-selective responses. After the rule-switch, category-selective neurons partly remapped, and previously non-selective neurons became responsive to the new categories. By experimentally decoupling other task parameters, like behavioral choice and reward, from category information, we identified uniquely category-modulated neurons that stably represented the category identity of stimuli across tasks. Together, these results demonstrate that mouse mPFC forms representations of learned categories and flexibly encodes changes in rules. We are currently using optogenetic manipulation of neuronal activity during the task to test for a causal role of the characterized representations in mPFC in different aspects of categorization behavior.

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Poster

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Title: Medial prefrontal cortex pyramidal cell ensembles form a stable reference frame of an olfaction guided spatial memory task

Authors: *H. MUYSERS[1,2], H.-L. CHEN[1], J.-F. SAUER[1], M. BARTOS[1];
[1]Inst. for Physiologie I, Med. Faculty, Univ. of Freiburg, Freiburg, Germany; [2]Univ. of Freiburg, Fac. of Biol., Freiburg, Germany

Abstract: The recall of long-term contextual memories involves the activation of neuronal populations in the medial prefrontal cortex (mPFC). However, how contextual memories are maintained on the long-term remains unknown. We addressed this question by using 1-photon calcium imaging in Thy1-GCaMP6f mice (10 males, 3 females), which express the calcium indicator predominantly in layer-V pyramidal cells of the mPFC. We trained mice in an olfaction guided spatial memory task, in which they needed to associate an odor (presented in the center-arm of an M-maze), with a specific reward location (left or right side-arm). In the first group of mice, we investigated the task representation over time and in the second group during learning on subsequent days. Our results show that a majority of active cells are spatially tuned. Moreover, cells kept stable and reliable spatial tuning across several weeks with only a mild drift (decay in Pearson's r correlation: ~ 0.006/day). Using decoder analysis, we were able to reliably predict trial identity (left/right trials) and location in the maze over the full experimental period of 24 days. Stability of spatial representation was unperturbed during introduction of breaks in task-exposure, during visual modification of the arena or switching of the cue-location pairing. Spatial tuning stability was lower in the initial phase of task exposure indicating that stability of the representation emerges during the initial learning phase. Such a coding scheme may provide a stable reference frame, which might support the animal's navigation in the task-context and to make quick task-related decisions. The cellular and circuit mechanisms supporting this stability in context representation remains to be further investigated.

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Poster

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Title: Structured credit assignment in mice

Authors: *K. MILLER[1], L. FREEMAN[2], Y. OH[2], M. M. BOTVINICK[1,2], K. D. HARRIS[2];
[1]DeepMind, London, United Kingdom; [2]Univ. Col. London, London, United Kingdom

Abstract: Reinforcement learning requires associating rewards with one or more of the states or actions that preceded them. The question of exactly which states or actions to associate with each reward is referred to as the "credit assignment problem", which must be addressed by both biological and artificial agents. Better solutions to this problem result in more efficient learning, and human subjects perform efficient credit assignment that is informed by knowledge of task structure. Here, we adapt a "structured" credit assignment task from the human literature for use with head-fixed mice. In this task, one type of reward ("controllable") depends causally on the mouse's actions, while another distinguishable type ("distractor") is independent of those actions. After experiencing a controllable reward, an optimal learner would assign credit to its recent action, while after experiencing a distractor reward the learner would not assign credit. We present behavioral evidence that mice, like humans, show a strategy that is partially structure-sensitive: they update their behavior based on both the controllable and the distractor reward, but they update more strongly to the controllable reward. We are currently collecting a neural recording dataset from these mice using high-density Neuropixel probes. We present preliminary results comparing responses in medial prefrontal cortex, orbitofrontal cortex, hippocampus, and striatum to rewards of each type.

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Poster

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Title: Sensory and behavioral correlates of avoidance learning in prefrontal cortex population activity

Authors: *B. EHRET, R. BOEHRINGER, E. A. AMADEI, M. R. CERVERA, C. HENNING, B. F. GREWE;
ETH Zurich, ETH Zurich, Zürich, Switzerland

Abstract: To survive in challenging environments, animals need to learn to adapt their behavior when exposed to threat-predictive sensory information. For this type of aversive learning, the medial prefrontal cortex (mPFC) has been suggested to link predictive stimuli and behavior execution. During learning, sensory-driven prefrontal responses emerge as stimuli gain behavioral relevance, and it has been shown that manipulations of mPFC activity can drive or inhibit the execution of learned behaviors. Yet, how prefrontal circuits link relevant sensory signals with specific behaviors has remained unclear. Here we investigate the role of mPFC in forming such a link using aversive auditory conditioning paradigms with dynamic stimulus-response mappings. We trained mice in two complementary active avoidance experiments in which we manipulated the contingencies between stimuli and conditioned behaviors. We achieved this manipulation by either changing the shock-predictive stimulus or by changing the action required to avoid the shock. In both cases, animals were required to learn a new mapping between sensory input and behavioral output. To investigate network-level information coding in mPFC, we used miniaturized microscopes and population calcium imaging in freely behaving mice. We employed a decoding approach to quantify how tones and avoidance-related behaviors are represented in mPFC activity, and how these representations change with learning. We found that prefrontal tone responses were tightly coupled to the behavioral relevance of a given stimulus. Moreover, mPFC activity contained predictive information on upcoming avoidance actions up to three seconds before action onset. However, this action-predictive activity did not change when animals had to adapt their avoidance actions. This indicates that mPFC activity does not encode specific aspects of upcoming actions, but rather reflects general action initiation signals related to a specific goal. Furthermore, we found that avoidance-predictive activity was not correlated with tone-evoked responses. These findings stand in contrast to related work that suggests joint coding of tone and behavior as a mechanism for linking sensory information to behavior execution. Taken together, our results motivate theoretical research into how links between sensory responses and action initiation signals could be achieved without such joint coding.

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Poster

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Title: Altered fyn signaling in the medial prefrontal cortex reduces resistance to stress-induced memory impairments

Authors: *Y.-J. JEON, Y.-S. JANG, N.-H. KIM, B.-R. CHOI, J.-S. HAN;
Konkuk Univ., Seoul, Korea, Republic of

Abstract: FYN is a 59 kDa member of the Src family of tyrosine-protein kinases associated with T-cell and neurodevelopmental signaling and regulation of cellular growth. It is located in the cytoplasmic leaflet of the plasma membrane and phosphorylates the tyrosine residue of target genes involved in various signaling transductions. When FYN is activated, it affects the downstream activation of molecular signals that induce the growth and motility of cells. FYN is highly expressed from embryonic to adult brains. Decreased FYN binding protein (FYB) levels have been reported in blood and brain samples from depressed and post-traumatic disorder (PTSD) patients. FYB is an adaptor molecule and is known to play a role in interacting with many other proteins and FYN. Our previous study showed that medial prefrontal cortex (mPFC) lesions decreased resistance to acute stress-induced memory impairments. Therefore, we examined the effect of FYN and FYB down-regulation in the mPFC on resistance to stress-induced memory impairment. Animals with viral-mediated knockdown of FYN in the mPFC were subjected to either a brief 20-min restraint plus 20 intermittent tail shocks (20-min stress), which is ineffective in inducing memory impairments, or a prolonged 60-min restraint plus 60 intermittent tail shocks (60-min stress) resulting in memory impairment. The cognitive status of these stressed rats was examined using a novel object recognition task. Recognition memory remained intact in control rats following the brief stress but was impaired in rats with FYN knockdown in the mPFC. Prolonged stress impaired recognition memory in both control rats and rats with FYN knockdown. We also observed that FYB down-regulation in mPFC reduced the resistance to stress-induced memory impairments as the FYN down-regulation in mPFC did. These findings indicate that altered mPFC FYN-related signaling is a potential mechanism for resistance to stress-induced cognitive impairment.

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Poster

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Topic: H.08. Learning and Memory

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Title: Training-dependent change in content of association in appetitive Pavlovian conditioning

Authors: H.-J. KIM[1], J. JANG[2], ***H.-Y. KOH**[2];
[1]Policy Res. Team, Korea Ctr. For Gendered Innovations For Sci. And Technol. Res., Seoul, Korea, Republic of; [2]Brain Sci. Inst., Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of

Abstract: In appetitive Pavlovian conditioning, experience with a conditional relationship between a cue (conditioned stimulus, CS) and a reward (unconditioned stimulus, US) bestows

CS with the ability to promote adaptive behavior patterns. Different features of US (e.g. identity-specific sensory, general motivational) can be encoded by CS based on the nature of CS-US relationship experienced (e.g. temporal factors such as training amount), and the content of association may determine CS's influence over behavior (e.g. mediated learning, conditioned reinforcement). The content of association changed with varying conditioning factors, thereby altering behavioral consequences, however, has never been addressed in relevant brain signals evoked by CS. Our previous study found that PLCβ1-KO mice display persistent mediated learning over the extended course of odor-sugar conditioning, and that wild-type (WT) mice lose mediated learning sensitivity after extended training. In this study, in order to see whether this behavioral difference between these two genotypes comes from a difference in the course of association content, we examined whether odor CS can evoke the taste sensory representation of an absent sugar US after minimal- and extended training in these mice. In contrast to WT which lost CS-evoked neural activation (c-Fos expression) in the gustatory cortex (GC) after extended training, KO displayed persistent association with the sensory feature of sugar, suggesting that sensory encoding is reliably linked to mediated learning sensitivity, and that there is a training-dependent change in the content of association in WT. PLCβ1 knockdown in the left mPFC resulted in mediated learning sensitivity and CS-evoked GC activation after extended training, proposing a molecular component of the neural system underlying this Pavlovian conditioning process. We also discuss how disruption of this process is implicated for hallucination-like behaviors (impaired reality testing).

Disclosures: H. Kim: None. **J. Jang:** None. **H. Koh:** None.

Poster

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Support: Swedish Research Council 2021-06645

Title: Selective and Lateralized Neuronal Population Activity in the Ventral Orbitofrontal Cortex During Autobiographical Recall and Self Judgement

Authors: *B. IRAVANI, J. PARVIZI;
Stanford Univ., Palo Alto, CA

Abstract: Selective and Lateralized Neuronal Population Activity in the Ventral Orbitofrontal Cortex During Autobiographical Recall and Self Judgement

Behzad Iravani and Josef Parvizi
The ventral surface of the orbitofrontal cortex (vOFC) is often hard to study with neuroimaging methods due to signal drop out, and its electrophysiological activity may not reach the scalp to be noted with non-invasive scalp electrodes. Consequently, our knowledge about the functional

roles of this region is largely rooted in observations in non-human primates or patients with lesions. Nevertheless, there is a body of evidence indicating that the vOFC is part of a large-scale intrinsic limbic network that is thought to play a significant role in processing emotion and memory. In the current work, we gained direct access to the human vOFC through direct recordings (i.e., intracranial electroencephalography, iEEG) using grids and strips or depth electrodes in patients with focal epilepsy (n = 27). The aim of the project was to probe the spatiotemporal functional organization of vOFC when human subjects were instructed to perform an experiment during which they had to engage in self-judgement (e.g., I am attractive) or autobiographical memory retrieval (e.g., "I had a seizure today). We relied on the power of high frequency broadband (HFB: 70 -170 Hz) as a measure of neuronal population activity. Our results clearly demonstrated localized neuronal population activity in both left and right vOFC during autobiographical and self-judgment conditions at the latest stage of autobiographical or self-judgment retrieval and decision. However, the localized activity showed a significant lateralization effect $F(1, 1080) = 8.871$, $p < .004$) in processing of autobiographical memory compared to self-judgment. Particularly, the right vOFC was found to be more involved in autobiographical recall compared to self-judgment whereas there was no significant difference across the hemisphere for the two conditions. In conclusion our result is further illuminating the spatial organization of vOFC for autobiographical memory recall and self-judgment task.

Disclosures: B. Iravani: None. **J. Parvizi:** None.

Poster

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Title: High-frequency activity in the orbitofrontal cortex is associated with expected outcome and changes in trial outcome during reversal learning

Authors: *R. STEVENSON[1], R. R. SONG[2], M. A. RUVALCABA[1], J. J. LIN[3], P. BRUNNER[4], R. T. KNIGHT[1];
[1]Helen Wills Neurosci. Inst., [2]Mol. and Cell Biol., UC Berkeley, Berkeley, CA; [3]Neurol., UC Davis, Davis, CA; [4]Dept. of Neurosurg., Washington Univ., Saint Louis, MO

Abstract: The orbitofrontal cortex (OFC) is thought to play a crucial role in representing and updating task-relevant information, including expected outcome. In reversal learning tasks in which outcome contingencies are dynamically altered, OFC activity has been shown to reflect expected outcome and to update these representations following changes in trial outcome, or 'reversals'. However, the ways in which this region contributes to reversal learning in humans is still poorly understood. We tested presurgical epilepsy patients (n=4) with electrodes implanted

along the medial-lateral axis of the OFC on a deterministic, three choice reversal learning task in which subjects learn associations between objects and locations through trial and error. Four object-location associations were learned concurrently, resulting in 90 'mini-blocks' of four objects pseudo-randomly shuffled. After a variable number of repetitions, or times a specific object was encountered (8-11), the correct location for that object was changed and subjects attempted to learn the new correct location for that object. After a variable number of post-reversal repetitions (4-6) the object was phased out and replaced by a new object. We fit subjects' behavior with a reinforcement learning (RL) model (a delta rule with counterfactual learning) to derive trial by trial estimates of expected outcome. This model fit subjects' behavior well, and simulations of the RL model captured subjects' patterns of behavior. During the pre-feedback period we found correlations between high-frequency activity (HFA; 50-150 Hz) and expected outcome in OFC electrodes in 3/4 subjects (FDR corrected for number of OFC electrodes). We also found increased OFC HFA at feedback for errors following reversals (compared to acquisition errors) in 2/4 subjects (FDR corrected). These results provide additional evidence supporting the role of the orbitofrontal cortex in representing and updating expected outcome during learning. Outside of the OFC, we found correlations between expected outcome and hippocampal HFA in 3/4 subjects as well as correlations between insula HFA and prediction error in all subjects. Future work will examine the dynamics of prefrontal and hippocampal interactions as novel associations are learned and updated.

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Poster

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The ministry of education and NRF BK21 program

Title: Task-specific neural firing patterns in the medial prefrontal cortex in a hippocampal-dependent scene-based working memory task.

Authors: *E.-H. PARK, I. LEE;
Dept. of Brain & Cognitive Sci., Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: It has been known that the medial prefrontal cortex (mPFC) plays a critical role in working memory (WM) to maintain task-related information during goal-directed behavior. Prior

studies suggest that its function is not only to hold the information but also to temporally organize the relevant pieces of information. Taking advantage of the presence of the unilateral monosynaptic projections from the intermedial hippocampus (iHPC) to the mPFC, we tested the functional significance of the visual contextual information presumably transmitted from the iHPC to the mPFC in a scene-dependent memory task in which the temporal organization of information according to task phase is critical. We inactivated the iHPC with muscimol (MUS) and recorded single-unit spiking activities (n = 88 in control sessions; n = 73 in MUS sessions) in the mPFC by using the hyperdrive equipped with 24 tetrodes in rats (n=5). A body-fixed rat was trained in a VR environment to run on a cylindrical treadmill to sample one of four visual scenes (Forest, City, Playground, Room) during a sample phase, and, after a 3-s delay phase, the rat must lick either the left or right licking port in a choice phase to obtain honey water as reward (30μL) in association with the sampled visual scene. MUS injections into the iHPC resulted in a severe performance deficit ($t_{(9)}$=5.67, p<0.001). Our preliminary analysis suggests that the neural firing correlates of task variables in the mPFC appear to be associated with specific task phases (sample, delay, test) and changed their firing correlates across multiple task phases in a dynamic fashion seemingly to facilitate information processing in each task phase. The dynamic shifts of task phase-specific firing patterns were more prominent in the infralimbic cortex (IL) than in the prelimbic cortex within the mPFC (Z=2.22, p<0.05). MUS injections into the iHPC decreased the proportion of these task-specific cells in the mPFC (X^2=26.96, p<0.0001). In addition, when MUS was injected into the iHPC, the mPFC population did not exhibit such dynamic firing patterns across multiple task phases to match the the temporal structure of the WM task. Overall, our findings suggest that the visual contextual information from the iHPC to the IL is critical for the mPFC network to temporally organize task-relevant information according to WM task demands in a goal-directed fashion.

Disclosures: E. Park: None. **I. Lee:** None.

Poster

405. Prefrontal Cortex Networks and Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 405.16

Topic: H.08. Learning and Memory

Support: Ministry of Science and Technology of China (2021ZD0203600)

Title: Neural responses in the lateral and medial prefrontal cortices during usage of item-location association memory

Authors: *X. ZHANG, C. YANG, S. XUE, Y. NAYA;
Peking Univ., Beijing, China

Abstract: Accumulating studies suggest involvements of both lateral and medial prefrontal cortices (lPFC and mPFC) for cognitive functions supported by long-term memory. However,

the relationship between these two brain regions remains unsolved. To address this problem, we compared neuronal activities between the lPFC and mPFC of non-human primates when retrieval of item-location association memory and its usage were required sequentially. We trained two macaques to associate eight visual items with four particular locations relative to a background map image; each two items were assigned to the same location. After the monkeys learned the associations, we recorded single-unit activities from both of the two brain regions during the item-location association task. In each trial, one of the eight visual items was presented as an item-cue first. After a delay, a randomly-tilted background image was presented as a background-cue. The monkeys were required to choose a target location according to a combination of the item-cue and background-cue. In total, we recorded 470 neurons from the lPFC and 322 neurons from the mPFC across two macaques. A recording region of the lPFC included the dorsolateral PFC, frontal eye field, dorsal and ventral part of principle sulcus while both dorsal and ventral parts were covered for the mPFC. To explore the information represented by the two PFC areas, we conducted the population decoding. Linear classifiers were trained and tested separately for lPFC and mPFC based on randomly re-sampled trials to reduce trial-to-trial noise. Results showed that the item-associated location and item identity can be decoded from the lPFC after item-cue presentation ($p < 0.001$, compared with chance level). After the background-cue presentation, the background-cue and target location can be decoded from the lPFC ($p < 0.001$, compared with chance level). On the contrary, only item-associated location and target location can be decoded from mPFC neurons after the item-cue and background-cue presentations, respectively ($p < 0.05$, compared with chance level). Moreover, these decoding accuracies were lower in the mPFC than the lPFC ($p < 0.001$). These results suggest that the lPFC is involved in retrieval of semantic-like memory and its usage more than the mPFC although the mPFC may also contribute to them.

Disclosures: **X. Zhang:** None. **C. Yang:** None. **S. Xue:** None. **Y. Naya:** None.

Poster

405. Prefrontal Cortex Networks and Behavior

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Program #/Poster #: 405.17

Topic: H.08. Learning and Memory

Support: NIH/NIMH R01MH116008
NIH/NIA RF1AG043640
NIH/NIMH K99/R00MH101234

Title: Somatodendritic distribution of cholinergic muscarinic receptors in inhibitory neuron subtypes of monkey anterior cingulate cortex revealed by 3D morphological analyses

Authors: M. SAKHARKAR, A. TSOLIAS, *M. MEDALLA;
Anat. & Neurobio., Boston Univ. Sch. of Med., Boston, MA

Abstract: The anatomical subcellular distribution of receptors is a large determinant of the mechanism of action of many neuromodulators. One neuromodulator, acetylcholine (ACh), robustly influences cortico-limbic circuit dynamics through a variety of presynaptic and postsynaptic receptors. In the cortex, muscarinic acetylcholine receptors (mAChR) m1 and m2 are the predominant subtypes, which have differential distributions across areas, layers and cell-types. Specifically, cholinergic modulation of the anterior cingulate cortex (ACC) and other limbic areas is robust and plays an important role in regulating arousal, affective states, and learning and memory. Our previous work has shown differential distribution of m1 and m2 mAChRs on subcellular compartments of excitatory pyramidal neurons in rhesus monkey ACC. The current study adapts a 3D Morphological MATLAB script used previously for microglial branching analyses (York et al., 2018, eNeuro), to quantify m1 and m2 receptor densities within somatodendritic compartments of individual inhibitory neurons expressing the calcium binding proteins, parvalbumin (PV), calbindin (CB), and calretinin (CR) in the ACC of adult rhesus monkeys (*M. mulatta,* n = 2 F, 4 M, 7-11y). The aim is to optimize a high-throughput, high-resolution method to investigate single-cell subcellular receptor distributions using immunolabeling, which can be applied to human brain sections. The segmentation was able to delineate somatic and dendritic ROIs based on calcium-binding protein labeling intensity, yielding similar ROI volumes across cell types. Significant differences in mAChR densities between compartments were found to be dependent on receptor and cell subtype. For CB and CR, but not PV, cells, m1+, but not m2+, receptor density was significantly greater in dendritic compared to somatic compartments (ANOVA two-way, $p < 0.05$), suggesting stronger m1 mediated potentiation of localized dendritic inputs. Further, within the soma of CB cells, a greater density of m1+ compared to m2+ was found, suggesting an even stronger potential for m1 mediated activation. These inhibitory neurons have been shown to be differentially activated by ACh in rodent cortex, and they each target specific neuronal compartments and cortical layers to mediate distinct modes of inhibition. Future analyses will assess regional and laminar differences in the compartmental distribution of mAChRs. Assessing cell-type specific compartmental distribution of receptors is highly important in elucidating the role of distinct neuromodulators on neural circuits, and how neurochemical imbalances in specific brain areas can lead to dysfunction.

Disclosures: **M. Sakharkar:** None. **A. Tsolias:** None. **M. Medalla:** None.

Poster

405. Prefrontal Cortex Networks and Behavior

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NIH/NIMH R01 MH116008
NIH/NIA RF1-AG062831

Title: Laminar electrophysiological and morphological properties of pyramidal neurons in distinct subdivisions of the rhesus monkey anterior cingulate cortex

Authors: *Y. ZHOU[1], M. HSIUNG[2], C. A. MOJICA[1], W. CHANG[1], A. TSOLIAS[1], T. L. MOORE[1], D. L. ROSENE[1], J. I. LUEBKE[1], M. MEDALLA[1];
[1]Anat. and Neurobio., Boston Univ. Sch. of Med., Boston, MA; [2]Boston Univ., Boston, MA

Abstract: The anterior cingulate cortex (ACC) -a major component of the limbic system and the prefrontal cortex- is a heterogenous region, consisting of functionally distinct cytoarchitectonic areas that have specialized roles in decision making and flexible behavior. Recent studies have shown overlapping but distinct roles of dorsal caudal ACC (dACC) area 24 versus ventral rostral ACC (rACC) area 32 in motor and cognitive control. However, lamina-specific cellular properties, and therefore mechanisms of signal processing, within these distinct ACC areas have not yet been elucidated in primates. In this study, we assessed the morphological and biophysical signaling properties of individual layer 3 (L3) and layer 5 (L5) pyramidal neurons in dACC area 24 and rACC area 32, using *in-vitro* whole-cell patch clamp recording with intracellular filling in brain tissue from young adult rhesus monkeys (*Macaca mulatta*; n=9; 6-13 years old; 3 females, 6 males). Intrinsic passive membrane (Vr, tau, and Rn) and action potential (AP) firing properties showed laminar differences independent of ACC subregion. Consistent with our previous work, L5 neurons exhibited greater excitability than L3 neurons in both ACC subdivisions, as indicated by the higher Rn, lower rheobase and higher AP firing frequency in response to current injections (*t*-test, $p < 0.05$, all comparisons). Further, dendritic morphology of recorded cells that were filled and reconstructed in 3D were relatively uniform between layers and areas. However, there is a small but significant laminar difference in apical dendritic complexity of dACC neurons, with L3 neurons exhibiting a higher number of branch points compared to L5 neurons ($p < 0.05$). Further, a significant regional difference in basal dendritic complexity was found in L5 neurons, with rACC L5 neurons having a higher number of basal branches per micron than L5 dACC neurons ($p < 0.05$). Our results show that these distinct ACC subdivisions exhibit similar layer-specific properties of pyramidal neurons in L3 and L5. These L3 and L5 pyramidal neurons participate in different cortical and subcortical pathways. The distributions of these layer-specific pyramidal neurons and their connectivity profiles with motor, limbic and cognitive areas likely reflect diverse communication of distinct ACC subdivisions with target brain areas.

Disclosures: Y. Zhou: None. **M. Hsiung:** None. **C.A. Mojica:** None. **W. Chang:** None. **A. Tsolias:** None. **T.L. Moore:** None. **D.L. Rosene:** None. **J.I. Luebke:** None. **M. Medalla:** None.

Poster

405. Prefrontal Cortex Networks and Behavior

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Support: NIH/NIMH K99/R00MH101234
NIH/NIMH R01 MH116008
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NIH/NIA RF1AG062831

Title: Distribution of muscarinic acetylcholine receptor m1 and m2 on subpopulations of excitatory and inhibitory neurons across subdivisions of the rhesus monkey anterior cingulate cortex

Authors: *A. TSOLIAS[1], M. SAKHARKAR[2], M. Z. TSOLIAS[1], C. A. MOJICA[1], Y. ZHOU[1], T. L. MOORE[1], D. L. ROSENE[1], M. MEDALLA[1];
[1]Anat. & Neurobio., Boston Univ. Sch. of Med., Boston, MA; [2]Boston Univ., Boston, MA

Abstract: The anterior cingulate cortex (ACC) is comprised of anatomically and functionally distinct dorsal (A24), rostral (A32), and ventral (A25) areas, which differ in their cortico-limbic connectivity profiles with higher order frontal areas and the amygdala (AMY). The ACC is densely innervated by cholinergic afferents that control excitatory and inhibitory circuit dynamics and facilitate attentional processing and cognitive-emotional integration. This cholinergic modulation depends on the localization of diverse receptors across layers and cell types, which is largely unknown for ACC areas and long-range ACC to AMY pathways in primates. The current study examines the laminar distribution of muscarinic acetylcholine receptors m1 and m2 on distinct excitatory and inhibitory neurons in ACC areas 24, 25, and 32 of adult rhesus monkeys (*M. mulatta*; n=2 females and 4 males, 7-11 yrs) using immunohistochemistry. To assess structural substrates of cholinergic modulation of cortico-limbic pathways, m1+ and m2+ expression of AMY-targeting projection neurons within ACC areas were examined via *in vivo* injections of bidirectional neural tracers into the basolateral amygdala (n=2 females and 4 males, 5-7 yrs). Our results demonstrate the laminar density of m1+ and m2+ expressing excitatory pyramidal (labeled with microtubule-associated protein, MAP2) and inhibitory neurons (labeled with calcium binding proteins: calbindin, CB; calretinin, CR; parvalbumin, PV) were dependent on area and cell-type. The density of m1+ and m2+ MAP2+ pyramidal neurons were significantly greater in upper (2-3) and deep (5-6) layers of A24 compared to A25 and A32 (*$p<0.05$, Two-Way ANOVA, all comparisons)*, which exhibited similar distributions. In all ACC areas, m1+ and m2+ CB+ and CR+ inhibitory neurons predominated in the upper layers, while PV+ inhibitory neurons were more prominent in the deep layers. Further, the density of m2+ receptors on presynaptic inhibitory axon terminals, labeled with vesicular GABAergic transporter (VGAT) was greater in the deep compared to the upper layers in all ACC areas. However, between-area comparisons showed a significantly greater density of m2+VGAT+ terminals in A25, suggesting more robust cholinergic suppression of inhibition, compared to A24 and A32. The density of m1+ and m2+ tracer+ AMY-targeting pyramidal neurons was greater in A25, suggesting stronger cholinergic modulation of amygdalar outputs. These findings reveal the anatomical substrate of diverse cholinergic modulation of local excitatory:inhibitory balance in distinct ACC areas and ACC outputs to the amygdala, in networks important for cognitive-emotional integration.

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Poster

405. Prefrontal Cortex Networks and Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 405.20

Topic: H.08. Learning and Memory

Support: ONR MURI N00014- 16-1-2832
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ONR N00014-22-1-2453

Title: Electric fields of Hebb's cell assemblies

Authors: *D. PINOTSIS[1], E. K. MILLER[2];
[1]U London/MIT, London, United Kingdom; [2]The Picower Inst. for Learning and Memory, MIT, Cambridge, MA

Abstract: Hebb introduced cell assemblies in his seminal work about 70 years ago. Today, cell assemblies are thought to describe groups of neurons coactivated when a certain memory, thought or percept is stored or processed. Here, we consider electric fields generated by cell assemblies. We show that they are more stable and reliable than neural activity. Fields appear to contain more information and to vary less across trials where the same memory was maintained. This stability can explain an open question in animal electrophysiology, known as representational drift: The exact neurons forming an assembly change from trial to trial. But something must stay the same. We here suggest that stability underlying memory maintenance is achieved at the level of the electric field. This is 'above' the brain, but still 'of' the brain. The field could direct the activity of participating neurons. To illustrate this idea, we analyzed local field potentials (LFPs) recorded during a working memory task. These were obtained using high resolution, multi-electrode arrays and allow one to capture details of neural activity at the microscopic level. During the task, the animals, were shown a dot in one of six positions on the edge of a screen that would then go blank. After the delay period, the animals saccaded to the position they just saw marked. Using deep neural networks and biophysical modeling, we obtained the latent space associated with each memory. Then, we mapped the latent state to the cortical patch occupied by the corresponding assembly. This allowed us to reconstruct the effective connectivity between different neuronal populations within the patch. Using a dipole model from electromagnetism, we predicted the electric field. Decoding analyses found that the field contained unique information associated with each remembered location and that training accuracy based on fields was higher compared to accuracy based on neural activity.

Disclosures: D. Pinotsis: None. **E.K. Miller:** None.

Poster

405. Prefrontal Cortex Networks and Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 405.21

Topic: H.08. Learning and Memory

Support: CIHR
CFI

Title: A neural subspace for encoding multiple associative memories in the primate lateral prefrontal cortex

Authors: *A. ROUZITALAB[1], C. BOULAY[2], J. C. MARTINEZ-TRUJILLO[3], A. J. SACHS[4]; [1]Univ. of Ottawa, Univ. of Ottawa, Ottawa, ON, Canada; [2]Ottawa Hosp. Res. Inst., Ottawa, ON, Canada; [3]Western Univ., Schulich Sch. of Med. and Dentistry, Robarts Institute, Western Univ., London, ON, Canada; [4]The Ottawa Hosp. Res. Inst., Ottawa, ON, Canada

Abstract: ***Introduction:*** The lateral prefrontal cortex (LPFC) of primates is thought to play a role in associativelearning. However, it remains unclear how neuronal ensembles in this area dynamically encode and storememories for arbitrary stimulus-response associations. We recorded the activity of neurons in the LPFC oftwo macaques during an associative learning task using multielectrode arrays. We assessed LPFC codingof associative memory for stimulus response associations (rules). First, we demonstrate neuronalpopulations show selectivity for rules once they are learned. We then used decoders applied to subsets ofthe data and found that the rule could be successfully decoded from the neural activity independently ofmovement parameters. Moreover, we showed that rules are encoded in low dimensional subspaces of neuralactivity, and the distance between rules in neural space correlates with the similarity between rules assessedbehaviourally. The distance in state space between the representations of the rules correlates with thesimilarity between the rules and behavioural performance.***Results:*** As animals learned a new rule over the course of few trials, the selectivity of multiunit clusters forthe learned rule increased (Fig. 1). After the cue presentation, information about the rule precededinformation about saccade direction (Fig. 2). We conducted a state space analysis and found that rules wereencoded in distinct neural activity subspaces, and that the distance between subspaces was correlated withthe similarity between rules and with the animals' performance (Fig. 3b). Thus, LPFC neuronal ensembleslearn new stimulus response associations mirroring behavioural performance during associative learningtasks (Fig. 3a). Such new rules are stored in neuronal subspaces in a manner that allow generalization ofprevious rule knowledge to novel associations as well as expansion of memory capacity.
LearnedUnlearned
Fig. 1 | Single Channel Analysis
Fig. 2 | Temporal Analysis of Rule and Saccade Decoding.ab
RSS
Fig. 3 | Impact of Rule Similarity on Learning

Disclosures: **A. Rouzitalab:** None. **C. Boulay:** F. Consulting Fees (e.g., advisory boards); Blackrock Neurotech. **J.C. Martinez-Trujillo:** None. **A.J. Sachs:** None.

Poster

405. Prefrontal Cortex Networks and Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 405.22

Topic: I.06. Computation, Modeling, and Simulation

Title: Implications of reduced inhibition in schizophrenia on human prefrontal microcircuit activity

Authors: *S. ROSANALLY, E. HAY;
Ctr. for Addiction and Mental Hlth., Toronto, ON, Canada

Abstract: Reduced performance when processing auditory oddball stimuli is commonly seen inschizophrenia and is associated with a reduced mismatch negativity in EEG signals from theprefrontal cortex. Post-mortem gene-expression studies indicate that a reduced inhibition byparvalbumin-expressing (PV) interneurons plays an important role in the functional and brainsignal deficits in schizophrenia, but this link remains to be established. We integrated humancellular, circuit and gene-expression data into detailed computational models of human corticalPFC microcircuit in health and schizophrenia, to mechanistically link the altered PV interneuroninhibition to deficits in oddball processing and EEG signals. We found a 25% significant increasein baseline activity in the schizophrenia microcircuit model due to reduced PV interneuroninhibition, and we differentiated the role of the two implicated mechanisms - reduced PVinterneuron inhibition onto other neurons vs reduced NMDA conductance onto PV interneurons.We then modelled the response activity during oddball processing to study the implications ofreduced PV interneuron inhibition on cortical processing, and to identify EEG biomarkers of thecircuit changes in schizophrenia. We found a 10% significant increase in pyramidal firing rates inthe schizophrenia model compared to the healthy model during the oddball response simulation,indicating a higher baseline activity and reduced mismatch negativity. Our study integrates diversehuman data to mechanistically link circuit changes to cortical function deficits and biomarkers inbrain signals.Figure 1. Detailed models of human prefrontal microcircuit baseline activity.

Disclosures: S. Rosanally: None. **E. Hay:** None.

Poster

406. Learning and Memory: Hippocampal: Cortical Interactions I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 406.01

Topic: H.08. Learning and Memory

Support: European Research Council (CoG, MECHIDENT, CIP)
BBSRC studentship (NLD DTP, BS)
Research and Training Supplementary Grant (BBSRC, BS)

Title: Transcranial ultrasound stimulation of primate hippocampus and prefrontal cortex modulates context-guided sequence learning

Authors: *B. SLATER[1], D. HOWETT[2], J. NACEF[1], A. EASTON[3], C. PETKOV[1,4], Y. KIKUCHI[1];
[1]Biosci. Inst., Newcastle Univ., Newcastle upon Tyne, United Kingdom; [2]Sch. of Psychological Sci., Univ. of Bristol, Bristol, United Kingdom; [3]Dept. of Psychology, Univ. of Durham, Durham, United Kingdom; [4]Dept. of Neurosurg., Univ. of Iow, Iowa, IA

Abstract: The hippocampus (HC) plays a key role in integrating spatial and temporal information. However, in natural scenarios different rules apply under different contexts. Contextual information could be hippocampal dependent, if it is integrated into a context-guided memory sequence (Eichenbaum, 2017: PMID 28655882). The prefrontal cortex could also be involved, if the memory is stored and accessed as a schema or when the rules change (Preston & Eichenbaum, 2013: PMID 24028960). The present study tested the effect of low-intensity focused Transcranial Ultrasound Stimulation (fTUS) in a primate model, using fTUS to perturb the anterior and posterior aspects of the hippocampus or medial prefrontal cortex during a context-guided sequence learning task. The task involved two spatial contexts implemented over double touch screen monitors attached to different parts of the macaque monkey's home unit. The background colours and physical locations established the two contexts and the rule with which a sequence of visual objects was to be identified in order (Context 1: 'A to B'; Context 2: 'C to D'). The macaque performed very well on the task (typically > 80% correct). Behavioural testing sessions and fTUS alternated with sham (no fTUS) conditions. During fTUS conditions, we used a protocol known to induce 'offline' fTUS effects in primates that can last for 1-2 hours (Verhagen et al., 2019: PMID 30747105). We used a Brain Sight (Rogue Research Inc) system for fTUS targeting of the anterior/posterior HC or the mPFC. Stimulation parameters were 40-s fTUS trains (250 kHz) comprising 30-ms bursts of ultrasound every 100 ms. After fTUS the animal was returned to their home cage and allowed to work on the task for approximately 2-3 hours. Preliminary results show that fTUS differentially modulated their behaviour depending on whether the anterior or posterior HC was stimulated. The stimulation of the anterior hippocampus increased performance under the fTUS condition compared to sham (Odds Ratio = 1.35, 95% Confidence Interval [1.10, 1.66], $p < .001$), whereas the stimulation of the posterior hippocampus decreased performance under the fTUS condition (OR = 0.70, 95% CI [0.52, 0.94], $p < .03$). fTUS of the mPFC is underway and we also aim to study the effects on reversal learning. These preliminary results indicate differential context-guided sequence learning effects during fTUS of the anterior/posterior axis of the hippocampus, and they help to advance the study of behaving primate fTUS with the potential for future study with humans (Kim et al., 2021: PMID 33935626). Support: European Research Council (CIP: ERC CoG, MECHIDENT); BBSRC studentship (NLD DTP; BS).

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Poster

406. Learning and Memory: Hippocampal: Cortical Interactions I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 406.02

Topic: H.08. Learning and Memory

Support: NIH Grant MH119391

Title: A Selective Role for the medial Prefrontal Cortex during Spatial Serial Reversal Learning

Authors: *R. M. GILLIS, K. S. KIDDER, S. J. Y. MIZUMORI;
Psychology, Univ. of Washington, Seattle, WA

Abstract: The medial prefrontal cortex (mPFC) and hippocampus (HPC) are known as critical structures in a network that supports spatial working memory and flexible decision making in rats. The HPC has traditionally been implicated in episodic and spatial memory, while the mPFC has been studied for its role in working memory, response inhibition, outcome evaluation, and implementation of task rules and strategies. Flexible decision making, or the ability of animals to alter their behavior in response to changing environmental contingencies, is often tested via reversal learning (RL) paradigms in rats, monkeys, and humans. However, the role of the mPFC in RL is debated and research investigating the mPFC in RL has produced conflicting results. In the present study, we elucidate the role of the mPFC in spatial RL by optogenetically disrupting the mPFC during specific task phases, or epochs, of a spatial RL task. Our data show that rats completed significantly fewer reversals when the mPFC is disrupted during the choice epoch of our task, and error analyses revealed an increase in regressive errors made by the rat during choice epoch mPFC disruption. Interestingly, error analyses also revealed that animals made significantly more perseverative errors when the mPFC was disrupted during the delay epoch of our task. These data suggest that the mPFC is involved in the active decision-making process at the choice point of our task, and that the mPFC has a role in updating strategies during the delay period of this task. These findings may explain how impaired behavioral flexibility could be caused by mPFC dysfunction in disorders of behavioral control such as depression, schizophrenia, and addiction.

Disclosures: R.M. Gillis: None. **K.S. Kidder:** None. **S.J.Y. Mizumori:** None.

Poster

406. Learning and Memory: Hippocampal: Cortical Interactions I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 406.03

Topic: H.08. Learning and Memory

Support: SFP1280

Title: Context-dependent single-unit correlates of extinction learning in the avian hippocampus and the nidopallium caudolaterale

Authors: *C. SEVINCIK[1], J. PACKHEISER[2], O. GÜNTÜRKÜN[1], R. PUSCH[1];
[1]Ruhr- Univ. Bochum, Ruhr Univ. Bochum, Bochum, Germany; [2]Netherlands Inst. for Neurosci., Amsterdam, Netherlands

Abstract: Extinction learning refers to the reduction of a conditioned response when the unconditioned stimulus or reinforcement is withheld. However, several relapse phenomena have been described that show that the initial memory trace is not vanished and that the conditioned response can reemerge under certain circumstances. Of these, the phenomenon of renewal is the context dependent reemergence of the conditioned behavior. In clinical settings, the existence of extinction and context-dependent renewal behavior can take up a major role in explaining pathological behaviors. Even though temporary and permanent lesion studies have stated that the hippocampus is crucial for context processing, real-time neural correlates of these phenomena have not been investigated. To reveal the cellular underpinnings of context processing in the avian extinction network, we recorded single-unit activity from the hippocampus of pigeons (*Columba livia*) with custom-made electrodes while the animals performed an extinction-learning paradigm. Our experimental task required the animals to first acquire a choice behavior under white ambient light conditions (context A). Subsequent extinction was performed under red ambient light conditions (context B). In a final test, light conditions changed back to the initial context to probe renewal behavior (known as ABA paradigm). 16% of the neurons we recorded (N=99) showed context-related activity changes at the transition from one context to another, either ramping their firing rate up or decreasing it. We compared these findings with recordings from the nidopallium caudolaterale (NCL), the avian equivalent of prefrontal cortex. We observed that only 5% of the cells (N=136) were reflecting this type of information. However, when we investigated the strength of these areas predicting the existence of the renewal behavior using receiver operating characteristics (ROC), the results were mirrored. While the area under the curve from the NCL was 65%, it was only 50% for the hippocampus. Therefore, our study provides evidence that the hippocampus signals context-related activity while the NCL exploits this information to govern proper behavioral output.

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Poster

406. Learning and Memory: Hippocampal: Cortical Interactions I

Location: SDCC Halls B-H

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Program #/Poster #: 406.04

Topic: H.08. Learning and Memory

Support: IBS-R015-D1

Title: Effective connectivity of the hippocampus and the medial entorhinal cortex following spatial learning measured by optogenetic fMRI

Authors: *C.-H. LEE[1], T. YOU[1,2,3], G. IM[1], S.-G. KIM[1,2,3];
[1]Ctr. for Neurosci. Imaging Res., Inst. for Basic Sci., Suwon, Korea, Republic of; [2]Dept. of Biomed. Engin., [3]Dept. of Intelligent Precisicion Healthcare Convergence, Sungkyunkwan Univ., Suwon, Korea, Republic of

Abstract: Recent studies reporting experience-dependent spatial representations in the early visual cortex (Saleem et al 2008, Diamanti et al., 2021), together with representations of navigation components in the parietal cortex (Sato et al., 2006) suggest a larger role for the neocortices in supporting spatial memory. However, little is known about how HPC and MEC, brain regions most known to be essential for spatial information processing, interact with multiple neocortical regions and how their interactions reflect spatial learning. In the current study, we combined optogenetics with functional magnetic resonance imaging (ofMRI) to investigate the effective connectivity (EC) of HPC and MEC in the whole brain. Wild-type C57BL/6 mice (male, n = 6) were injected with a retrograde AAV (AAV-hSyn-hChR2(H134R)-eYFP) in the right HPC (AP: -2.2 mm ML: 1.6 mm, DV: 1.3 - 2 mm) and implanted with optic fibers (diameter: 200 mm, NA = 0.37) in the left HPC and the right MEC (AP: -4.2 mm, ML: 3.4 mm, DV: 3.2 mm). Six weeks post-surgery, mice underwent an ofMRI scan (TR/TE = 1000/11.5 ms, 0.132 X 0.132 mm^2, 18 0.5-mm slices) in the 15.2T MRI scanner, where optogenetic stimulation (3-5mW, 30 Hz, 20% duty cycle) of the HPC or MEC was given in a block design (20s stimulation, 60s recovery, repeated twice per trial, 10 trials per condition). After the scan, mice were trained on the active place avoidance task (APAT) using a custom-built maze (Lee et al., 2022). On each day, two 30-minute trials were conducted with a 90-minute rest period between trials. In each trial, the animal was placed in the rotating maze and a foot shock (0.2mA, 60Hz) was given whenever the animal entered the pre-defined shock zone. Shock-zone configurations were changed when the animal had experienced at least four trials and shown less than five shocks in the last three 5-minute intervals. Each mouse learned four different shock-zone configurations for 2-3 weeks. Once the training was completed, the second ofMRI scan was conducted. In addition to the 30 Hz stimulation condition, 1 Hz and 5 Hz stimulation conditions were added, separately for HPC and MEC to investigate whether there were frequency-dependent EC differences. Based on our data, HPC EC was stronger with sensory and parietal cortices while MEC EC was stronger with higher-order cortices (e.g. mPFC). Within the HPC, there was frequency-dependent modulation to HPC stimulation ($F=35.58$, $p=0.001$, repeated measures ANOVA), with 30 Hz stimulation showing stronger EC ($p=0.001$, Wilcoxon signed-rank test). Our results suggest a way to map potentially differentiated connections between HPC and MEC to neocortices in a frequency-dependent manner using an exploratory fMRI approach.

Disclosures: C. Lee: None. **T. You:** None. **G. Im:** None. **S. Kim:** None.

Poster

406. Learning and Memory: Hippocampal: Cortical Interactions I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 406.05

Topic: H.08. Learning and Memory

Support: NSF T32 1829071

Title: Lateral entorhinal cortex temporoammonic projections to CA1 encode odor-specific information during working memory

Authors: *C. DORIAN[1], J. TAXIDIS[2,4], S. CHEUNG[3], P. GOLSHANI[5];
[1]Univ. of California, Los Angeles Interdepartmental Ph.D. Program In Neurosci., Los Angeles, CA; [2]Neurol., [3]UCLA, Los Angeles, CA; [4]SickKids Res. Inst., Toronto, ON, Canada; [5]UCLA Dept. of Neurol., Los Angeles, CA

Abstract: Hippocampal CA1 neurons generate sequential firing during the performance of a working memory task, encoding both odor and the passage of time. How does CA1 integrate its various inputs to give rise to sensory and temporal representations? Lateral entorhinal cortex (LEC) neurons receive olfactory inputs, project to CA1 distal dendrites through the temporoammonic path, and have been shown to represent the passage of time over long durations. We asked whether LEC projections to CA1 encode odor and temporal specific information during working memory performance. We therefore conducted 2-photon calcium imaging of LEC axons expressing GCaMP7s within the stratum lacunosum moleculare of CA1, while mice performed an olfactory-based delayed non-match to sample working memory task with a 5 second delay. Our preliminary data shows that 16% of axons have significant firing fields during the first odor presentation or delay period in our task. Of those, 73% fire maximally during odor presentation, 18% during the 1st second of the delay, and the other 9% during the remaining 4 seconds of the delay. Compared to CA1 sequential representations, there are much fewer temporal representations tiling the entire delay between the 2 odors. This suggests LEC inputs contribute to odor specific firing during the odor presentation period, but most likely do not play a significant role in encoding the passage of time during the delay period. We are currently performing experiments to gauge the effects of LEC inhibition on hippocampal sensory and temporal representations. Based on our initial axon imaging results, we predict that CA1 multimodal representations will become disrupted and be less stable when inhibiting EC inputs to CA1.

Disclosures: C. Dorian: None. **J. Taxidis:** None. **S. Cheung:** None. **P. Golshani:** None.

Poster

406. Learning and Memory: Hippocampal: Cortical Interactions I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 406.06

Topic: H.08. Learning and Memory

Title: Cognitive deficits in mouse model of Fragile X Syndrome can be attributed to specific dysfunction in recruitment of memory ensembles in the ventral hippocampus-PreLimbic cortex axis

Authors: K. BHANDARI[1], P. CARONI[2];
[1]Friedrich Miescher Inst. for Biomed. Res., Basel, Switzerland; [2]Neurosci., FMI - 1066.3.38, CH-4002 Basel, Switzerland

Abstract: Fragile X Syndrome (FXS) is the most common form of intellectual disability and a leading monogenic cause of autism spectrum disorder (ASD). The protein whose expression is lost in FXS (FMRP) is expressed ubiquitously in the brain, where it has important roles for synaptic function and plasticity. Whether the defects in FXS, and their potential therapeutic alleviation, reflect ubiquitous loss of function or deficits specific to particular brain circuits has remained unclear. Here we show using brain area specific conditional knockout of FMRP (KO) in principal neurons, that area-specific loss in adult wildtype (WT) mice behaviorally mimics acute silencing of the same area. However, in Fmr1(y/-) mice despite the ubiquitous absence of FMRP, behavioral and immediate early gene (IEG) expression analyses, revealed that cognitive functions of most brain areas were unaffected by the absence of FMRP, with the exception of ventral Hippocampus (vH) and Prelimbic cortex (PreL). Using conditional restoration of FMRP in Fmr1(y/-), we found that the specific behavioral deficits of Fmr1(y/-) mice can entirely be accounted for by absence of FMRP in a vH-PreL axis. Mechanistic investigations revealed that despite of apparently normal re-expression of cFos at recall in vH neurons of Fmr1(y/-) mice, vH cFos+ ensemble recruitment was insufficient for cognitive function.

Disclosures: K. Bhandari: None. **P. Caroni:** None.

Poster

406. Learning and Memory: Hippocampal: Cortical Interactions I

Location: SDCC Halls B-H

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Program #/Poster #: 406.07

Topic: H.08. Learning and Memory

Support: Whitehall Foundation Research Grant 2018-08-88
Brain and Behavior NARSAD Young Investigator Grant 27668
Alzheimer Association Research Grant AARG-21-850571
NIH Grant 5R01MH06364917

Title: Non-waking sounds during sleep impair hippocampal memory consolidation

Authors: *K. SALGADO-PUGA[1], E. NELSEN[1], G. ROTHSCHILD[1,2];
[1]Psychology, Univ. of Michigan, Ann Arbor, MI; [2]Dept. of Otolaryngology - Head and Neck Surgery, Kresge Hearing Res. Inst., Ann Arbor, MI

Abstract: Sleep is critical for the process of memory consolidation- the stabilization of recent labile memory traces into long-term memories. A key neurophysiological mechanism supporting memory consolidation are hippocampal sharp-wave ripples (SWRs)-brief oscillatory bursts of hippocampal activity that occur during non-rapid eye movement sleep (NREMs) and waking quiescence. SWRs during sleep facilitate bidirectional communication between the hippocampus and cortical regions and causally support memory consolidation. Although sleep is often referred to as an "offline" state, conducive of internally-generated activity patterns such as SWRs, the brain is not fully disconnected from the environment in this state. In particular, sounds heard during sleep evoke robust responses throughout the auditory pathway. Although the consequences of sound processing during sleep beyond the auditory pathway is less understood, anatomical and functional studies have demonstrated that sounds can influence hippocampal activity. However, whether and how incoming sounds during sleep impact hippocampal SWRs and their memory consolidation function remains largely unresolved. To address this gap, in this study we used a closed-loop system in rats to detect SWRs in the dorsal CA1 region of the hippocampus during sleep and pair them with brief non-waking broad-band noise (BBN) bursts. We found that exposure to BBN bursts during sleep suppressed the ripple power and reduced the rate of SWRs. Furthermore, BBN triggered during SWRs (On-SWR-BBN) suppressed ripple power significantly more than BBN triggered 2 seconds after SWRs (Off-SWR-BBN). Next, we used this system to test the influence of BBN presentation during sleep on memory consolidation. To this end, SWR-triggered BBN bursts were applied during sleep sessions following learning of a conditioned place preference paradigm, in which rats learned a place-reward association. The influence of sound presentation during sleep on memory was quantified by comparing memory retention performance following the SWR-BBN paradigms to that of no BBN exposure. Interestingly, we found that On-SWR-BBN pairing during sleep impaired memory consolidation, as evidenced by a dramatic reduction in memory retention 24h following learning. Moreover, Off-SWR-BBN pairing also impaired memory retention at 24h, but to a lesser extent. These findings suggest that sounds heard during sleep interfere with SWR-facilitated memory consolidation.

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Poster

406. Learning and Memory: Hippocampal: Cortical Interactions I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 406.08

Topic: H.08. Learning and Memory

Support: R01 MH060941
T32 MH106454

Title: Negative emotional overlap in events may impede inference through differentiation

Authors: *A. NADIADWALA[1,4], J. E. DUNSMOOR[1,4,2], A. R. PRESTON[1,4,3];
[1]Neurosci., [2]Psychiatry, [3]Psychology, Univ. of Texas at Austin, Austin, TX; [4]Ctr. for Learning and Memory at Univ. of Texas at Austin, Austin, TX

Abstract: Everyday, we experience events that share people, places, and objects with past events. It is adaptive to combine such distinct but related experiences to build generalized knowledge structures. Likewise, it is oftentimes important to differentiate between the details of related experiences to limit overgeneralization. Understanding how emotion may affect the tradeoff between integration and differentiation is important, as it may reveal how the brain organizes memory across emotional experiences to guide behavior in both adaptive and maladaptive ways. Here, we investigated whether memory integration is enhanced or reduced in emotional events by behaviorally indexing inference judgment between overlapping individual memories. We used an associative inference paradigm wherein subjects learned an object-face association (AB) followed by a new object-face association comprising an overlapping face stimulus and a new object (BC). Critically, the face linking the two distinct objects was either neutral or expressing fear. In the final inference test, subjects were tasked with inferring a relationship between the two objects (AC) that had never been directly paired. Results from three online experiments (total N = 108) showed that AC inference was less accurate for objects linked by a common fearful face as compared to a common neutral face. Further, inference differences remained when we sequentially presented stimuli in image pairs to rule out the possibility of attentional capture by the negative content. An additional online experiment (N = 36) indicated no difference in associative inference for objects linked by a common happy face relative to those linked by a neutral face, suggesting that fear, in particular, exerts a powerful influence on how memories are organized. These results suggest that distinct experiences linked by a negative emotional element, such as fear, promote memory differentiation rather than integration. These findings provide a new perspective that negative emotional elements may protect memory from interference introduced by future events, thereby preserving the details of these experiences as distinct episodes in long-term memory. Such an organization may be adaptive, as it may allow individuals to avoid the precise event elements in the future that were associated with fearful experiences in the past.

Disclosures: A. Nadiadwala: None. **J.E. Dunsmoor:** None. **A.R. Preston:** None.

Poster

406. Learning and Memory: Hippocampal: Cortical Interactions I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 406.09

Topic: H.08. Learning and Memory

Support: NIH Grant MH124004
NIH Shared Instrumentation Grant S10OD020039
NSF GRFP Grant DGE1745303

Title: Within-individual assessment of parallel networks along the hippocampal long axis

Authors: *P. A. ANGELI[1], L. M. DINICOLA[1], N. SAADON-GROSMAN[1], R. L. BUCKNER[1,2];
[1]Harvard Univ., Cambridge, MA; [2]Massachusetts Gen. Hosp., Boston, MA

Abstract: Functional specialization along the long axis of the hippocampus has been extensively studied in both animal models and humans. One approach to gain traction on this issue in humans is to use functional connectivity network assignment as a clue to functional subdivisions of the hippocampus. Using such an approach, Zheng et al. (2021 *PNAS*) recently discovered that the human anterior and posterior hippocampus display functional connectivity to different cortical networks: the Default Network (DN) and Parietal Memory Network (PMN) respectively. To study this novel dissociation and its functional implications, we explored data from two independent studies (n = 9, n =11) that each included individuals repeatedly scanned at high resolution during fixation (for functional connectivity analysis) and also while performing a battery of cognitive tasks (including episodic remembering and prospection). Functional connectivity analysis in Study 1 replicated in all individuals the previous finding from Zheng et al. (2021) of DN connectivity to the anterior hippocampus and PMN connectivity to the posterior hippocampus. The anterior hippocampus is primarily associated with DN-A, a cortical network within the canonical Default Network recruited for episodic projection and scene construction (DiNicola et al. 2020 *J Neurophys*, 2022 *bioRxiv*), and less so other networks including DN-B. Cortical connectivity patterns seeded from the anterior and posterior hippocampal regions exhibit robust differences, including diagnostic features of DN-A and PMN respectively, confirming dissociation between the two. Utilizing the task data, direct analysis of the anterior hippocampal region showed a strong response to episodic remembering and prospection, with the response level across trials robustly tracking scene construction (Hassabis & Maguire 2009 *Phil Trans R Soc B*). There was minimal or no response variation to self-oriented processing demands. Prospective replication of these results is now underway for the Study 2 data. Our initial findings replicate Zheng et al. and further show that the anterior hippocampal region responds to scene (spatial) information suggesting a connection to rodent and non-human primate neurophysiological studies.

Disclosures: P.A. Angeli: None. **L.M. DiNicola:** None. **N. Saadon-Grosman:** None. **R.L. Buckner:** None.

Poster

406. Learning and Memory: Hippocampal: Cortical Interactions I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 406.10

Topic: H.08. Learning and Memory

Title: Cognitive and tryptophan catabolism alterations in two models of prenatal stress in mice

Authors: *J. CHÁVEZ, D. RAMÍREZ, V. PÉREZ;
Natl. of Neurol. and Neurosurg. Inst., Mexico City, Mexico

Abstract: Lots of evidence demonstrate that prenatal stress (PS) leads to neuropsychiatric disorders in humans, including depression, schizophrenia and cognitive dysfunction. The last one has been mostly related with changes in glucocorticoids at cerebral level in fetus and adult offspring; nevertheless, the molecular mechanisms involved in this process have not been well elucidated. In this context, kynurenic acid (KYNA), a tryptophan metabolite, has been linked with cognitive dysfunction in several experimental models. The aim of this study was to characterize the cognitive and tryptophan catabolism alterations in two mice models of PS. We carry out two experimental models of PS on C57BL/6 mice; in the first one, the dams were exposed to the odor predator (PS-P) at 14 to 19 days of gestation for 60 min daily, and in the second one, the dams were placed in an acrylic holder (PS-I) for 45 min daily three times per day at 14 to 21 days of gestation. Cognitive function and glutamate, glutamine, KYNA and 3-hydroxykinurenine (3-HK) brain levels were evaluated in offspring at 60 postnatal day (PND). A deficit in long term memory at the novel object recognition paradigm and elevated T-maze in both models were found. Interesting, there was an increase in 3-HK and glutamine, while glutamate brain levels decreased compared to control group. No changes in KYNA brain levels neither in PS-P and PS-I models compared to control were found. These data suggest that the PS models analyzed induce cognitive impairment and neurotransmitter and tryptophan catabolism alterations. Further studies are needed to elucidate the mechanisms and cognitive alterations involved in both PS models.

Disclosures: **J. Chávez:** None. **D. Ramírez:** None. **V. Pérez:** None.

Poster

406. Learning and Memory: Hippocampal: Cortical Interactions I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 406.11

Topic: H.08. Learning and Memory

Title: A ventromedial visual stream to the hippocampus for ‘where’ scene representations in humans, with idiothetic update by a parietal cortex dorsal visual stream, for memory and navigation

Authors: *E. T. ROLLS;
Computat. Neurosci., Oxford Ctr. For Computat. Neurosci., Coventry, United Kingdom

Abstract: The human hippocampus is involved in episodic memory and navigation, but the information used for this, and how it reaches the hippocampus, is still not clear and is considered in this research. The effective (directed, causal) connectivity between 360 cortical regions defined in the Human Connectome Project Multimodal Parcellation atlas (HCP-MMP) was measured in 171 HCP participants, and complemented with functional connectivity and diffusion

tractography.
A Ventromedial Visual 'Where' Stream for scene representations has effective connectivity in the pathway V1 - V2 - V3 - V4 - Ventromedial Visual regions VMV1-3 and medial parahippocampal regions PHA1-3 which is the Parahippocampal Scene Area (PSA or PPA) where spatial view cells are found in macaques. It is proposed that scene representations are formed in this pathway by overlapping visual features in scenes that form a continuous attractor network representation of scenes.
A Dorsal Visual Stream connects via V2 and V3A to MT+ Complex regions (including MT and MST), which connect to intraparietal regions (including LIP, VIP and MIP) involved in visual motion and actions in space. This stream performs coordinate transforms for idiothetic update, and has effective connectivity to the Parahippocampal Scene Area, where it is proposed to implement the idiothetic update of spatial view cells as discovered in macaques.
A Ventrolateral Visual 'What' Stream for object and face recognition projects hierarchically from V1 - V2 - V3 - V4 - FFC (Fusiform Face Cortex) - inferior temporal cortex TE regions, and has effective connectivity to the human hippocampus via lateral parahippocampal cortex TF. The human hippocampal system can then form combinations of these 'What' inputs from the ventrolateral stream with 'Where' inputs from the Ventromedial Visual Stream and Reward Inputs from the orbitofrontal cortex to implement episodic memory, and navigation from landmark to landmark.
Novel features for the human hippocampal system compared to rodents are the use of visual scene information facilitated by the foveal design of the primate visual system; path integration for eye position, head direction etc performed extra-hippocampally in the dorsal visual stream; and connectivity from the orbitofrontal cortex and anterior cingulate cortex to the cholinergic basal forebrain nucleus that connects to neocortex and the septal nuclei that connect to the hippocampus that are important in long-term memory consolidation and contribute to the memory deficits associated with vmPFC damage.

Disclosures: E.T. Rolls: None.

Poster

406. Learning and Memory: Hippocampal: Cortical Interactions I

Location: SDCC Halls B-H

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Program #/Poster #: 406.12

Topic: H.08. Learning and Memory

Support: R01 AG076198
Harlan Scholars Program
Harlan Scholars Program

Title: Optogenetic Dissection of Rearing and Spatial Memory: Function and Neurophysiology

Authors: *D. LAYFIELD, S. CASSITY, N. SIDELL, K. BLANKENBERGER, E. L. NEWMAN;
Dept. of Psych. and Brain Sci., Indiana Univ. Bloomington, Bloomington, IN

Abstract: Exploration is critical for spatial memory and navigation. In rodents one form of exploratory behavior is rearing, where rats briefly stand on their hind legs and visually scan their environment. A behavior theorized to aid in spatial information gathering from distant sources. While rearing has been used as a measure of novelty and exploration, the behavior itself and its links to spatial learning and memory remain poorly understood. In a series of experiments, we aim to further elucidate the behavior of rearing, including its function and neural underpinnings. We first tested the hypothesis that hippocampal activity during rearing events is critical for spatial memory. To test this hypothesis, we assessed performance in rats performing an 8-arm maze delayed-win-shift task (DWST) when the dorsal hippocampus was optogenetically inactivated in a closed-loop fashion controlled by rearing behavior. We find that hippocampal inactivation during rearing impairs memory performance. Previously it has been hypothesized that hippocampal control of rearing is largely cholinergic, with acetylcholine promoting an exploratory mode. To test this hypothesis, we again utilized the DWST, and optogenetically inactivated the cholinergic neurons of the medial septum which provide the main cholinergic input to the hippocampus. We find that spatial memory performance and rearing frequency is unaffected. Our findings provide evidence that hippocampal activity during rearing can be critical for spatial memory and, acetylcholine can modulate rearing but an assumed reduction in hippocampal acetylcholine is not sufficient for preventing rearing behavior. We propose that 1. Rearing can be critical for spatial memory where distal cues can be utilized to guide behavior and 2. Cholinergic neurons of the medial septum do not promote exploration. Together these findings demonstrate that hippocampal activity occurring during rearing events can be critical for spatial memory and identify rearing behavior as a target for increasing our understanding of hippocampal function, exploration and memory.

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Poster

406. Learning and Memory: Hippocampal: Cortical Interactions I

Location: SDCC Halls B-H

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Program #/Poster #: 406.13

Topic: H.08. Learning and Memory

Support: NIH Grant (1U01NS108923-01)
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NSF Career Award (1944415).

Title: Hippocampal-cortical coupling differentiates long-term memory processes

Authors: *P. DAHAL[1], O. J. RAUHALA[2], D. KHODAGHOLY[2], J. N. GELINAS[3]; [1]Columbia Univ., [2]Columbia Univ., New York, NY; [3]Columbia Univ. Irving Med. Ctr., New York, NY

Abstract: Newly formed memories become more stable and durable through memory consolidation. Reactivation of a consolidated memory can reopen a window of lability, after which the memory can be strengthened, updated, or weakened depending on the nature of the retrieval experience. Naturalistic behaviors strongly hinge on this flexible modulation of long-term memory because the salience of environmental stimuli frequently changes. Although coupling of hippocampal and cortical activity patterns facilitate initial memory consolidation, whether and how these network patterns are involved in post-reactivation memory processes and enable this behavioral flexibility is not known. In this study, we monitored the hippocampal-cortical network as rats performed cycles of spatial and nonspatial long-term memory tasks that required repeated consolidation, reconsolidation, and updating within a consistent behavioral schema. Neurophysiologic sleep recordings were performed after each behavior session, and we analyzed the trajectory of oscillatory coupling during NREM sleep. We show that interactions between hippocampal sharp wave-ripples, cortical spindles, and cortical ripples are jointly modulated in the absence of memory demand, but independently recruited depending on stage of memory and task type. Reconsolidation of memory after retrieval is associated with an increased and extended window of coupling between hippocampal sharp wave-ripples and cortical ripples compared to the initial consolidation. Furthermore, this coupling is temporarily suppressed when reward associations are updated. Hippocampal sharp wave-ripple and cortical spindle interactions are preferentially engaged during memory consolidation. This coupling is strongly modulated by this spatial, but not nonspatial task and regulated independently of hippocampal ripple-cortical ripple coupling, suggesting separate network functions. These results suggest that specific, time-limited patterns of oscillatory coupling can support the distinct memory processes required to flexibly manage long-term memories in a dynamic environment. Our findings may be useful in identifying markers of different ongoing memory processes and understanding mechanisms that lead to both impaired and over-active information retention.

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Poster

406. Learning and Memory: Hippocampal: Cortical Interactions I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 406.14

Topic: H.08. Learning and Memory

Title: Hippocampus as a Novelty Detector for Episodic Memory

Authors: *T. TO, H. YOO, A. M. HASSIEN, R. TAN, J. KRIEGEL, B. C. LEGA; Neurolog. Surgery, UT Southwestern Med. Ctr., Dallas, TX

Abstract: Models of episodic memory processing posit complementary roles for the anterior and posterior hippocampus during the processing of novel stimuli. These models, developed principally from non-invasive data, suggest that the anterior hippocampus and associated rhinal areas exhibit elevated activity for the encoding of novel items, linked with a more general role in affective processing. However, the specific electrophysiological patterns that support longitudinal differences in novelty processing have not been determined using human intracranial recordings. We sought to compare activity in the theta frequency band between associative versus absolute novelty using the associative recognition memory paradigm. We hypothesized that the anterior hippocampal regions would exhibit increased theta oscillatory activity during processing of completely novel items as well as "rearranged" items, which exhibit novelty only in terms of associative information. By contrast, we predicted that the posterior hippocampus would exhibit theta oscillatory responses during associative novelty exclusively. Epilepsy patients consented to undergo an associative memory recall task, in which word pairs were presented to the patient during the 2-second encoding phase. During the recall phase, word pairs were then again shown to the patients who determined whether these pairs were new, rearranged, or intact. EEG data was collected from the patient, and various filtering techniques such as kurtosis were used to remove noisy electrodes. Linear mixed-effect modelling was then used to consider random effects due to subjects and electrodes in analyzing power. We additionally analyzed the phase-reset of these signals as well as traveling waves in hopes of obtaining directionality of these observed signals. Through these models, we not only found differences between novelty and associative misses, but also differential activity between associative novelty (word pairs that were rearranged) and absolute novelty. Our results support updated conceptions of models of hippocampal longitudinal specialization such as "gist/detail" models and affective models.

Disclosures: T. To: None. **H. Yoo:** None. **A.M. Hassien:** None. **R. Tan:** None. **J. Kriegel:** None. **B.C. Lega:** None.

Poster

406. Learning and Memory: Hippocampal: Cortical Interactions I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 406.15

Topic: H.08. Learning and Memory

Support: NSERC

Title: Representational drift of contextual fear engrams across the brain

Authors: T. WANG, B. DUNGATE, ***J. SNYDER**;
Univ. of British Columbia, Vancouver, BC, Canada

Abstract: The physical manifestation of memory is an "engram", the population of neurons that is activated during a learning experience and, when reactivated, contributes to the process of

memory retrieval and subsequent behaviour (Josselyn & Tonegawa, 2020). To maintain stable memories, it has long been assumed that neural representations must also be stable (Guzowski et al., 1999; Reijmers et al., 2007). However, memories are dynamic and recent investigations also suggest that neural representations are more fluid than formerly thought. In the hippocampus, a structure that is critical for daily event memory, neurons previously recruited during an experience show variation through time, a process called representational drift (Sweis et al., 2021). This phenomenon has gone unnoticed because traditional electrophysiological approaches often only record from identified neurons on a timescale of minutes to hours (Leutgeb et al., 2005). However, modern mouse models (or Ca2+ imaging), which permanently label activated neurons, suggest variable reactivation rates in the hippocampus over days (Ramirez et al., 2015; Redondo et al., 2014). How representations drift over even longer intervals remains unclear, but is important to understand how short- vs long-term memories are used to guide future behavior. Since perception and memory rely on many brain regions, and drift has been identified in areas outside of the hippocampus (Rule et al., 2019), true understanding of representational stability requires a broad network-level approach. A recent study stresses this idea, demonstrating that activity in individual regions fail to correlate with fear memory retrieval (Santos et al., 2021). Here, to characterize representational drift across sensory and associational regions of the brain, FosTRAP mice and activity-dependent tagging are used to indelibly label activated neuronal populations during two identical contextual fear conditioning events, at recent and remote timepoints. This work will help to identify the extent to which representations drift in individual brain regions and brain-wide networks that are involved in perception and memory.

Disclosures: **T. Wang:** None. **B. Dungate:** None. **J. Snyder:** None.

Poster

406. Learning and Memory: Hippocampal: Cortical Interactions I

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Program #/Poster #: 406.16

Topic: H.08. Learning and Memory

Support: ERC-2018-COG 819814

Title: Human hippocampal sharp wave ripples tune cortical responses in uncertain visual contexts

Authors: *D. FRANK[1], S. MORATTI[2], J. SARNTHEIN[3], A. GIL-NAGEL[4], R. TOLEDANO[4], B. A. STRANGE[5];
[1]Univ. Politécnica De Madrid, Madrid, Spain; [2]Univ. Complutense Madrid, Univ. Complutense Madrid, Pozuelo De Alarcón (madrid), Spain; [3]Dept. of Neurosurg. Univ. Hosp. and Univ. of Zurich, Zurich, Switzerland; [4]Hosp. Ruber Internacional, Hosp. Ruber Internacional, Madrid, Spain; [5]Univ. Politécnica de Madrid, Madrid, Spain

Abstract: To adaptively encode information into memory, the hippocampus should be able to extract statistical regularities in the environment and generate possible future events. Indeed, previous fMRI findings show that the anterior hippocampus tracks the expected uncertainty of an event in a given context (Strange et al., 2005). Such generative hippocampal responses can then inform cortical processing of upcoming sensory inputs. Here, we used intracranial EEG in human patients to examine the pre- and post-stimulus temporal dynamics underlying hippocampal and visual cortex sensitivity to contextual uncertainty (Shannon's entropy) and surprise (a given event's improbability). Fifteen medication-resistant epilepsy patients participated in the experiment. All patients had depth electrodes implanted in the anterior hippocampus, eight patients also had contacts in occipital cortex, and seven patients had contacts in the fusiform gyrus. On each trial, patients were presented with a coloured shape, and performed a visuo-motor task. Two colours and two shapes were combined to form four possible stimuli per block. In each block, there were likely and unlikely stimuli, varying surprise within-block; the entropy of stimuli varied across blocks (different probability distribution of the four stimuli in each block). Time-frequency analysis utilized a trial-wise GLM approach, with two predictors of interest: entropy and surprise. Furthermore, we detected hippocampal sharp wave ripples (80-120 Hz) and examined their frequency of occurrence as a function of entropy and peri-stimulus time. We found increased hippocampal ripple rate in high entropy contexts around 800 ms pre-stimulus, as well as a negative association between entropy and gamma power (47-97 Hz) around the same time. Pre-stimulus hippocampal ripples also modulated cortical activity in the occipital cortex by suppressing gamma power (35-135 Hz). Pre-stimulus ripples were associated with an earlier latencyfusiform gamma response (40-95 Hz) to surprise, compared to trials without a ripple. These findings point to a role for hippocampal ripples as a mechanism through which predictions are generated and propagated to cortex to minimize prediction error. This mechanism may facilitate episodic memory by preparing hippocampal-cortical dynamics for encoding as a function of environmental unpredictability.

Disclosures: D. Frank: None. **S. Moratti:** None. **J. Sarnthein:** None. **A. Gil-Nagel:** None. **R. Toledano:** None. **B.A. Strange:** None.

Poster

406. Learning and Memory: Hippocampal: Cortical Interactions I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 406.17

Topic: H.08. Learning and Memory

Support: NIH Grant 5R01NS112366-03

Title: Overlap between events has different consequences for learning and memory when events overlap in location versus content information

Authors: *B. CHALOUPKA, D. ZEITHAMOVA;
Psychology, Univ. of Oregon, Eugene, OR

Abstract: Overlap with prior knowledge can facilitate new learning via integration across experiences, but also hinder learning and memory through interference. Research on facilitation and research on interference also implicate two distinct hippocampal codes to benefit memory. Facilitation research has shown that overlapping events can be integrated, accompanied by similar neural representations. In contrast, interference research has shown that overlapping events tend to get separated or represented as exceedingly dissimilar to resolve interference. We designed a task that allowed us to investigate these two disparate effects simultaneously. Participants learned a grid of object-location associations, then they learned a second grid that overlapped with the first in objects, locations, both objects and locations, or did not overlap with the first (new objects, new locations). Behaviorally, we found that content overlap (re-use of the same objects) hindered learning of the second grid, while location overlap (re-use of the same locations) facilitated learning of the second grid, as compared to no-overlap baseline. We found no interaction, suggesting that these effects are independent and additive. To determine how overlap is represented in the brain, we scanned participants while they were engaged with the grid task and used pattern similarity analysis to measure hippocampal representations of each grid. We found that in anterior hippocampus, grids that overlapped in locations showed greater neural pattern similarity, in line with facilitation of overlapping events. Grids that overlapped in objects were not represented as any more or less similar than grids that contained different objects, suggesting pattern-separated representations. These results indicate that overlap between events may be represented differentially in the brain under conditions of facilitation versus interference. No reliable effects were found in the posterior hippocampus. Taken together, our results demonstrate the nuances of information overlap. Some types of overlap cause facilitatory effects on learning and memory while others cause detrimental effects. Additionally, neural representations of complex stimuli may differ depending on the behavioral relevance of different types of overlap.

Disclosures: **B. Chaloupka:** None. **D. Zeithamova:** None.

Poster

406. Learning and Memory: Hippocampal: Cortical Interactions I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 406.18

Topic: H.08. Learning and Memory

Support: ONR MURI N00014-16-1-2832
ONR DURIP N00014-17-1- 2304
NSF BCS 1625552

Title: Hierarchical gradients in prefrontal cortex and hippocampus support context-dependent rule learning

Authors: *T. M. MORIN[1], M. F. DUNNE[1], A. E. CHANG[2], C. E. STERN[3];
[1]Grad. Program for Neurosci., [2]Psychological and Brain Sci., [3]Cognitive Neuroimaging Ctr., Boston Univ., Boston, MA

Abstract: Recent work from our lab has provided evidence that the human hippocampus exhibits an anterior-posterior gradient of organization for the retrieval of hierarchical task rules (Brown et al., 2021). Similarly, prefrontal cortex is organized along a rostro-caudal axis with more rostral regions important for incorporating abstract information and more caudal regions implementing immediate motor actions (Badre & Nee, 2018). In this study, we demonstrate that successfully learning context-dependent rules is associated with systematic changes in functional connectivity along the gradient organization of the hippocampus and prefrontal cortex. We used an fMRI dataset collected from twenty-nine naïve human participants as they learned context-dependent rules through trial and error (Morin et al., 2021). For each subject, we manually segmented the hippocampus into head, body, and tail seed-regions, and examined dynamic functional connectivity during learning. The results demonstrated that the hippocampal head showed increased functional connectivity with lateral frontal pole and the caudate during successful learning. The hippocampal body and tail showed less widespread increases in functional connectivity with more caudal regions of prefrontal cortex. Additionally, the hippocampal head (but not the body and tail) showed reduced functional connectivity with posterior regions of the default network (precuneus and posterior cingulate) during successful learning. The observed functional connectivity changes were strongest during the cue period of the task, when subjects used contextual information to determine which rule to use. This study demonstrates that increased functional connectivity between hippocampal head and the lateral frontal pole/caudate supports the representation of successfully learned contexts when implementing context-dependent rules. We show that this connectivity pattern emerges in successful learners as they learn, but not in a natural control group of unsuccessful learners. We suggest that representations of higher order contexts and rules are supported by hierarchically organized functional connectivity between the hippocampus and prefrontal cortex.

Disclosures: T.M. Morin: None. **M.F. Dunne:** None. **A.E. Chang:** None. **C.E. Stern:** None.

Poster

406. Learning and Memory: Hippocampal: Cortical Interactions I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 406.19

Topic: H.08. Learning and Memory

Support: Leibniz Institute for Neurobiology, Magdeburg, Germany

Title: Bridging further human and animal memory research using 9.4T fMRI in awake rats

Authors: *L. MAHNKE[1], C. CHWIESKO[3], P. WENK[2], M. HOEHN[4], F. ANGENSTEIN[5], M. SAUVAGE[1];

[1]Functional Architecture of Memory Dpt, Leibniz Inst. for Neurobio., Magdeburg, Germany; [2]Leibniz Inst. for Neurobio., Combinatorial NeuroImaging Core Facility, Germany; [3]Neurobio. and behavior, UCI, Irvine, CA; [4]Inst. 3 for Neurosci. and Med. (INM-3), Res. Ctr. Jülich, Jülich, Germany; [5]Functional Neuroimaging Group, Deutsches Zentrum Für Neurodegenerative Erkrankungen (DZNE), Magdeburg, Germany

Abstract: Retrieving memories is crucial for daily-life events. Damage to the Medial Temporal Lobe (MTL), which includes the hippocampus (HIP), leads to severe memory deficits. Most MTL studies investigating memory and its neural substrates in humans rely on 3T functional magnetic resonance imaging (fMRI). Studies with lesions circumscribed to a specific MTL area (or HIP subfield) are very scarce, hindering the characterization of the specific contribution of these regions to memory function. The MTL cytoarchitecture and function are conserved across a wide range of species including rodents for which controlled targeted invasive approaches are possible. However, fMRI-compatible tasks taxing memory function are not available in rodents to date nor is scanning rats awake or analyzing these data in a comparable manner as in humans. Here, we report in awake rats BOLD patterns comparable to those reported in humans for recognition memory, which suggest a preponderant role of the HIP and the cingulate cortex (Cg) in this process. For this purpose, a 9.4T fMRI compatible memory task involving the presentation of a high number of stimuli was used. Additionally, BOLD responses to the presentation of stimuli for which a memory could be formed and BOLD responses to new stimuli were compared at the group level using SPM (i.e., 'old' vs 'new' contrast). Furthermore, we brought evidence that scanning animals awake within this framework is necessary, as scanning animals sedated under the same conditions did not yield any relevant clusters. Taken together, we report similar findings in rats and humans for recognition memory using similar experimental conditions in both species (awake scanning, group analysis, and similar contrasts). Moreover, we underline the necessity of scanning animals awake to maximize the likelihood of detecting BOLD signal. This approach contributes to further bridging human and animal memory research and paves the way for future investigations aiming at combining fMRI in awake rats and invasive approaches (such as permanent or transient inactivation of targeted brain areas and *in-vivo* electrophysiology) with the aim of gaining a broader understanding of memory function or other brain/bodily functions that would require fMRI in awake small rodents.

Disclosures: L. Mahnke: None. **C. Chwiesko:** None. **P. Wenk:** None. **M. Hoehn:** None. **F. Angenstein:** None. **M. Sauvage:** None.

Poster

406. Learning and Memory: Hippocampal: Cortical Interactions I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 406.20

Topic: H.08. Learning and Memory

Support: SFB 1089
ERC grant MicroSynCom

Title: The 6-zone track: A novel spatial memory test for awake head-fixed Ca^{2+}-imaging

Authors: *M. MITTAG, F. FUHRMANN, F. MUSACCHIO, S. POLL, M. FUHRMANN; German Ctr. for Neurodegenerative Dis. (DZNE), Bonn, Germany

Abstract: Two-photon Ca^{2+}-imaging is one of the state-of-the-art methods to record the activity of large neuronal populations in awake and behaving animals. Despite the emergence of miniature microscopes – which allow Ca^{2+}-imaging in freely moving animals – many experimental systems still rely on head-fixation for better spatial resolution. However, memory tests assessing spatial learning in head-fixed mice and combine it with Ca^{2+}-imaging are still rare. Here, we introduce the novel 6-zone track, a spatial memory test that allows testing for different aspects and features of spatial memory processing in awake head-fixed mice. For this, we utilize a 360 cm long linear treadmill equipped with textures, separating the track into six reward zones with unique textual patterns and uniform transition zones. The local cues guide the mice along the track and allow them to identify zones associated with reward. The 6-zone track can be flexibly set-up in different ways – for example by introduction of fake reward zones or a delayed start – in order to test for working memory or different types of reference memory. Food-restricted and habituated male mice were able to learn the tasks within a period of five days and showed significant improvements in several performance parameters like reward success rate, total number of obtained rewards in a given time, as well as licking outside of reward zone. Furthermore, these parameters could be used to identify and characterize different behavioral patterns and learning strategies in terms of efficacy and specificity. This allowed us to distinguish between different behavioral phenotypes. For these reasons we are convinced that the 6-zone track will be a an efficient tool to routinely perform spatial memory tests in head-fixed mice in combination with functional *in vivo* two-photon Ca^{2+}-imaging of large neuronal populations.

Disclosures: M. Mittag: None. **F. Fuhrmann:** None. **F. Musacchio:** None. **S. Poll:** None. **M. Fuhrmann:** None.

Poster

406. Learning and Memory: Hippocampal: Cortical Interactions I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 406.21

Topic: H.08. Learning and Memory

Support: Departamento de Ciencias de la Salud UAM Lerma

Title: Prolonged exposure to novelty increases tyrosine hydroxylase levels in the hippocampus, improving memory and synaptic plasticity in cognitively impaired animals due to chronic exposure to a high-fructose and high-fat diet.

Authors: *E. S. GUTIÉRREZ-LÓPEZ[1], F. BERMÚDEZ-RATTONI[2], K. R. GUZMAN-RAMOS[3];

[1]Inst. De Fisiologia Celular, UNAM, Mexico City, Mexico; [2]Inst. de Fisiología Celular, UNAM, Ciudad de México, Mexico; [3]Ciencias de la Salud, Univ. Autónoma Metropolitana, Mexico, Mexico

Abstract: Metabolic deregulations like the excessive accumulation of body fat, hyperglycemia, and overweight are considered risk factors for developing cognitive and neuronal plasticity impairment. The mechanisms underlying these declines are unknown. However, several studies indicate that chronic exposure to high-calorie diets affects spatial memory and neuronal plasticity and reduces the catecholaminergic levels in the hippocampus. Increasing catecholaminergic levels with intra-hippocampal microinjection of nomifensine in rats that were chronically exposed to high-calorie diets recover memory and synaptic plasticity. However, it is unknown whether implementing an alternative treatment that increases catecholamines in the hippocampus, such as novelty, reverses the adverse effects of chronic consumption of these diets on synaptic plasticity and memory. To test this hypothesis, a group of male mice was exposed to a high-fructose and high-fat diet for six months compared to a control group of male mice exposed to a standard diet for the same number of months. Cognitive performance was evaluated with the object location memory task, neuronal plasticity was assessed by long-term potentiation on the perforating to dentate gyrus pathway, and tyrosine hydroxylase (TH) in the hippocampus was measured by western blot. The high-fructose and high-fat diet caused an increase in adiposity and glucose intolerance, constituting a model of metabolic deregulation. The behavioral results indicate an impairment of spatial memory and a deficit in the induced long-term potentiation in mice with high-fructose and high-fat diets. However, a 6-month exposure to novelty consisting of every other day replacing toys of different sizes, colors, shapes, and textures in mice with high-fructose and high-fat diets recover synaptic plasticity and spatial recognition memory like controls. Furthermore, animals with a high-fat diet exposed to novelty showed TH levels in the hippocampus similar to controls. These results show that the novelty prevents the deterioration of recognition memory and long-term plasticity and reestablishes TH in the hippocampus. These results will help develop a treatment for the decline of memory associated with chronic consumption of a hypercaloric diet.

Disclosures: E.S. Gutiérrez-López: None. **F. Bermúdez-Rattoni:** None. **K.R. Guzman-Ramos:** None.

Poster

406. Learning and Memory: Hippocampal: Cortical Interactions I

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Topic: H.08. Learning and Memory

Support: NIH Grant R01NS110806
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NIH Grant R01MH112523

Title: Spectral dynamics θ oscillons in wake rats

Authors: *M. ZOBAER[1], C. DOMENICO[2], L. PEROTTI[3], D. JI[2], Y. A. DABAGHIAN[1]; [1]Neurol., The Univ. of Texas Hlth. Sci. Ctr. at Houston (UTHealth), Houston, TX; [2]Dept. of Neurosci., Baylor Col. of Med., Houston, TX; [3]Physics, Texas Southern Univ., Houston, TX

Abstract: A recently developed computational technique—Discrete Padé Transform (DPT)—opens a newperspective on the structure and the dynamics of the oscillatory extracellular fields. Analyses ofhippocampal and cortical local field potential (LFP) recorded in wild type rats reveal that the brainrhythms are comprised of a few phase-modulated waves—*oscillons*—enveloped in weak noisebackgrounds. The frequency domains occupied by the individual oscillons roughly correspond to thefrequency domains attributed to the consensual θ- and γ-waves, although oscillons' structure issubstantially more elaborate than the structure of the Fourier-defined "brain waves."In this study, we focused on the lowest-frequency, highest-amplitude, θ-oscillon that is most salientduring active navigation and persists in quiescence. First, we found that the mean frequency and theamplitude of the hippocampal and the cortical θ-oscillons are coupled to the animal's speed. Theseproperties of θ-oscillon match the known of the conventional θ-rhythms, thus linking our analyses tothe traditional results established via Fourier decompositions. Next, we studied properties specific toDPT method, e.g., the LFP's noise component, and found that hippocampal and cortical noise arealso coupled to speed and exhibit complex dynamics during transitions between wakefulness andquiescence. The embedded frequencies of the θ-oscillons—the *spectral waves*—exhibit mostcomplex behaviors that include both slow frequency changes and rapid, abrupt dynamics, suggestiveof external inputs into hippocampo-cortical network. These results qualitatively expand the knownscope of θ-rhythms' properties and shed new light on the structure and functionality of synchronizedneuronal activity in hippocampo-cortical circuit.

Disclosures: M. Zobaer: None. **C. Domenico:** None. **L. Perotti:** None. **D. Ji:** None. **Y.A. Dabaghian:** None.

Poster

406. Learning and Memory: Hippocampal: Cortical Interactions I

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Program #/Poster #: 406.23

Topic: H.08. Learning and Memory

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AHA 831442 to J.K.

Title: Coordination of sleep slow oscillations and hippocampal sharp-wave ripples during manifold exploration in motor cortex

Authors: *J. KIM[1,3], A. JOSHI[2], L. FRANK[2], K. GANGULY[1,3];
[1]Neurol. department, [2]HHMI and Departments of Physiol. and Psychiatry, Univ. of California San Francisco, San Francisco, CA; [3]Neurol. and Rehabil. Service, San Francisco Veterans Affairs Med. Ctr., San Francisco, CA

Abstract: Systems consolidation - a process for long-term memory stabilization - has long been hypothesized to occur in two-stages. Whereas newly acquired memories require the hippocampus, they become integrated into cortical networks over time, making them increasingly independent of the hippocampus. How hippocampal-cortical dialogue precisely evolves during this process and what informs the transitions between stages are not clear. Moreover, how cortical representations precisely evolve during systems consolidation is unknown. Here, we use a skill learning task to monitor the temporal dynamics of cross-area coupling during NREM sleep along with changes in primary motor cortex (M1) representational stability during learning. Our results indicate that the precise temporal dynamics of cross-area coupling between hippocampus, prefrontal cortex (PFC) and M1 can demarcate two stages of cortical processing. We specifically find that each animal demonstrates a sharp increase in PFC and M1 sleep slow oscillation (SO) coupling that is linked to stabilization of task performance. This sharp increase then predicts a drop in hippocampal sharp-wave ripple (SWR)-M1 SO coupling - suggesting feedback to inform hippocampal disengagement and transition to a second stage. Notably, the first stage shows significant increases in hippocampal SWR-M1 SO coupling in the post-training sleep and is closely associated with rapid learning and variability of the M1 low-dimensional manifold. Strikingly, even after consolidation, inducing new manifold exploration by changing task parameters reengages hippocampal-M1 coupling. We thus find evidence for dynamic hippocampal-cortical dialogue that is associated with exploration of cortical representations during both motor learning and adaptation.

Disclosures: J. Kim: None. **A. Joshi:** None. **L. Frank:** None. **K. Ganguly:** None.

Poster

406. Learning and Memory: Hippocampal: Cortical Interactions I

Location: SDCC Halls B-H

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Program #/Poster #: 406.24

Topic: H.08. Learning and Memory

Support: NIH (1R01NS109498)
MOTI-VATE graduate school, Faculty of Medicine, University of Freiburg

Title: The cultured mouse hippocampal formation forms organotypic projections to the medial prefrontal cortex in vitro

Authors: *P. STÖHR, M. LENZ, A. VLACHOS;
Inst. of Anat. and Cell Biology, Fac. of Medicine, Dept. of Neuroanatomy, Freiburg im Breisgau, Germany

Abstract: The hippocampal formation and medial prefrontal cortex (mPFC) have well-established roles in memory encoding and retrieval. Considerable research supports the idea that a direct pathway from the hippocampal formation to the mPFC is involved in regulating cognition, emotion, and behavior in health and disease. However, the interactions between the hippocampus and mPFC are not fully understood. Here, we established a new organotypic *in vitro* model and studied synaptic properties of hippocampal projections to the mPFC. Horizontal slices containing the entorhinal cortex and the hippocampus — prepared from mice of both sexes — were cultured adjacent to frontal sections of the mouse mPFC. Using anterograde and retrograde labeling techniques, electrophysiology, and ultrastructural analysis, we assessed 3-weeks old entorhinal-hippocampal-mPFC cultures. These complex cultures were viable and maintained their organotypic cyto- and fiber-architecture. In addition to the previously reported entorhino-hippocampal and entorhino-ammonic projections, a direct pathway from hippocampal area CA1 and the subiculum to the mPFC was readily observed. In turn, no direct projections from the mPFC to the hippocampus were detected, similar to what is observed *in vivo*. Indeed, CA1 pyramidal neurons formed functional synapses with excitatory and inhibitory neurons in the mPFC. We conclude that organotypic functional projection pathways form in vitro, making complex cultures suitable tools for studying signal propagation/integration and interactions between the hippocampal formation and the mPFC *in vitro*.

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Poster

406. Learning and Memory: Hippocampal: Cortical Interactions I

Location: SDCC Halls B-H

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Program #/Poster #: 406.25

Topic: H.08. Learning and Memory

Support: 4R00MH120343
4R00MH122582

Title: CA2 synchronous events anticorrelated with sharp-wave ripples during memory consolidation

Authors: *L. A. KARABA, A. FERNANDEZ-RUIZ, A. OLIVA;
Neurobio. and Behavior, Cornell Univ., Ithaca, NY

Abstract: Hippocampal population activity during spatial navigation and declarative memory tasks has been extensively studied over the past few decades, primarily in the CA1, CA3, or dentate gyrus regions. More recently, additional focus has been placed on the CA2 region of the hippocampus despite its comparatively weaker place-related activity. Recent studies uncovered the significant role CA2 plays in social memory and in the initiation of sharp-wave ripples (SWRs - synchronous network events that support memory consolidation). In an effort to further understand the contributions of dorsal CA2 to memory processes, we recorded hippocampal and

cortical regions with silicon probes in both mice and rats during spatial and social memory paradigms. We describe a novel population event in the hippocampus wherein CA2 pyramidal neurons display a 'barrage' of action potentials. We identified and exploited the unique characteristics of these events in order to develop an offline detection pipeline, allowing us to flag and isolate this activity for further analysis. We find that these barrages are characterized by an increase in activity in a small subset of CA2 pyramidal neurons reaching firing rates up to 200 Hz. This increase in firing is coupled with a simultaneous inhibition of all other hippocampal cell types. CA2 barrages tend to exceed 300 ms in duration, occur during non-REM sleep, and are anticorrelated with SWRs. These events also increase in both rate and duration following learning. To elucidate their functional role, we further examined CA2 barrages' relationship with other cortical and hippocampal patterns across brain states and behaviors. Individual hippocampal cells displayed distinct patterns of modulation in CA2 barrages and SWRs after learning. We hypothesize that both events play complementary roles for memory consolidation.

Disclosures: L.A. Karaba: None. **A. Fernandez-Ruiz:** None. **A. Oliva:** None.

Poster

406. Learning and Memory: Hippocampal: Cortical Interactions I

Location: SDCC Halls B-H

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Program #/Poster #: 406.26

Topic: H.08. Learning and Memory

Support: 4R00MH120343
4R00MH122582

Title: Dynamics of social memory ensembles across the dorsoventral hippocampal axis

Authors: *P. PAUDEL, A. FERNÁNDEZ-RUIZ, A. OLIVA;
Neurobio. and Behavior, Cornell Univ., Ithaca, NY

Abstract: The ability to recognize other individuals is an important feature to form social structures in most animal communities. House mice have been reported to use various external cues like odors, ultrasonic vocalizations and other sensory information to interact with each other and identify their conspecifics. However, how mice form a social cognitive map that includes individual identity and information about the context within which they interact remains elusive. Hippocampal sharp-wave ripples (SWRs), highly synchronous network events that facilitate consolidation of recent experience, have been shown to be involved in social memory consolidation. In addition, several studies have reported that neuronal activity in the dorsal CA2 and ventral CA1 are necessary for the formation of social memories. Do these different brain regions represent distinct features of social episodes? Do SWRs, that propagate across the dorsal-ventral axis of the hippocampus, coordinate these representations? To address these questions, we designed a novel behavioral paradigm to analyze behavior and neural representations while mice interact with familiar or novel conspecifics in different spatial contexts. We implanted

high-density silicon probes spanning the entire proximal-distal axis (CA1, CA2, CA3) of both dorsal and ventral hippocampus simultaneously. We recorded extracellular electrophysiological signals during the task and subsequent sleep epochs. Our findings show that mice can discriminate the identity of multiple conspecifics. Mice spent more time interacting when the conspecific stimuli were displaced, or novel stimuli were introduced, suggesting that subjects form and maintain a social memory of other conspecifics. Individual place cells and neuronal assemblies responded to either social or contextual variables or a combination of both, in a region-specific manner, and later reactivate during SWRs. We propose that the hippocampus generates multiplexed neuronal ensembles that encode various task features, that are integrated during SWRs to form unified episodic representations.

Disclosures: **P. Paudel:** None. **A. Fernández-Ruiz:** None. **A. Oliva:** None.

Poster

406. Learning and Memory: Hippocampal: Cortical Interactions I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 406.27

Topic: H.08. Learning and Memory

Support: 4R00MH120343
4R00MH122582

Title: Selective perturbation of theta sequences during experience impairs consolidation in replay

Authors: *C. LIU, R. TODOROVA, A. OLIVA, A. FERNANDEZ-RUIZ;
Neurobio. and Behavior, Cornell Univ., Ithaca, NY

Abstract: Replay is the phenomenon by which sequences of hippocampal place cells are reactivated in the same order as they were active along spatial trajectories the animal experienced. However, there is evidence that trajectories experienced only indirectly (e.g. shortcuts or inaccessible corridors) may also be replayed. This suggests that once a cognitive map of an environment is successfully formed, replay may be thought of as a generative process exploring that map. However, recent evidence points at fast timescale sequences nested in individual theta cycles (theta sequences) taking place during active behavior as critical for subsequent replay. Together, these results suggest the hypothesis that theta sequences are only required to form the cognitive map of a particular environment, from which all types of trajectories would be replayed in sleep. An alternative hypothesis posits that theta sequences of each individual trajectory would be required to support replay of that trajectory. Medial entorhinal cortex inputs have been shown to be crucial for the temporal organization of CA1 ensemble dynamics. Therefore, to test our hypotheses, we optogenetically entrained interneurons in the medial entorhinal cortex to perturb the temporal structure of entorhinal outputs. Our manipulation was applied only for selective trajectories while the rats traveled on different

mazes. Place cell properties at the behavior timescale were largely maintained during stimulation epochs, but the oscillation frequency of CA1 pyramidal neurons was reduced and their preferred theta phases of firing were shifted. Theta phase precession and theta sequences were significantly impacted only in the stimulated trajectory. Therefore, within the same maze experience, on one trajectory the animal had the correct theta scale encoding, while on the other trajectory theta sequences were disrupted. We compared the pre-to-post experience enhancement of hippocampal place cell replay sequences encoding the two trajectories during sleep, and we found that replay for the disrupted trajectory was significantly impaired. Our results provide evidence that the precise encoding at theta timescale for each individual trajectory is required for the genuine reinstatement of memory during replay in sleep consolidation.

Disclosures: C. Liu: None. **R. Todorova:** None. **A. Oliva:** None. **A. Fernandez-Ruiz:** None.

Poster

406. Learning and Memory: Hippocampal: Cortical Interactions I

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Program #/Poster #: 406.28

Topic: H.08. Learning and Memory

Support: 4R00MH120343
4R00MH122582

Title: Selective memory encoding, routing and replay by segregated hippocampal pyramidal cell populations

Authors: *R. E. HARVEY, A. OLIVA, A. FERNANDEZ-RUIZ;
Cornell Univ., Ithaca, NY

Abstract: The hippocampus replays behaviorally relevant information during sharp-wave ripples (SWRs). SWRs broadcast hippocampal memory representations to multiple brain areas and support memory-guided behavior. In this process, the hippocampus is traditionally described as a uniform processing module. However, recent evidence has shown the existence of large cellular diversity among its principal cells. In this regard, the exact extrinsic and intrinsic mechanisms that shape the computational and functional roles of these diverse cell populations remain unclear. To investigate how this cellular diversity contributes to the flexible computational capabilities of the hippocampus, we used high-density silicon probes to capture laminar-specific hippocampal and cortical dynamics during sleep and awake periods as rats completed a variety of spatial tasks. We discovered that the laminar-specific anatomical distribution of CA1 pyramidal cells is a major organizing principle of hippocampal assembly dynamics and the experience-dependent emergence of memory replay. Further, superficial and deep CA1 pyramidal cell populations encode either trajectory and choice-specific information or keep track of changes in reward configuration, and selectively route these representations to different downstream cortical targets. These findings reveal the existence of functionally specialized

hippocampo-cortical sub-circuits, providing a cellular mechanism that supports the computational flexibility and memory capacities of these structures.

Disclosures: R.E. Harvey: None. **A. Oliva:** None. **A. Fernandez-Ruiz:** None.

Poster

406. Learning and Memory: Hippocampal: Cortical Interactions I

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Program #/Poster #: 406.29

Topic: H.08. Learning and Memory

Support: 4R00MH120343
4R00MH122582

Title: Boosting sleep sharp-wave ripples enhances behavioral performance in cortical dependent manner

Authors: *H. L. ROBINSON[1], R. TODOROVA[1], G. A. NAGY[2], A. GRUZDEVA[1], A. OLIVA[1], A. FERNANDEZ-RUIZ[1];
[1]Dept. of Neurobio. and Behavior, Cornell Univ., Ithaca, NY; [2]Inst. of Exptl. Medicine, Hungarian Acad, Inst. of Exptl. Medicine, Hungarian Acad, Budapest, Hungary

Abstract: After experience, memories must be consolidated for future use. This “two-stage” model of memory is based on an initial encoding phase during experience and a second consolidation phase during sleep. Hippocampal sharp-wave ripples (SWRs) mediate the immediate consolidation of recent memories and their transfer to the neocortex for longstanding consolidation. Yet, the mechanisms of how SWRs coordinate neuronal ensemble reactivation across structures are still not well understood. We investigated this using the Spatial Object Recognition (SOR) memory task. We implemented a system to perform simultaneous closed-loop stimulation or silencing or SWR-associated neuronal activity in hippocampus and medial prefrontal cortex (mPFC). We found that closed-loop inhibition of mPFC during SWRs impaired memory performance in this task. Furthermore, we observed that optogenetic enhancement of hippocampal SWRs during post learning sleep improved memory performance. However, this effect was abolished by simultaneously silencing mPFC. No effect on behavior was observed when optogenetic stimulation was delivered outside SWRs, highlighting the importance of a precise temporal coordination between both structures for memory consolidation. Together these results support the hypothesis that cortical ensemble reactivation during SWRs is necessary for memory consolidation.

Disclosures: H.L. Robinson: None. **R. Todorova:** None. **G.A. Nagy:** None. **A. Gruzdeva:** None. **A. Oliva:** None. **A. Fernandez-Ruiz:** None.

Poster

406. Learning and Memory: Hippocampal: Cortical Interactions I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 406.30

Topic: H.08. Learning and Memory

Support: 4R00MH120343
4R00MH122582

Title: Boosting sleep sharp wave-ripples enhances the hippocampo-cortical dialogue

Authors: *R. TODOROVA[1], H. L. ROBINSON[2], A. OLIVA GONZÁLEZ[3], A. FERNANDEZ-RUIZ[3];
[1]Cornell Univ., [2]Dept. of Neurosci. and Behavior, [3]Neurobio. and Behavior, Cornell Univ., Ithaca, NY

Abstract: Hippocampal sharp wave-ripples are believed to play a key role in the transfer of memories from the hippocampus to the neocortex. In sleep following a learning experience, ripple amplitude is enhanced, and in response to ripples, cortical task-related ensembles are reactivated and followed by a cortical delta wave. Here, we take advantage of a novel technique to boost ripples detected on-line (Fernandez-Ruiz et al., 2019). We applied optogenetic activation of hippocampal pyramidal neurons in mice to boost endogenous ripples during sleep following training on a memory task. To gauge the effects of the stimulation, we also recorded local field potentials and spiking activity of hippocampal area CA1 and the medial prefrontal cortex. Our results show that the stimulation triggered the recruitment of hippocampal neurons and the reactivation of task-related hippocampal assemblies and improved behavioral performance. The stimulation therefore resulted in an enhanced ripple activity which closely mimicked the enhancements observed following natural learning. We moreover recorded the cortical responses to these boosted events, and we report that the stimulation resulted in an enhanced cortical assembly reactivation and hippocampo-cortical coordination consistent with an artificially induced memory consolidation. Optogenetic silencing of the cortical activity following hippocampal ripples silenced the ripple-associated reactivation in the prefrontal cortex and prevented memory consolidation, indicating that the reactivation of task-related cortical assemblies during ripples is necessary for memory consolidation.

Disclosures: R. Todorova: None. **H.L. Robinson:** None. **A. Oliva González:** None. **A. Fernandez-Ruiz:** None.

Poster

407. Hippocampal Physiology III

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

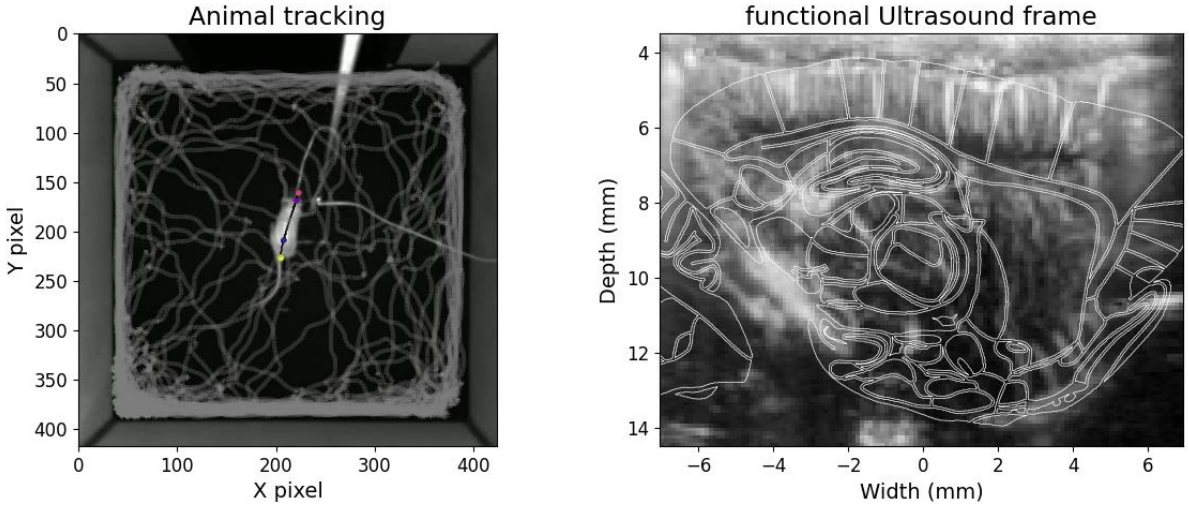
Program #/Poster #: 407.01

Topic: H.09. Spatial Navigation

Title: Chronic functional ultrasound imaging combined with behaviour tracking on freely moving rats

Authors: *F. CYBIS PEREIRA[1], N. IALY-RADIO[1], S. BHATTACHARYA[2], B.-F. OSMANSKI[2], S. PEZET[1], M. TANTER[1];
[1]Lab. 'Physics For Medicine', ESPCI-PSL, Inserm, CNRS, Paris, France; [2]Iconeus, Iconeus, Paris, France

Abstract: Brain-wide functional images of freely moving animals are key to understanding how cognitive behaviors may emerge from dynamic activation across different areas of the underlying neural circuitry. Functional Ultrasound (fUS) imaging has recently been demonstrated to robustly record brain-wide cerebral blood volume (CBV) dynamics as an indirect measure of neural activity over several weeks or months in freely moving rodents.Hardware and software interface to couple unrestrained cognitive experiments and fUS data is essential and can provide reliable use of this technique in a wide variety of behavioral paradigms.Here we propose studying freely moving rats in naturalistic behavior exploring an open arena using fUS. Spatial information is extracted using DeepLabCut library and custom pipeline. fUS imaging technology is optimized using a very light 15MHz piezo composite probe and highly flexible cable driven by an Ultrafast Ultrasound scanner (Iconeus One, Iconeus, France). With minimal preprocessing methods on the fUS data, we show encouraging results correlating changes in the CBV of specific brain regions, such as the dorsal hippocampus, with the speed of the animal during its exploration of the environment. In depth analysis of the signal suggests a sequence of activation within these brain regions during animal locomotion. Functional Ultrasound emerges as an interesting tool for unconstrained deep brain imaging during spatial navigation in rodents.



Disclosures: **F. Cybis Pereira:** A. Employment/Salary (full or part-time):; Iconeus. **N. Ialy-Radio:** None. **S. Bhattacharya:** A. Employment/Salary (full or part-time):; Iconeus. **B. Osmanski:** A. Employment/Salary (full or part-time):; Iconeus. **S. Pezet:** None. **M. Tanter:** E.

Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Iconeus.

Poster

407. Hippocampal Physiology III

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 407.02

Topic: H.08. Learning and Memory

Support: NIH-R01 1R01NS113782-01A1

Title: Transcranial electrical stimulation induced synaptic plasticity in freely moving rats

Authors: *M. VOROSLAKOS, K. MCCLAIN, A. ROTHSTEIN, G. BUZSÁKI;
NYU, New York, NY

Abstract: In recent years, researchers' interest has grown extensively in low-intensity, non-invasive brain stimulation methods, such as transcranial direct current stimulation (tDCS). tDCS can modulate cortical excitability which can persist after the stimulation, suggesting that the lasting changes may be mediated by synaptic plasticity, the mechanism(s) that bring about lasting neuronal plasticity are not fully understood. In our experiments, we set out to investigate the mechanisms through which tDCS can induce long-term neuroplasticity at synaptic, single neuron, circuit, and network levels in rodents. We developed a biocompatible stimulation system and combined it with high-channel count electrophysiology in behaving rats. We recorded single unit activity and local field potential (LFP) from hippocampus and visual cortices simultaneously using Neuropixels probes. We collected one-hour baseline, stimulation at various intensities (25, 50, 100 and 200 μA), with different polarities and durations (1, 3, 5 and 10 minutes), and 2 hours of post stimulation baseline data in the homecage. tDCS was applied once every 24 hours. Animals also received 2 sham stimulation sessions. We then examined the long-term functional rearrangement in hippocampal circuits and representation using a linear maze running task. The rats ran 55 laps on the track with or without electrical stimulation. On stimulation sessions, after 20 baseline trials, stimulation (500-ms Gaussian pulse) was given for 15 trials at a fixed position and running direction.To date, we stimulated 286 of 795 recorded neurons from the visual cortex and hippocampus (n=3 rats). We found that stimulation was more effective in changing the firing pattern of hippocampal cells, compared to visual cortical cells. We also recorded place cells during track running (n=2 rats). In one session, we directly stimulated neurons on the track while inducing more than 1 V/m electric fields. While these directly stimulated neurons did not show persisting place fields on the track, we found other (non-directly stimulated) pyramidal cells that did develop new place field after electrical stimulation. Our results indicate that tDCS can induce long-lasting changes in neuronal activity provided that sufficient current intensity is used.

Disclosures: M. Voroslakos: None. **K. McClain:** None. **A. Rothstein:** None. **G. Buzsáki:** None.

Poster

407. Hippocampal Physiology III

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 407.03

Topic: H.08. Learning and Memory

Support: NIH Grant 1R01NS113782-01A1
NIH Grant 2TL1TR001447-06A1

Title: Effects of transcranial radio-frequency energy exposure on neural activity in-vivo

Authors: *O. YAGHMAZADEH[1], G. BUZSAKI[2];
[1]New York Univ. Neurosci. Inst., New York, NY; [2]Neurosci., New York University, Langone Med. Ctr., New York, NY

Abstract: We examined the effect of non-invasive exposure of radio-frequency (RF) energy on ongoing neural activity in rodents' brains in-vivo. RF stimulation was applied at around 1GHz using commercially available patch or custom-made stub antennas. Optical calcium imaging was used for monitoring neural activity. First, we examined whether RF energy exposure could induce direct and non-thermally-induced changes in neural activity. To do so, we modified a miniature head-mounted imaging system (UCLA Miniscope V3) and developed a fiber-coupled RF-interference-free 1Photon imaging apparatus. Our results, comparing stimulated and sham conditions, for RF energy exposure with induced in-situ electric field strengths up to 230 V/m (and corresponding specific absorption rate (SAR) of 28 W/Kg), demonstrate that RF exposure does not affect the ongoing activity of hippocampal CA1 neurons in a statistically significant manner. Hence, RF exposure higher than levels that are allowed by regulatory limits in real-life scenarios does not affect neuronal activity in a direct and non-thermal manner. Nevertheless, we show that by adjusting parameters of the RF stimulation (e.g. power and duty cycle) and using custom-made stub antennas we are able to increase the brain temperature (and not the body temperature) in a controlled manner, thereby altering neuronal activity. Affecting brain activity by means of RF stimulation within a safety zone provides various possibilities for research and clinical applications. Thus, we suggest that Transcranial RF Stimulation can be used as a novel neuromodulation technique and help expand the spectrum of non-invasive tools for brain stimulation.

Disclosures: O. Yaghmazadeh: None. **G. Buzsaki:** None.

Poster

407. Hippocampal Physiology III

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 407.04

Topic: H.08. Learning and Memory

Support: EU Horizon 2020 Marie Skłodowska-Curie grant agreement No 892957

Title: Functional coupling of dorsal midline thalamic neurons to the prelimbic cortex and the ventral subiculum

Authors: *G. KOMLÓSI[1,2], L. ACSADY[2], G. BUZSAKI[1];
[1]Neurosci. Inst., NYU Langone Hlth., New York, NY; [2]Inst. of Exptl. Med., Budapest, Hungary

Abstract: The dorsal midline thalamus (dMT) has long been implicated in motivated behaviors and being associated with high arousal states. By receiving afferents from multiple hypothalamic and brainstem regions it is ideally positioned to monitor the internal states and needs. dMT also receives prominent glutamatergic input from two forebrain regions the prelimbic cortex (PL) and the ventral subiculum (vS) and sends its axon collaterals back to them. However, little is known how dMT activity is related to these two regions. Neurons in the dMT selectively express calretinin (CR), and we recently showed that activity of calretinin (CR) containing neurons in the dMT (CR-dMT) positively correlates with arousal level and their selective stimulation reliably leads to behavioral arousal. We performed large-scale unit recordings from optogenetically identified CR-dMT neurons of the anterior dMT during unrestrained sleep-wake behavior, while simultaneously recorded population activity from the PL and vS. To discriminate CR-dMT neurons from neighboring non-CR expressing neurons, we transduced neurons in the dMT of CR-Cre mice with Channelrhodopsin-2 and implanted optic fibers both into the somatic and terminal fields (PL and vS) of CR-dMT neurons. Using brief optical pulses in these structures, while monitoring neuronal activity within the dMT, enabled us to further discriminate CR-dMT neurons connected to these two regions. CR-dMT and putative non-CR expressing neurons showed distinct firing characteristics during sleep and wakefulness. Optical stimulation within the terminal fields (PL, vS) evoked either antidromic spikes within CR-dMT neurons and/or disynaptic excitation emerged from indirect synaptic activation of dMT projecting PL/vS neurons. Since in many cases it was difficult to differentiate between these two types of response, we simplified our categorization to PL-coupled and vS-coupled CR-dMT neurons. In our recordings PL-coupled CR-dMT neurons were more abundant and distributed more evenly across the anterior dMT than vS-coupled CR-dMT neurons, corroborating anatomical findings. We found that both CR-dMT and non-CR thalamic neuronal activity were coupled to hippocampal/subicular ripples and cortical slow-wave activity. The relationship between thalamic unit and ripple/slow wave activity was heterogenous across the population. Identifying how dMT interacts with the PL and the vS, two key limbic regions, at the neuronal level might help us understand how dMT plays role in motivated behaviors. *This project has received funding from the EU Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 892957.*

Disclosures: G. Komlósi: None. **L. Acsady:** None. **G. Buzsaki:** None.

Poster

407. Hippocampal Physiology III

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 407.05

Topic: H.08. Learning and Memory

Support: 1U19NS107616-01

Title: In-vivo oxytocin modulation of neural network in the hippocampus

Authors: *Y. ZHANG[1], J. LIU[1], H. WANG[2], Y. LI[2], R. TSIEN[1], G. BUZSÁKI[1];
[1]nyu grossman school of medicine, NEW YORK, NY; [2]Peking Univ., Beijing, China

Abstract: Neuromodulatory control by oxytocin is essential for a wild range of social, maternal, and stress-related behaviors. In addition to acetylcholine (ACh), oxytocin is becoming another possible treatment for Alzheimer's disease. However, how oxytocin receptor activity regulates neuronal networks of the hippocampus remains largely unknown. Several lines of research found that activating oxytocin receptors in hippocampal slices induced firing in fast-spiking interneurons and sharpened synaptic transmission (*Owen, S. et al, 2013*), but direct evidence for real-time oxytocin action on neural activities in freely behaving animals during different behavioral states is lacking. Here we combined fiber photometry and Cre recombinase for enabling the selective and fast monitoring oxytocin and acetylcholine levels with optogenetic manipulation of ACh and oxytocin-expressing neurons. Hippocampal network activity was monitored by high-density electrode arrays simultaneously from multiple regions and layers of the hippocampus. In contrast to acetylcholine, whose level increased to a high level during REM sleep, oxytocin activity fell to a minimum, similar to changes of DA, NE and 5-HT. Within nonREM sleep, oxytocin activity showed an inverse slow co-fluctuation with power in the sleep spindle band (10 Hz~20 Hz) and showed peak coherence at 0.01~0.1 Hz. ACh and oxytocin showed different and complimentary patterns surrounding hippocampal sharp wave ripples (SPW-R). The oxytocin signal ramped up 10-20 sec before SPW-R occurrence, and showed a large and rapid decrease thereafter. In contrast, the largest probability of SPW-Rs coincided with the minima of the acetylcholine signal. During waking, oxytocin signal varied inversely with low gamma (30-80 Hz) oscillation power. Optogenetic activation of oxytocin neurons in the paraventricular nucleus of hypothalamus (PV) suppressed SPW-Rs. Future experiments are planned to clarify how different constellation of Ach and oxytocin levels allow or prevent the occurrence of other network patterns.

Disclosures: Y. Zhang: None. **J. Liu:** None. **H. Wang:** None. **Y. Li:** None. **R. Tsien:** None. **G. Buzsáki:** None.

Poster

407. Hippocampal Physiology III

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 407.06

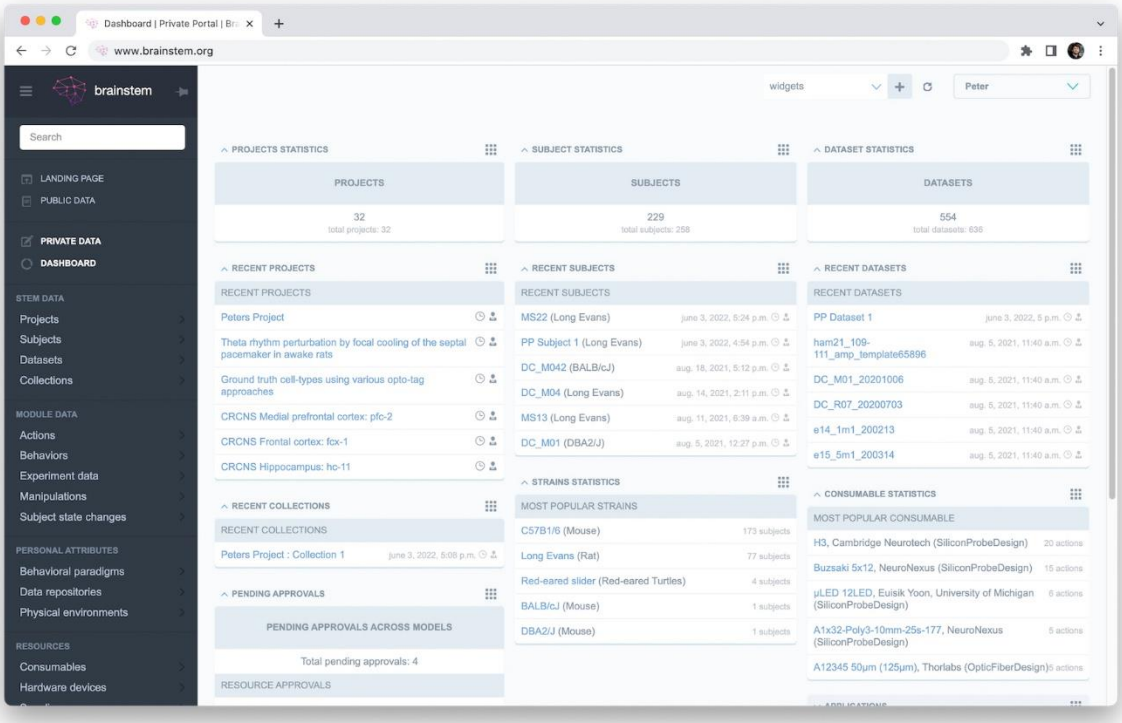
Topic: H.08. Learning and Memory

Support: 5U19NS107616

Title: Brainstem: A collaborative electronic lab notebook for experimental neuroscience

Authors: *P. C. PETERSEN[1], R. AMADUCCI[2], A. SURKIS[3], G. BUZSAKI[4];
[1]Neurosci. Inst., New York University, Langone Hlth., New York, NY; [2]NYU Neurosci. Inst., [3]New York Univ., New York, NY; [4]Neurosci., New York University, Langone Med. Ctr., New York, NY

Abstract: BrainSTEM is a collaborative electronic lab notebook solution that is tailored to experimental neuroscience, with a focus on large-scale single-cell neurophysiology as captured using intracellular, extracellular, and two-photon recording techniques.
Research data from published papers on large-scale single neuron research is rarely shared. This data often has considerable potential for reuse and reinterpretation in different studies and provides transparency and reproducibility to already-published studies. Further, data shared on public repositories is often difficult to discover, lacking standardized metadata, limiting its degree of reusability. To address these current shortcomings, we are building BrainSTEM (Brain Structured Experimental Metadata).
BrainSTEM provides three key benefits as an electronic notebook solution for experimental neuroscience:
1. **It has a very low barrier to use**
- Data can be entered through web-based intuitive forms and organized in a user-friendly UI.
- Data can be shared with collaborators or publicly with a single click.
2. **It is centralized**
- It requires no technical know-how to use or set up.
- Keeps metadata from becoming fragmented across various sources.
- Easily discover and organize data through the relational data structure.
3. **It is standardized**
- We built a standardized yet flexible language to accommodate current methods and techniques and it is built to accommodate future needs.
- It promotes a rich level of metadata making experimental data more interpretable.
- It is machine-readable that allows for tools to be built with programmable access via a REST API.
BrainSTEM has the potential to become the standard metadata model within neurophysiology, make data FAIR, promote standardization, data sharing, and provide better integration across datasets, both within and across collaborative labs and for published datasets.
We are looking for pilot groups - please visit our poster or our website www.BrainSTEM.org to learn more.



Disclosures: **P.C. Petersen:** None. **R. Amaducci:** None. **A. Surkis:** None. **G. Buzsaki:** None.

Poster

407. Hippocampal Physiology III

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 407.07

Topic: H.08. Learning and Memory

Title: Interrogation of multiplexed hippocampal output to the posterior hypothalamus

Authors: *N. NITZAN, G. BUZSÁKI;
New York Univ. Langone Med. Ctr., New York Univ., New York, NY

Abstract: The hippocampus is a brain structure critical for the processing of episodic memories and for spatial navigation. Yet it does not fulfil its attributed role in memory in isolation, but is embedded in a much larger network of cortical and subcortical structures known as the ‘extended hippocampal system’. The majority of current work investigating hippocampal output examines how information processed by the hippocampus is transmitted back to the neocortex. However, approximately the same number of hippocampal output fibers target subcortical structures and the significance and physiological relevance of these outputs are much less investigated. Evidence from lesion and patient case studies suggests that various nuclei in the mammillary

region, including the medial and lateral mammillary bodies, the supramammillary nucleus and the tuberomammillary nucleus are of particular relevance for spatial navigation and memory. However, how these areas communicate with the hippocampus to support those functions is not clear. To address this question, we performed paired silicon probe recordings from freely behaving mice in the dorsal hippocampus and various subcortical nuclei in the posterior hypothalamus. We found that various nuclei in the mammillary regions are characterized by idiosyncratic activity patterns such as local-field potentials signatures and spike-time relationships with hippocampal neurons, which can be used as physiological marker to delineate them. Moreover, we show that hippocampal-hypothalamic interactions are dominated by anatomical gradients affecting both theta and sharp-wave ripples states. In addition, we found that the activity of many hypothalamic cells is modulated by various spatial variables such as position, translational movement and direction.

Disclosures: **N. Nitzan:** None. **G. Buzsáki:** None.

Poster

407. Hippocampal Physiology III

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 407.08

Topic: H.08. Learning and Memory

Support: NIH Grant U19NS104590-01

Title: Developmentally structured coactivity in the hippocampal trisynaptic loop

Authors: *R. HUSZÁR[1], D. HUILGOL[2], J. LIU[1], J. HUANG[2], G. BUZSAKI[3];
[1]New York Univ., New York, NY; [2]Duke Univ. Sch. of Med., Durham, NC; [3]Neurosci., New York University, Langone Med. Ctr., New York, NY

Abstract: The hippocampus is a key player in learning and memory. Research into this brain structure has long emphasized its plasticity and flexibility (McClelland et al., 1995), though recent reports have come to appreciate its remarkably stable firing patterns (Mizuseki et al., 2013). How plasticity updates networks maintaining their dynamics remains an open question, largely due to a lack of experimental access points into network stability. Development may provide one such access point (Cossart & Khazipov, 2022). We showed that CA1 pyramidal neurons of the same embryonic birthdate exhibit prominent cofiring across different brain states, including behavior in the form of overlapping place tuning. These features could partially be explained by structured connectivity between pyramidal cells and local interneurons, suggesting a developmentally installed circuit motif (Huszár et al., 2022). Prior anatomical work showed that same birthdate excitatory neurons across hippocampal subregions are likely synaptic partners (Deguchi et al., 2011) displaying highly clustered input on common dendritic branches (Druckmann et al., 2014). This suggests that local connectivity and structured input could jointly explain the cofiring of same birthdate CA1 neurons. Here, we investigate the latter influence at a

functional level. First, we studied the role of shared embryonic birthdate in shaping firing patterns of the CA3 and DG. We achieved ChR2 expression in birthdate-defined populations of DG granule cells by delivering tamoxifen to timed-pregnant Tbr2-CreER X Ai32 dams. Neural ensembles were monitored with high density silicon probes and birthdate-defined populations were identified optogenetically. Same birthdate granule cells displayed prominent coactivity in dentate spikes, mirroring our observations in the CA1. Moreover, CA3 neurons that discharged upon mossy-fiber stimulation tended to cofire during spontaneous activity bursts. This suggests that same-birthdate granule cells shape the activity of downstream CA3 partners, which is consistent with their synaptic interconnectedness (Deguchi et al., 2011). To directly explore the interaction of these populations across synaptically connected areas, we birthdated populations of DG and CA3 neurons with intrauterine virus injection of AAV5-Camkii-Cre, followed by adult injection of pAAV-CAG-DIO-ChR2 into DG and CA3. Ongoing experiments involve simultaneous monitoring of same birthdate populations in DG and CA3, with an emphasis on the logic of activity rearrangements in a hippocampus dependent learning task. This study is aimed to provide experimental insights into the stability-plasticity dilemma.

Disclosures: **R. Huszár:** None. **D. Huilgol:** None. **J. Liu:** None. **J. Huang:** None. **G. Buzsaki:** None.

Poster

407. Hippocampal Physiology III

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 407.09

Topic: H.08. Learning and Memory

Title: Physiological and representational structure of CA1 activity along the longitudinal axis of the hippocampus

Authors: *K. MCCLAIN[1], M. VÖRÖSLAKOS[1], Y. JIN[1], G. BUZSAKI[2];
[1]NYU Neurosci. Inst., New York, NY; [2]Neurosci., New York University, Langone Med. Ctr., New York, NY

Abstract: The hippocampus (HPC) is thought to enable memory consolidation through wide spread communication with distal brain regions. Hippocampal signaling is organized along the longitudinal axis of HPC, which segregates distinct topography of long-range projections (e.g., RSC receives projections from dorsal HPC, while PFC receives projections from ventral HPC). In the CA1 subregion of HPC, a wide range of neurological features have been shown to vary along this axis, including transcriptional profile, topography of CA3 recurrent collaterals, magnitude of LTP, and density of vascularization. The resulting longitudinal structure of CA1 physiology and its implications for how information is routed to various partner regions is not well understood. A handful of studies have identified disparate trends: a gradient of place field width along dorsal hippocampus, a shift in theta phase from one end to the other, sharp-wave ripples traveling in a variety of patterns through dorsal hippocampus. However, these pieces are

not yet assembled into a unified picture of longitudinal structure in hippocampal physiology. In this study we performed simultaneous high-density recordings from multiple locations along the longitudinal axis, ranging from dorsal (dCA1) to intermediate (iCA1) to ventral (vCA1). We aim to quantify 1) spatial coding properties during behavior as a function of longitudinal position and how they dictate representational structure of encoded variables 2) organization of offline reactivation and 3) network structure exhibited by fine timescale spiking (i.e., sequences) and their interaction with large scale dynamics such as sharp-wave ripples and theta oscillations. We have identified a gradient of place cell features as well as spatial information from pole to pole, which is observable even within dCA1. We have also observed a marked discontinuity in large scale dynamics between dCA1 and i/vCA1. Exploration of these effects as they relate to network structure and sequential spiking is ongoing, as well as the representational structure of reactivation. Overall, we characterize the longitudinal organization of dCA1 activity as a step toward understanding the role of the hippocampus in global brain signaling.

Disclosures: K. McClain: None. **M. Vöröslakos:** None. **Y. Jin:** None. **G. Buzsaki:** None.

Poster

407. Hippocampal Physiology III

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 407.10

Title: WITHDRAWN

Poster

407. Hippocampal Physiology III

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 407.11

Topic: H.09. Spatial Navigation

Support: R01NS113557

Title: Precise behavioral performance with an imprecise hippocampal code on a dynamic, multi-step linear maze

Authors: *J. WIDLOSKI[1], D. J. FOSTER[2];
[1]Univ. of California Berkeley, Berkeley, CA; [2]Psychology, Univ. of California, Berkeley, Berkeley, CA

Abstract: The recent surge in the development of planning-based strategies in artificial intelligence holds great promise for elucidating the mechanisms of planning in the brain.

However, for tractability reasons, existing animal decision-making tasks tend to be relatively impoverished in complexity, both with few decision points and relatively rigid task contingencies, making it difficult to differentiate between competing planning models. To address this, we developed a novel spatial task that enhances both types of complexity. Rats were trained to make a series of left-right, memory-guided decisions across 5 identical blind-alley choice points arranged on a linear track. The track had high walls to occlude distal cues. Rats ran the maze repeatedly while being rewarded with chocolate milk at the end of each run. To preclude the use of a reflexive strategy, the correct path changed randomly from session to session, so that efficient movement through the maze required adaptation to the changing spatial contingencies. In addition, rats were implanted with tetrode arrays targeting the dorsal hippocampus in order to track neural substrates of spatial learning in the task. Rats learned to adapt rapidly to each new configuration, achieving, on average, near error-free performance within a few trials regardless of the size of the perturbation. Correct performance persisted even when the maze segments were shuffled but keeping the configuration the same, showing that behavior was memory-guided and not based on the discrimination of location-specific odor, visual, or tactile cues. Moreover, while rats exhibited on average the distinctive goal-gradient behavior common to spatial sequence learning in rats (maze segments nearer the reward were learned faster), the emergence of correct behavior over time and across maze segments could be highly idiosyncratic depending on the maze configuration. Strikingly, these error patterns were conserved across rats for most sessions. In contrast to the rat's spatially precise behavior, hippocampal place cell responses were largely spatially ambiguous, with many cells showing repeated fields across segments of the maze. Together, these results suggest a potentially powerful new spatial paradigm for discriminating different planning models, but also challenge the notion that the hippocampus is well suited to subserve planning in spatial sequence learning.

Disclosures: **J. Widloski:** None. **D.J. Foster:** None.

Poster

407. Hippocampal Physiology III

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 407.12

Topic: H.08. Learning and Memory

Support: RIKEN Center for Brain Science
KAKENHI-20K06935
KAKENHI-18K14856
The Takeda Science Foundation
The Uehara Memorial Foundation

Title: Locus coeruleus noradrenergic control of schema-based behavior in contingency reversal

Authors: *S. AMEMIYA[1], T. J. MCHUGH[2];
[1]RIKEN Ctr. for Brain Sci., RIKEN Ctr. for Brain Sci., Saitama, Japan; [2]RIKEN Ctr. for Brain Sci., Toshima-ku, Japan

Abstract: In decision making the inferential process that allows the deliberation and anticipation of current state and possible outcomes requires schema, knowledge structure of task and environment. Thus, the context-dependent reference and manipulation of schema is crucial for adaptive behavior and decision making, however, its neural mechanism is still unclear. Previous studies have reported that locus coeruleus noradrenergic neurons (LC-NA) are involved in controlling cognitive processes such as memory, attention and decision making in accordance with current demands, suggesting LC-NA may be involved in schema-related processes. Here, we examined the involvement LC-NA in schema-based inference by combining reversal learning paradigms in the T-maze with chemogenetic inhibition of activity of LC-NA in mice. Inhibitory Gi DREADD (designer receptor exclusively activated by designer drug) receptors were expressed in LC-NA and subject mice were trained a maze task where reward contingency (correct side has 2 pellets at 90%, and incorrect side was not rewarded) switched multiple times (every 15-25 correct trials) in each session for three weeks; enhanced correct choice across the training indicated mice established schema of the task. After training, mice ran same task following daily injection of the DREADD ligand deschloroclozapine (DCZ) or saline as control. Inhibition of LC-NA, compared to the control condition, increased correct choices 15 trials after the reversal as a result of persistent choice of the correct side, indicating the inhibition of LC-NA suppressed predictive choices mediated by schema-based inference of contingency reversal. Next, we examined effect of inhibition of LC-NA on learning a new schema. Mice were trained on a T-maze with one arm baited with 3 pellets and the other baited with 1 for four consecutive days, then the reward condition was reversed and mice ran the reversal condition for six consecutive days. LC-NA inhibition impaired learning of the high reward side both during the initial learning and following reversal and decreased mice's head orienting behavior ("vicarious trial-and-error" (VTE)) at the choice point which reflect schema-based deliberation, indicating the inhibition LC-NA impaired schema learning and manipulation. Together, our data suggest that LC-NA plays a role in schema-based processes.

Disclosures: S. Amemiya: None. **T.J. McHugh:** None.

Poster

407. Hippocampal Physiology III

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 407.13

Topic: H.08. Learning and Memory

Support: Leon Levy Neuroscience Fellowship
1U19NS104590
U19NS107616
R01MH122391

Title: Hippocampal tuning to task-relevant features in an acoustic-cue guided navigation task

Authors: *I. ZUTSHI[1], E. BALZANI[2,3], T. DOHI[1], C. SAVIN[2,3], G. BUZSÁKI[1];
[1]New York Univ. Sch. of Med., New York, NY; [2]Ctr. for Neural Sci., [3]Ctr. for Data Sci., New York Univ., New York, NY

Abstract: While most often studied for their reliable spatial tuning, neurons within the hippocampus flexibly switch their tuning to any relevant modality under the right conditions, including odors, auditory tones, rewards, action, and self-movement. However, such tuning to sensory modalities is most often evaluated separately from a spatial context (O'Keefe and Krupic, 2021). We sought to address how spatial and sensory tuning converges within the hippocampus by designing a freely moving behavior task that requires flexible switching of attention between spatial and sensory cues while traversing the same environment.
We implemented an '*acoustic cue-guided navigation task' (ACgN)* where mice switch between non-acoustic and acoustic trials on a linear track with multiple reward ports (up to 7 ports). During acoustic trials, mice must attend to an ascending auditory tone (from 1 kHz to 22 kHz) that is played in closed loop and driven by the mouse's spatial location. Importantly, the gain between location and frequency is randomized such that for every trial the target frequency, i.e., 22 kHz, coincides with a random reward port on the track. Mice thus must actively pay attention to the auditory tone as they run on the track, and lick at the correct reward port (corresponding to 22 kHz for that trial) to receive a reward.
In our first implementation of the task, mice chose between three reward ports on the track and reached 80% accuracy. Hippocampal recordings from CA1 specifically during auditory trials demonstrated tuning to a variety of task-relevant features, such as space, reward locations, trajectories, but surprisingly less tuning to pure auditory tones (Aronov et al., 2017). We further validated the tuning of single neurons to these multiple external variables by using a Poisson Generalized Additive Model (Balzani et al., 2020). Next, we implemented a more difficult version of the task, with six possible reward ports. Mice reached 65-70% accuracy within 2-3 weeks of training. Preliminary data on the more difficult version of the task indicates that increased attentional demand alters the representation of task features within CA1 pyramidal cells.
Our recordings from the ACgN task thus reveal rich task-relevant tuning in the hippocampus. However, the circuit mechanisms that generate such a confluence of spatial and task-relevant firing in the hippocampus are currently unknown. By performing optogenetic manipulations, we are further addressing how inputs to CA1, either from the medial prefrontal cortex, or the medial entorhinal cortex, might contribute to independent aspects of task-relevant firing such as sensory, reward, or trajectory-specific tuning.

Disclosures: I. Zutshi: None. **E. Balzani:** None. **T. Dohi:** None. **C. Savin:** None. **G. Buzsáki:** None.

Poster

407. Hippocampal Physiology III

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 407.14

Topic: H.08. Learning and Memory

Support: Simons Foundation (542955)
McKnight Endowment Fund for Neuroscience
National Science Foundation Graduate Research Fellowship
NIMH (R01 MH117777)

Title: Task structure governs activity in the monkey hippocampus

Authors: *J. RUECKEMANN[1,3], Y. BROWNING[2], A. J. MALLORY[1], B. KIM[1], C. I. O'LEARY[1], A. L. FAIRHALL[1], E. A. BUFFALO[1,3];
[1]Physiol. and Biophysics, [2]Grad. Program in Neurosci., Univ. of Washington, Seattle, WA;
[3]Washington Natl. Primate Ctr., Seattle, WA

Abstract: Hippocampal neurons have been most often studied in rodents running mazes, resulting in a rich history characterizing their activity as spatial correlates. However, these neurons have been recently shown to respond in a variety of tasks without an explicit spatial component, suggesting that hippocampal activity more generally reflects the progression of experience through salient task events.

To determine how task structure shapes hippocampal activity in primates, we analyzed data from three rhesus macaques performing a delayed alternation task in a virtual maze - a task chosen to facilitate comparison across species. Neurons were recorded from the full anatomical extent of the hippocampus using chronically-implanted drives housing 124 independently movable electrodes. We find that neurons are selectively active during each phase of the task and reliably fire across trials, resulting in a sequence of ordered activity that robustly tracks progression across the spatial and non-spatial components of the task. Population-level analysis reveals that the task is parcellated by salient events, creating distinct representations of the start zone and post-choice portions of the maze as well as the reward period and the intertrial interval.

For comparison to spatial models of hippocampal function, spatial specificity was assessed in two reference frames: the avatar position in the maze and the location of the monkey's gaze in the virtual world. Using a GLM framework and an information theoretic approach, we find that 30% of neurons exhibit reliable activity relative to avatar position in the maze, and a majority of these neurons are also reliably predicted by gaze location. This seemingly incongruous result is attributable to the many neurons that cover wide expanses of a maze arm, which corresponds to a separation of the maze into task phases. These large firing fields contrast to the canonical punctate place fields seen in rats and strongly suggest that precise location is not the best predictor of the neuronal activity.

We also examined neural activity in distinct environments by changing the visual appearance of the maze. While some neurons "remap" their responses, the majority of neurons maintain their task phase-specific responses irrespective of the visual cues defining the environment. The stability of activity across distinct contexts demonstrates the primacy of task-based responses and suggests that the hippocampus is operating on a schematized representation of this well-learned task. In combination with the parcellation of activity by salient events, these data indicate that representing task structure is foundational to activity in the hippocampus.

Disclosures: **J. Rueckemann:** None. **Y. Browning:** None. **A.J. Mallory:** None. **B. Kim:** None. **C.I. O'Leary:** None. **A.L. Fairhall:** None. **E.A. Buffalo:** None.

Poster

407. Hippocampal Physiology III

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 407.15

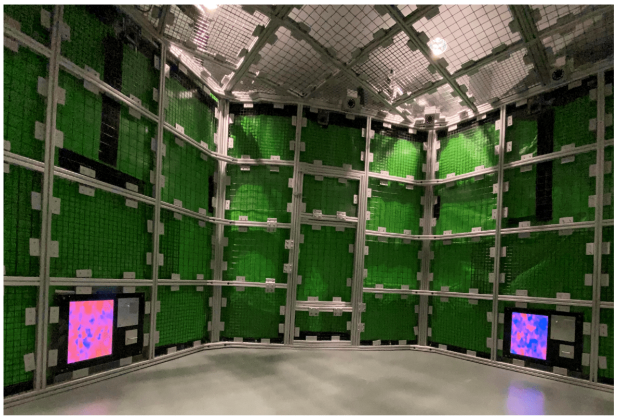
Topic: H.09. Spatial Navigation

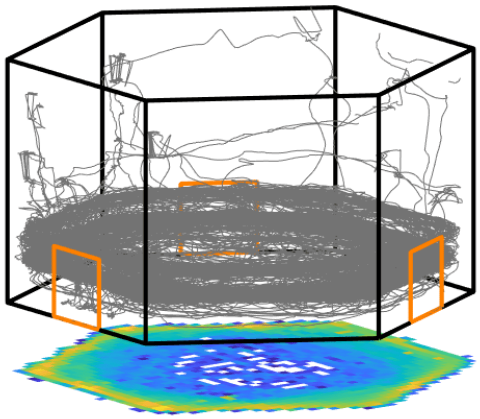
Support: NIH Grant 1RF1 NS127122-01

Title: Freely moving monkeys forage under sensory uncertainty in a naturalistic task

Authors: ***P. ALEFANTIS**[1], T. KILINDRIS[1], B. WOODRUFF[1], B. CAZIOT[2], A. STAVROPOULOS[1], X. PITKOW[3], D. E. ANGELAKI[1];
[1]Ctr. for Neural Science, New York Univ., New York, NY; [2]Dept. of Neurophysics, Univ. of Marburg, Marburg, Germany; [3]Dept. of Neuroscience, Baylor Col. of Med., Houston, TX

Abstract: Within a finite time horizon, animals must distribute their behavior optimally relative to the available resources to maximize their chance of survival. To achieve this, they must collect information from noisy environmental cues, make decisions under uncertainty among a multitude of choices, and navigate to a goal — a natural behavior that is also known as foraging. Technological limitations of the past constrained the study of foraging to oversimplified proxy tasks with unclear ecological validity. Here, we provide a naturalistic framework where unconstrained primates navigate in a 3-dimensional, relatively spacious environment foraging for a juice reward whose availability is signaled by uncertain environmental cues. Specifically, monkeys are exposed to 3 concurrent variable interval (VI) schedules, while the instantaneous probability of reward availability is cued by a color gradient whose transition from blue to red reflects the probability range from 0 to 1. Our results demonstrate that subjects exhibit approximate matching behavior, and foraging policy is significantly affected by stimulus reliability. This task which can be performed both in virtual reality and the real world, allows exploration of naturalistic behaviors that replicate the complexity of the real world while keeping behavioral computation tractable.





Disclosures: **P. Alefantis:** None. **T. Kilindris:** None. **B. Woodruff:** None. **B. Caziot:** None. **A. Stavropoulos:** None. **X. Pitkow:** None. **D.E. Angelaki:** None.

Poster

407. Hippocampal Physiology III

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 407.16

Topic: H.09. Spatial Navigation

Support: FCT Scholarship SFRH/BD/138752/2018
Wellcome Trust Grant HMR02820

Title: Rapid structure learning for route planning in mouse spatial navigation

Authors: *B. S. GODINHO, T. E. J. BEHRENS, M. E. WALTON, T. AKAM;
Univ. of Oxford, Oxford, United Kingdom

Abstract: Behavioural flexibility is a hallmark of human and animal intelligence. It relies on internal models of the world, or cognitive maps, which allow the behavioural consequences of new information to be inferred, rather than learned from trial and error. Rodent spatial navigation is a powerful model system for studying the neural basis of such cognitive maps, due both to its ethological validity and our precise knowledge of how neurons represent space. However, most recordings come from simple behaviours and environments, so a major open question is how spatial representations support model-based behaviours such as planning, in complex dynamic environments. We developed a novel goal-directed navigation task which quantifies route planning while requiring animals to flexibly adapt to changes in both goal location and environment structure. Mice navigated to visually cued goal locations on an elevated maze comprised of 7x7 grid of towers, each with reward port and stimulus light, interconnected by bridges to form a complex graph. Each trial started by illuminating one tower, visually cuing it as the goal. The subject navigated to the goal to receive a water reward, then another randomly

selected tower become the new goal on the next trial. The large number of possible goals, and their non-repeating sequence, precludes habitual strategies, while the complex maze structure allows route planning to be dissociated from vector navigation. Subjects ran two sessions per day, with one bridge added or removed after the first session, such that the maze structure gradually evolved over weeks (n=8, 18 maze structures, 416 sessions, 15611 trials). The sequence of maze configurations was optimised to both maximally decorrelate shortest path distances between successive mazes and discriminate route planning from vector navigation within each maze. Using a mixture of strategies model, we found mice used knowledge of the maze structure to preferentially select shortest paths to the current goal, but were also influenced by a vector navigation strategy using direction to goal. Additionally, mice rapidly adapted their route planning to changes in maze structure, showing a strong influence of structural changes within the first session. We have recorded neural activity in dorsal CA1 to probe mechanisms of route planning and structure learning in this behaviour (n=5, unilateral 128 channel silicon probes, 29 maze structures, 264 sessions, 7051 trials, 2200 cells), and are currently analysing this neural data.

Disclosures: **B.S. Godinho:** None. **T.E.J. Behrens:** None. **M.E. Walton:** None. **T. Akam:** None.

Poster

407. Hippocampal Physiology III

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 407.17

Topic: H.09. Spatial Navigation

Support: Ministry of Education Tier 3 Research Fund (MOE2017-T3-1-002)
National Medical Research Council (OFIRG21JUN-0064)

Title: Mixed spatial selectivity in the non-human primate hippocampus

Authors: H. TAN[1], T. P. Y. NG[1], C. OWENS[1], C. LIBEDINSKY[2,1], ***S.-C. YEN**[1];
[1]The N.1 Inst. for Hlth., [2]Dept. of Psychology, Natl. Univ. of Singapore, Singapore, Singapore

Abstract: The hippocampus and parahippocampal areas support spatial memory across many animal species, including rodents, bats, non-human primates (NHPs), and humans. They contain multiple neurons responsive to spatial variables such as occupied location, head direction, and borders. In rodents, selectivity of hippocampal neurons to single spatial variables is most prevalent, while mixed selectivity for two or more variables can be found in the surrounding areas. However, studies in NHPs and humans have found mixed selectivity to be more prevalent than single selectivity (Wirth et al., 2017; Mao et al., 2021). This discrepancy may be due to differences in the geometry of the recording environments, such as the use of linear tracks in NHP experiments versus open fields in rodent experiments, and analytical methods that have yet to untangle sampling correlations between spatial variables in NHP experiments. We sought to

account for these differences by recording from the NHP hippocampus in a male macaque during navigation in a semi-open field virtual maze, and analyzing the independent activity of place, view, and head direction. Preliminary analysis of 351 cells showed 24 cells exhibiting pure selectivity to a single variable, with 16 cells selective only for place, 8 selective only for view, and none for head direction. Another 16 cells were mixed-selective, with 7 selective for place and view, 2 for place and head direction, 2 for view and head direction, and 7 jointly-selective for place, view, and head direction. Mixed selectivity may be as common as pure selectivity, but place-only selectivity remains a property of a significant proportion of hippocampal units.

Disclosures: H. Tan: None. **T.P.Y. Ng:** None. **C. Owens:** None. **C. Libedinsky:** None. **S. Yen:** None.

Poster

407. Hippocampal Physiology III

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Program #/Poster #: 407.18

Topic: H.09. Spatial Navigation

Support: ERC Consolidator award (SOUNDSCENE)
Rosetrees Trust Seedcorn (M762)

Title: Hippocampal activity in the ferret (Mustela putorius furo)

Authors: *S. L. DUNN, D. A. BENDOR, J. K. BIZLEY;
Univ. Col. London, London, United Kingdom

Abstract: The hippocampal formation is critical for episodic memory and spatial navigation in mammals. The majority of our understanding of the physiology of the hippocampal formation is from numerous seminal works performed in rats and mice. Here, place-, grid-, and head direction cells have been described, along with larger organising principles through network level phenomena such as theta oscillations and sharp-wave ripple events. While these findings have been used to generate models of hippocampal function, significant differences have recently been found across species in distinct mammalian orders, for example bats and primates. To further investigate differences in hippocampal physiology across species we have recorded hippocampal activity in freely moving ferrets (*Mustela putorius furo*). Ferrets are predators of the order *Carnivora* which share many locomotor behaviours with rodents but with sensory strategies that rely on distal senses more akin to those used by primates. Recently we have shown that ferrets have robust theta oscillations during locomotion, but at a lower frequency of 4-7 Hz[1]. We also found a very high proportion of theta oscillations during immobility, in stark contrast to both rodents and primates. This immobility-related theta was abolished by administration of atropine, suggesting it is analogous to Type 2 theta - a relatively rare phenomenon in the rat. To expand on these findings we have performed the first hippocampal single unit recordings from the freely moving ferret. Here we present data collected using both single- and 4-shank

Neuropixels probes during foraging on a large open field (2 x 3 m), while running on a linear track (3 m length), and during sleep.
1. Dunn et al., 'Behaviourally Modulated Hippocampal Theta Oscillations in the Ferret Persist during Both Locomotion and Immobility'. bioRxiv (2021)

Disclosures: S.L. Dunn: None. **D.A. Bendor:** None. **J.K. Bizley:** None.

Poster

408. Hippocampal/Entorhinal Physiology II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 408.01

Topic: H.09. Spatial Navigation

Support: ERC Synergy (Kiloneurons): 951319
Centre of Excellence grant: 223262
Kavli Foundation
NORBRAIN, grant number 295721

Title: The internal direction signal in medial entorhinal cortex and parasubiculum is qualitatively different from the head direction signal in upstream regions

Authors: *A. VOLLAN, R. GARDNER, M.-B. MOSER, E. I. MOSER;
Kavli Inst. Systems Neurosci, Trondheim, Norway

Abstract: A large fraction of cells in the medial entorhinal cortex and parasubiculum (MEC/PaS) encode an internal direction (ID) signal with theta-rhythmic, left-right-alternating dynamics that differs from head direction (HD) cells (Gardner et al, same session). It is unknown where and how the ID signal is generated and whether ID coding is unique to MEC/PaS. We therefore asked if we could find features of ID at earlier stages of the classical HD circuit. To address this question, we recorded neural activity from many hundreds of cells with Neuropixels probes in MEC/PaS and either presubiculum (PrS) or anterodorsal thalamus (ADn) in rats during open field foraging, linear track running and sleep.
We extracted the directional signals encoded by ensembles of cells in each region by applying dimensionality reduction methods to population spike counts. The directional signal in MEC/PaS displayed left-right-alternating dynamics, while the directional signals in ADn/PrS reliably tracked HD. During exploration and REM sleep, we observed occasional 'decoupling events' where the directional signals in ADn/PrS and MEC/PaS drifted independently. Except for these 'decoupling events', the ID signal in MEC/PaS was centred around the HD signal in ADn/PrS, and the anchoring between the two signals remained unchanged during sleep and after rotations of the environment.
Lastly, during sleep-to-wake transitions, the firing of ID cells in MEC/PaS was markedly reduced, while HD cells in ADn remained highly active. The brief suppression of ID cell firing might be required to switch from an internally generated to a sensory driven representation of the

animal's surroundings during awakening. This inverse relationship in population activity also supports the notion that HD and ID representations are partially independent.
In conclusion, there is a functional split between the directional codes in ADn/PrS, which faithfully track HD, and the ID signal in MEC/PaS. The two signals are correlated but can transiently decouple and differ on the theta-timescale. HD and ID are similarly anchored across brain states, suggesting that they might be distinct directional signals operating within the same cognitive map.

Disclosures: A. Vollan: None. **R. Gardner:** None. **M. Moser:** None. **E.I. Moser:** None.

Poster

408. Hippocampal/Entorhinal Physiology II

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 408.02

Topic: H.09. Spatial Navigation

Support: ERC Synergy Grant 951319
RCN FRIPRO Grant 286225
Centre of Neural Computation 223262
Eccellenza Grant PCEGP3_194220
ERC Starting Grant 850769

Title: Minute-scale oscillatory sequences in medial entorhinal cortex

Authors: *S. GONZALO COGNO[1], H. OBENHAUS[1], R. JACOBSEN[1], F. DONATO[2], M.-B. MOSER[1], E. MOSER[1];
[1]Kavli Inst. for Systems Neuroscience, NTNU, Trondheim, Norway; [2]Biozentrum, Univ. of Basel, Basel, Switzerland

Abstract: The medial entorhinal cortex (MEC) hosts many of the brain's circuit elements for spatial navigation and episodic memory, operations that require neural activity to be organized across long durations of experience. While location is known to be encoded by a plethora of spatially tuned cell types in this brain region, little is known about how the activity of entorhinal cells is tied together over time. Among the brain's most powerful mechanisms for neural coordination are network oscillations, which dynamically synchronize neural activity across circuit elements. In MEC, theta and gamma oscillations provide temporal structure to the neural population activity at subsecond time scales. It remains an open question, however, whether similarly powerful coordination occurs in MEC at behavioural time scales, in the second-to-minute regime. Here we show that MEC activity can be organized into a minute-scale oscillation that entrains nearly the entire cell population, with periods ranging from 10 to 100 seconds. Throughout this ultraslow oscillation, neural activity progresses in periodic and stereotyped sequences. This activity was elicited while mice ran at free pace on a rotating wheel in darkness, with no change in its location or running direction and no scheduled rewards. The oscillation

sometimes advanced uninterruptedly for tens of minutes, transcending epochs of locomotion and immobility. Similar oscillatory sequences were not observed in neighboring parasubiculum or in visual cortex. The ultraslow oscillation of activity sequences in MEC may have the potential to couple its neurons and circuits across extended time scales and to serve as a scaffold for processes that unfold at behavioural time scales, such as navigation and episodic memory formation.

Disclosures: S. Gonzalo Cogno: None. **H. Obenhaus:** None. **R. Jacobsen:** None. **F. Donato:** None. **M. Moser:** None. **E. Moser:** None.

Poster

408. Hippocampal/Entorhinal Physiology II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 408.03

Topic: H.09. Spatial Navigation

Support: Forskningsrådet SFF (10399101)

Title: Egocentric modulation of hippocampal place cells

Authors: *J. CARPENTER[1], J. BLACKSTAD[1], D. TINGLEY[2], E. I. MOSER[1], M.-B. MOSER[1], B. A. DUNN[3];
[1]Kavli Inst. for Systems Neurosci., Trondheim, Norway; [2]Endocrinol., Beth Israel Deaconess Med. Ctr., Boston, CA; [3]Mathematical Sci., NTNU, Trondheim, Norway

Abstract: Navigation relies on the integration of incoming sensory information with a stable schema that can be applied to map the current environment. This integration results in a dynamic map of the animal's position based on coordinate systems which have origins defined either by the navigator (egocentric) or the assembly of distal external cues (allocentric). In recordings from the hippocampus, neurons represent pure allocentric space (place cells), but their firing rates may also show tuning to angular variables, defined in either an allocentric or an egocentric reference frame.
Here, we focus on systematic firing rate changes in place cells that correlate to a preferential egocentric orientation relative to reference points within an animal's local environment. In agreement with previous work, we find indications for modulation of place cell activity by egocentric variables using a previously defined method in data from open field environments. The applied method models spatially dependent egocentric tuning curves as cosine functions to determine an optimal egocentric reference point and compares the results to a cyclically permuted null distribution. In the present study, we further tested the possibility that the activity in hippocampal neurons can sometimes be falsely attributed to angular variables. By creating a large population of simulated neurons composed of functional cell types with various forms of spatial tuning and by applying classical statistical approaches, we find that while simulated units with true tuning to egocentric variables are identified with high accuracy, simulated 'pure' place

cells are often misclassified as being significantly tuned to egocentric variables. We find that these false positive rates vary across cell types, behavioral sessions, noise levels, and firing field sizes, pointing to factors that might potentially account for misattribution to egocentric tuning in experimental studies. Lastly, we employ a more conservative cross-validated generalized linear model approach to compare models with place, egocentric bearing and head direction. We find that a markedly smaller proportion of recorded hippocampal cells are stably modulated by egocentric bearing than expected from previous reports. Our results suggest that, at least in the case of open field recordings, egocentric modulation of hippocampal neurons may be over-estimated.

Disclosures: **J. Carpenter:** None. **J. Blackstad:** None. **D. Tingley:** None. **E.I. Moser:** None. **M. Moser:** None. **B.A. Dunn:** None.

Poster

408. Hippocampal/Entorhinal Physiology II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 408.04

Topic: H.09. Spatial Navigation

Support: ERC Grant 951319
RCN Grant 286225
RCN Grant 300394
RCN Grant 286225
RCN Grant 223262
RCN Grant 295721
EMBO Grant 1078-2020

Title: An all-optical approach for characterizing functional connectivity among cell types in medial entorhinal cortex

Authors: *W. ZONG[1], R. VALE[1], N. L. DE JONG[1], M.-B. MOSER[2], E. I. MOSER[2];
[2]Kavli Inst. Systems Neurosci, [1]Kavli Inst. Systems Neurosci, Trondheim, Norway

Abstract: The medial entorhinal cortex (MEC) is thought to create an internal map of self-position that animals use for spatial navigation. This map contains several spatially tuned cell types active at specific locations or heading-directions: grid cells, border cells, object-vector cells, and head-direction cells. How these cells interact as a network to enable self-localization and navigation is yet to be determined. The most popular theoretical framework for dynamic representation of location builds on the idea of continuous attractor networks (CAN), where specific recurrent synaptic connectivity constraints the joint activity of neurons to a low-dimensional manifold, in one dimension for head direction cells and in two dimensions for grid cells. However, predictions from CAN models have been difficult to test due to small cell samples in experimental data. Recent recordings with high-throughput silicon probes support

CAN models by showing that populations of grid cells operate on a toroidal manifold (Gardner et al 2022). Yet these recordings leave a key prediction of CAN models untested: most CAN models predict strong excitatory connectivity between functionally similar cells and weaker or inhibitory connectivity between functionally dissimilar cells. This functional connectivity determines the formation of a neural activity bump that can move around on the manifold in accordance with the animal's location or direction. Here we set out to measure, with a new all-optical interrogation approach, the probability and amplitude of the functional connectivity among spatially tuned MEC cells as proposed by many CAN models. We follow a three-step procedure: i) we first use our recently developed miniature two-photon (2P) microscope-MINI2P (Zong, et.al., 2022) to identify functionally tuned cells in freely-moving mice; ii) we then align the field-of-view (FOV) from the MINI2P with the FOV of a benchtop 2P photostimulation system and photo-activate opsin-positive functionally characterized neurons that were identified in the first step, and iii) we measure stimulation-triggered responses in untargeted neurons to measure the functional connectivity between functionally identified cells. We shall present methods of MINI2P vs. benchtop 2P photostimulation system alignment, protocols for detecting and stimulating functional cell types, as well as data on the efficiency of 2P photostimulation in MEC onto target cells. The method shall allow us to directly test connectivity predictions of CAN models for grid cells and head direction cells, as well as the organization of connections between of grid cell modules, head direction cells, and other spatial cell types.

Disclosures: W. Zong: None. **R. Vale:** None. **N. L. de Jong:** None. **M. Moser:** None. **E.I. Moser:** None.

Poster

408. Hippocampal/Entorhinal Physiology II

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Program #/Poster #: 408.05

Topic: H.09. Spatial Navigation

Support: ERC Synergy grant 286225 'KILONEURONS'
Research Council of Norway grant 295721 'NORBRAIN'
Research Council of Norway Center of Excellence grant 223262
Kavli Foundation

Title: Sweep generation in grid-cell circuits via a velocity integration mechanism

Authors: *R. J. GARDNER, A. Z. VOLLAN, T. WAAGA, M.-B. MOSER, E. I. MOSER; Kavli Inst. For Systems Neurosci., Trondheim, Norway

Abstract: During alert states, position-tuned cells in the hippocampal formation activate in rapid sequences which encode "sweeps" - highly stereotyped prospective trajectories traveling outwards from the animal's location once every theta cycle (Johnson & Redish, 2007, O'Neill 2017, Kay et al, 2020). While the mechanistic origin of sweeps is unknown, continuous attractor

network (CAN) models of grid cells provide a candidate mechanism for generating smooth, sweep-like trajectories, independently of spatial context. Crucially, an input directional signal is required to "drive" the trajectories - therefore, the hypothesis that sweeps originate from this mechanism predicts the existence of a neural code for sweep directions. We therefore searched for the latter, using Neuropixels probe recordings from 100s-1000s of cells in medial entorhinal cortex of rats.
We discovered a covert, theta-entrained population code which we term the "internal direction" (ID). During navigation, ID rhythmically alternated between two principal angles offset ~45° either side of the animal's head direction. Alternation was strongest during high-speed runs on a linear track, revealing a dissociation between ID and route planning. Remarkably, ID correlated rigidly with directions of grid-cell sweeps in all behavioral contexts (including REM sleep), such that individual ID-tuned cells represented fixed directions on the grid-cell torus manifold.
Finally, we simulated a grid-cell CAN model's response to input from the empirically observed ID signal. The model generated sweeps which shared key spatiotemporal features with real grid-cell sweeps. First, the model predicted that each grid-cell module generates sweeps of a stereotypical length proportional to the grid spacing. The lengths of real grid-cell sweeps conformed to this prediction; furthermore, sweeps never travelled further than half the grid spacing - meaning that all possible locations spanned by a sweep are represented unambiguously by the grid module. Second, the model predicted that grid-cell sweeps may traverse all nearby locations regardless of familiarity or accessibility. We tested this prediction by applying latent-variable analysis to grid- and place-cell activity, which confirmed that cells in both categories were tuned to spatial locations outside the arena boundaries.
The reported directional population code is a plausible candidate for driving grid-cell sweeps via a velocity integration mechanism. Sweep-generation may be a fundamental function of grid-cell circuits, connected to the latter's role as a spatial metric, in contrast to earlier speculation that sweeps support route-planning.

Disclosures: **R.J. Gardner:** None. **A.Z. Vollan:** None. **T. Waaga:** None. **M. Moser:** None. **E.I. Moser:** None.

Poster

408. Hippocampal/Entorhinal Physiology II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 408.06

Topic: H.09. Spatial Navigation

Support: Wellcome Trust Senior Research Fellowship, 220886/Z/20/Z
ERC Consolidator Award 'DEVMEM'

Title: Functional development of the grid cell network in the entorhinal cortex

Authors: L. MUESSIG[1], F. CACUCCI[2], *T. WILLS[2];
[1]Cell and Developmental Biol., [2]Univ. Col. London, London, United Kingdom

Abstract: Grid cells are principal cells in layers II/III of the medial entorhinal cortex (mEC) whose activity in rodents, as animals explore an open field environment, is characterised by local peaks of high activity arranged in a repeating pattern of equilateral triangles. This pattern can be described by its wavelength (distance between peaks), orientation (angle of peak locations to an arbitrary axis) and phase (peak location). On the population level groups of grid cells are organised into modules whereby cells in the same module have similar wavelengths and orientations but a different phase. In contrast, cells in different modules have different wavelengths, with increasing period from dorsal to ventral locations in the mEC, and mostly different orientations. Previous work in rats has shown that in early life adult-like grid cell firing emerges during the 4th week after birth. Because the existing data was acquired using tetrode techniques, which make it challenging to track individual cells across days as well as to record the activity of large ensembles of grid cells, very little is known about how the firing patterns of individual cells emerge and how the modular organisation of grid cells on the population level might develop. Here we use neuropixels probes to record large groups of grid cells from the mEC in 3-5 week old rats in an attempt to track the emergence of grid cell firing in young animals and characterise how and when the population level organisation of grid cells into modules occurs. In particular, it is unclear whether different modules emerge sequentially or in parallel. One hypothesis is that short wavelength modules might emerge first as younger animals will navigate along shorter distances and it has previously been shown that in the mEC there is a dorso-ventral gradient in the maturation of principal cells during the first 3 weeks after birth. Our preliminary results indicate that different modules emerge concurrently and grid cells in all modules show experience-dependent rescaling upon repeated exposure to an environment. The combination of the population signal of several modules is a theoretical prerequisite for an unambiguous estimate of self-location based on grid cell activity. Other than place cells whose population level properties, e.g. their organisation into theta sequences or offline hippocampal replay, appear &gt 1 week after their location specific firing, our results indicate that grid cells show the hallmarks of their population organisation from the time point of their emergence. This occurs broadly within the developmental window during which hippocampal dependent memory first emerges.

Disclosures: **L. Muessig:** None. **F. Cacucci:** None. **T. Wills:** None.

Poster

408. Hippocampal/Entorhinal Physiology II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 408.07

Topic: H.09. Spatial Navigation

Support: NINDS, NIH intramural research program

Title: A consistent environmental map of the medial entorhinal cortex contributes to spatial memory

Authors: *T. J. MALONE[1], N.-W. TIEN[1], L. CUI[1], S. LYU[1], M. V. MYROSHNYCHENKO[2], D. A. KUPFERSCHMIDT[2], J. A. GORDON[2], Y. GU[1];
[1]Natl. Inst. of Neurolog. Disorders and Stroke, Bethesda, MD; [2]Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: The medial entorhinal cortex (MEC) plays a crucial role in spatial navigation and the encoding of spatial memory. This role is evidenced by spatial memory impairment in rodents after entorhinal dysfunction and in human patients with medial temporal lobectomy or damage. The MEC is also one of the first areas affected in Alzheimer's disease when significant spatial memory decline emerges. However, whether and how the neural dynamics of the MEC relate to the encoding of spatial memory during learning have not been fully investigated. Here, we performed cellular-resolution calcium imaging of the MEC while mice learned to navigate a novel virtual environment over multiple days. We utilized this technique to investigate the relationship between MEC activity and spatial learning performance, which varied among individual mice. Based on spatial learning performance, we categorized mice as good-performers and bad-performers. We found that good-performer mice had fewer active cells, which exhibited stronger neural activity, higher and continuously improved inter-day and intra-day activity consistency, enhanced representation of the spatial environment, and more accurate spatial decoding by population activity. Together, these data suggest that the consistent spatial map in the MEC in good performers can better encode the spatial environment during learning. Optogenetically disrupting this consistent MEC map significantly reduced a mouse's performance in a learned environment, supporting a causal link between the consistent MEC map and spatial memory. Finally, good performers showed increased numbers of c-Fos$^+$ MEC cells in novel environments, indicating the encoding of spatial memory in the local MEC network. Overall, our study demonstrates the important role of a consistent spatial map in the MEC for spatial memory.

Disclosures: T.J. Malone: None. **N. Tien:** None. **L. Cui:** None. **S. Lyu:** None. **M.V. Myroshnychenko:** None. **D.A. Kupferschmidt:** None. **J.A. Gordon:** None. **Y. Gu:** None.

Poster

408. Hippocampal/Entorhinal Physiology II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 408.08

Topic: H.09. Spatial Navigation

Support: University of St Andrews
Alfred Dunhill Links Foundation

Title: Reduction in grid cell regularity is associated with increased error in distance estimation

Authors: *S. DUNCAN[1], M. V. KURUVILLA[2], B. THOMPSON[3], D. BUSH[4], J. A. AINGE[3];
[1]Indiana Univ., Bloomington, IN; [2]Wicking Dementia Res. and Educ. Ctr., Univ. of Tasmania,

Hobart, Australia; [3]Univ. of St Andrews, Univ. of St Andrews, St Andrews, United Kingdom; [4]Inst. of Cognitive Neurosci., Univ. Col. London, London, United Kingdom

Abstract: Grid cells in the medial entorhinal cortex (MEC) fire in a regular hexagonal grid pattern as an animal passes through its environment - providing a putative measure of distance travelled that could be used for path integration. The regular hexagonal firing pattern of grid cells has been shown to distort in polarized environmental geometries such as trapezoids, suggesting that path integration should also be impaired. The present study made *in-vivo* electrophysiological recordings from grid cells in MEC in rats performing a distance estimation task. Rats (n=4) were trained to run a specific distance in a rectangular box. The box was then transformed into a trapezoid and the rats required to estimate the same distance. Rats consistently over-estimated the distance they had travelled in trapezoid relative to rectangle environments. Grid cells were defined statistically relative to a random distribution of gridness scores produced using shuffling analysis of rotational symmetry scores. Grid cells were significantly less regular in the trapezoid open field compared to the rectangle open field. Interestingly, grid regularity did not immediately return to normal in the rectangle open field following the trapezoid open field suggesting that distortions in the grid pattern caused by altering environmental geometry take some time to be reset. Grid regularity was also examined in the trials by assessing differences in spike density distributions between rectangle and trapezoid trials and their respective open fields. Results indicated that grid cells were distorted to a significantly greater extent in the trapezoid trials than in the rectangle trials. Furthermore, the grid distortion measured during the trials was positively correlated with distance estimation error. These data are consistent with grid cell regularity being necessary for distance estimation.

Disclosures: S. Duncan: None. **M.V. Kuruvilla:** None. **B. Thompson:** None. **D. Bush:** None. **J.A. Ainge:** None.

Poster

408. Hippocampal/Entorhinal Physiology II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 408.09

Topic: H.09. Spatial Navigation

Support: KAKENHI 21K15611
PRESTO JPMJPR21C2
KAKENHI 18H05213
KAKENHI 19H04994

Title: From grid cells to language: generalized spatial representation model of entorhinal cortex

Authors: T. HAGA[1], Y. OSEKI[2], T. FUKAI[1];
[1]Okinawa Inst. of Sci. and Technol. Grad. Univ., Onna-son, Okinawa, Japan; [2]Dept. of Language

and Information Sciences, Grad. Sch. of Arts and Sci., Univ. of Tokyo, Meguro-ku, Tokyo, Japan

Abstract: In medial entorhinal cortex (MEC), a neural population of grid cells represents a position in the physical space and supports vector-based spatial navigation. Recent experiments additionally suggest that grid-like representations in MEC are created not only for physical space but also for conceptual space if there is a 2-dimensional structure, and those representations are the basis of vector-based conceptual inference (Constantinescu et al., 2016; Bao et al., 2019; Park et al., 2021). However, the computational principle behind such universal neural processing of physical and conceptual space is still unclear, and whether the neural processing mechanism in 2-D can be generalized to complex conceptual space beyond 2-D is not known. Here we propose a model which we call disentangled successor information (DSI). DSI is an extension of successor representation (SR) (Dayan, 1993; Stachenfeld et al., 2017), which stems from a theory of reinforcement learning and became one of promising computational models of hippocampus and MEC. Like dimension-reduced SR, DSI in 2-D physical space yield grid-like codes which quantitatively reproduces experimentally observed properties of grid cells in MEC. In addition, DSI can be applied to natural language by regarding words as a sequence of states, and in that case, DSI generates semantic representations that resemble “concept cell” which has been found in human medial temporal lobe (Quiroga, 2012; Reber et al., 2019). This result indicates that DSI model can learn biologically plausible representations for 2-D space and semantic concepts. Furthermore, we also found that DSI enables vector-based computation in both physical and conceptual space. In a 2-D space, vector-based navigation using DSI representations mathematically approximates value-based decision making with additional constraints (Todorov, 2009). In a conceptual space learned from natural language, vectorial calculation enables analogical inference like word embedding models in natural language processing (e.g. Mikolov et al., 2013). For example, if we calculate “king” - “man” + “woman” using DSI vectors, the resulting vector is close to “queen” vector, which is inference of a new concept by combining other concepts. Our model reveals a new theoretical relationship between models in computational neuroscience and machine learning, and suggests a hypothesis that spatial processing mechanism in MEC can be generalized to vector-based inference of complex conceptual space in language.

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Poster

408. Hippocampal/Entorhinal Physiology II

Location: SDCC Halls B-H

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Program #/Poster #: 408.10

Topic: H.09. Spatial Navigation

Support: R01 NS039456
T32 AG027668

Title: Generation of Hippocampal Landmark Vector Activity: Effects of visual input and dependence on allocentric vs. egocentric bearing inputs

Authors: *V. PULIYADI[1], R. P. JAYAKUMAR[2], S. S. DESHMUKH[4], J. J. KNIERIM[3]; [1]Psychological and Brain Sciences, Zanvyl Krieger Mind/Brain Inst., Johns Hopkins Univ., Baltimore, MD; [2]Zanvyl/Krieger Mind Brain Institute; Lab. for Computat. Sensing and Robotics, Johns Hopkins Univ., BALTIMORE, MD; [3]Zanvyl Krieger Mind/Brain Institute, Solomon H. Snyder Dept. of Neurosci., Johns Hopkins Univ., Baltimore, MD; [4]Ctr. for Neurosci., Indian Inst. of Sci., Bangalore, India

Abstract: For spatial navigation, the presence of landmarks provides precise information about an animal's location. Previous work has shown that distance and direction from landmarks are critical for finding hidden goals (Collett et al. 1986). McNaughton et al. (1995) proposed a vector encoding model to explain this behavior, suggesting that individual place fields can generate a vector with orientation and distance from a specific landmark. Instances of such landmark vector encoding were identified in previous recordings from our lab in the CA1 hippocampal subregion (Deshmukh and Knierim, 2013). Landmark vector cells are defined as cells with place fields that maintain a specific and consistent vector relationship to specific landmarks in the environment. In this experiment, we aimed to investigate how these vector representations may be computed. Since these fields are object-related but located away from the objects, visual cues would be expected to perform an important role in the generation and maintenance of these landmark vectors. To investigate the necessity of visual information for landmark vector cells, the neural activity of CA1 place cells was recorded while rats foraged on a circular platform in darkness with three objects. The objects were placed either at the center of the apparatus to allow for vector fields to form in all directions around the object, or at the perimeter of the apparatus to use as a control. Based on data from 458 units in 7 male Long-Evans rats, we observed a greater proportion of neurons with vector activity when objects were placed near the center of the environment than at the perimeter of the environment, suggesting that these vector relationships can be computed without visual information. Within this data set we also observed instances of spontaneous rotation of fields between experimental sessions, as previously reported by Knierim et al. (1995). While place cells during these sessions used the center of the apparatus as the axis of rotation, landmark vector cells rotated at the same angle but maintained a relationship to the objects, using each object as an axis of rotation. We provide a new model of how place fields and landmark vector cells can be generated using a common computational mechanism by which allocentric head direction cells and egocentic bearing cells (Wang et al., 2018) converge to generate allocentric place fields bound either to local landmarks (i.e., landmark vector cells) or to the center of the environment (i.e., place cells; O'Keefe, 1990).

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Poster

408. Hippocampal/Entorhinal Physiology II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 408.11

Title: WITHDRAWN

Poster

408. Hippocampal/Entorhinal Physiology II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 408.12

Topic: H.09. Spatial Navigation

Support: NSF GRFP DGE # 1746891
NIH R01 NS039456

Title: Leveraging place field repetition to understand positional versus nonpositional inputs to hippocampal field CA1

Authors: *W. HOCKEIMER[1,2], R.-Y. LAI[1], M. NATRAJAN[2], W. SNIDER[1,2], J. J. KNIERIM[1,2];
[1]Krieger Mind/Brain Inst., [2]Solomon H. Snyder Dept. of Neurosci., Johns Hopkins Univ., Baltimore, MD

Abstract: The hippocampus is believed to encode episodic memory by binding information about the content of experience within a spatial framework encoding the location of that experience. Previous work implies a distinction between positional inputs to the hippocampus, which provide information about an animal's location, and nonpositional inputs, which provide information about the content of experience, both sensory and navigational. Here we leverage the phenomenon of "place field repetition" to better understand the functional dissociation between positional and nonpositional inputs to CA1 as rats navigated freely on a novel city-block maze, which combined elements of open-field foraging and linear-track tasks. The fields of neurons with multiple fields were significantly more likely to share their orientation (i.e., being located in vertical versus horizontal alleys) than expected by chance (mean orientation alignment score = 0.71, 95th percentile shuffle = 0.64), demonstrating a positional input determining where the cell fires that was preserved across locations. Unlike typical results in open-field foraging, many place fields (30% identified via Mann-Whitney test; 36% identified via generalized linear model (GLM)) were directionally tuned on the maze, even though the animal's behavior was not constrained to 1-D trajectories. Repeating fields from the same cell tended to have the same directional preference when the fields were aligned along a linear corridor of the maze (directional tuning correlation $r^2 = 0.237$), but they showed uncorrelated directional preferences when they were unaligned across different corridors (directional tuning correlation $r^2 = 0.017$). Lastly, individual fields displayed complex time dynamics (28% of fields with significant effect of time within GLM). This resulted in the population activity changing continuously over the course of minutes (average slope per data set through population vector correlations from increasingly spaced time windows = -0.06 ± 0.022, two-sided t-test compared to 0, $t(9) = 7.88$, p

= $2.49*10^{-5}$). These temporal dynamics were evident across repeating fields of the same cell. These results demonstrate that the positional inputs that drive a cell to fire in similar locations across the maze can be behaviorally and temporally dissociated from the nonpositional inputs that alter the firing rates of the cell within its place fields, thereby increasing the flexibility of the system to encode episodic variables within a stable, spatial framework provided by place cells.

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Poster

408. Hippocampal/Entorhinal Physiology II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 408.13

Topic: H.08. Learning and Memory

Title: Lateral entorhinal cortex involvement in episodic-like memory in mice

Authors: F. TOZZI[1,2], M. MAINARDI[1], A. CATTANEO[2], *N. ORIGLIA[1];
[1]CNR- Neurosci. Inst., Pisa, Italy; [2]Bio@SNS, Scuola Normale Superiore, Pisa, Italy

Abstract: Increasing evidence indicates that the Lateral Entorhinal Cortex (LEC) might play a fundamental role in forming and recalling episodic memories. Here, we aimed to clarify the contribution of the LEC to the recall of previously acquired episodic-like associative memories. To study episodic memory in a non-human animal, we took advantage of a behavioral paradigm called the novel object-place-context recognition test (OPCRT) that allowed us to investigate mice ability to discriminate between novel and familiar object-place-context associations. Using immunofluorescence staining, we observed similar recruitment of the lateral and medial subdivisions of the EC during memory processing in 3 month old wild-type C57BL/6 mice. However, electrophysiological recordings in brain slices obtained from mice, 12h following the execution of OPCRT, revealed a specific long-lasting change in long-term forms of synaptic plasticity, such as long-term potentiation (LTP) and long-term depression (LTD) in the LEC intrinsic circuitry. In particular, LTP was occluded and LTD was enhanced after OPCRT respect to controls and to slices obtained from mice exposed only to the context. These synaptic plasticity changes were observed in a time window comparable to that of memory expression, suggesting that the LEC might retain a trace of past experiences that could be useful for subsequent memory recall. By combining activity-dependent mapping with chemogenetic manipulation, we identified the LEC neuronal ensembles engaged during the encoding phase of a new episodic memory and we demonstrated that their re-activation is necessary and sufficient to elicit successful memory recall, confirming that the LEC plays a crucial role in this process. Finally, cfos expression analysis confirmed that the LEC, differently from the MEC, is recruited during successful memory recall and it is not engaged by failed retrieval, further strengthening the link between LEC neuronal activity and memory expression at the behavioral level. Taken together, our results suggest that the LEC can store internal representations of past episodes

during the ongoing experience into the activity of neuronal ensembles whose re-activation is necessary and sufficient for the subsequent recall of previously acquired information

Disclosures: F. Tozzi: None. **M. Mainardi:** None. **A. Cattaneo:** None. **N. Origlia:** None.

Poster

408. Hippocampal/Entorhinal Physiology II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 408.14

Topic: H.09. Spatial Navigation

Support: INT/WT-ISSF/Cambridge Joint RG
Wellcome Trust Sir Henry Dale fellowship
Pathfinding Bridging Fund (School of Biology, Cambridge)

Title: Error correction in the hippocampal-medial-entorhinal cognitive map

Authors: T. VARGOVA[1], ***P. KEREKES**[1], M. BAUZA[2], J. KRUPIC[1];
[1]Univ. of Cambridge, Cambridge, United Kingdom; [2]Univ. Col. London, London, United Kingdom

Abstract: Hippocampal place cells and medial entorhinal grid cells, border cells and head direction cells are thought to support cognitive map-based navigation. However, they are also known to accumulate error and recent studies have shown that environmental cues have a complex influence on their firing patterns.
We investigated the effect of environmental landmarks on error correction in spatially modulated cells during one dimensional navigation in a virtual reality environment, using in vivo chronic multi-tetrode recordings from mouse hippocampus and medial entorhinal cortex. Increasing the number of available visual cues resulted in an increase in the number of firing fields, decrease in the field size and variability, and increase in peak firing rate. A cell-type specific preference for field position relative to cue location was observed. Average drift in field position decreased significantly with increasing number of cues, while precision with which cells corrected their drift significantly increased. This effect was stronger in fields located closer to the visual cues. The presence of a non-visually cued fixed reward location decreased drift, suggesting that error in spatial cell firing can also be modulated by non-visual cues. Increasing the spatial information content of the presented visual cues further increased these stabilizing effects. The above changes resulted in the animal's increased precision in anticipating the reward location. These results suggest that increasing the number and information content of cues in the environment helps correct error in the firing of spatial cells by stabilizing their firing fields and increasing their spatial resolution.
To further understand the role of visual cues in correcting the map error, we set randomly chosen sections of the linear track to be fully deprived of visual cues (the virtual reality monitors displaying only a dark background). We are currently analysing the extent of error accumulation

as a function of the relative position of these dark epochs compared to the field position. With these experiments we aim at reporting systematically the effect of gradually increasing visual cue information on the cognitive map stabilization.

Disclosures: **T. Vargova:** None. **P. Kerekes:** None. **M. Bauza:** None. **J. Krupic:** None.

Poster

408. Hippocampal/Entorhinal Physiology II

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 408.15

Topic: H.09. Spatial Navigation

Support: St. Olaf College CURI funding

Title: Head direction cell activity is disrupted during anesthesia, and shows properties consistent with a ring attractor network model during recovery

Authors: *G. MUIR[1], H. CABASCO[2], I. AARRESTAD[3], N. WEITERMANN[1];
[1]St. Olaf Col., Northfield, MN; [2]Allen Brain Inst., Seattle, WA; [3]UC Davis, Davis, CA

Abstract: Head direction (HD) cells in the rat provide orientation information by firing only when the rat's head points in a specific direction in the horizontal plane. In this study, we investigated the effects of inhalable (isoflurane) and injectable (sodium pentobarbital) anesthesia on HD cell activity by recording from HD cells in the anterior dorsal thalamus (ADN) while rats were unconscious and as they 1) recovered from, or 2) became unconscious under, anesthesia. Methods: During the baseline session, HD cells were isolated while rats foraged in a gray cylinder with a white cue card on the wall. Following anesthesia administration, HD cells were recorded while the rat lay unconscious on a slowly rotating platform, and, in the case of isoflurane, during recovery in a cylinder with, or without, the original cue card present. Results: In all cases, HD cells ceased to fire directionally while the rat was unconscious under anesthesia. Additionally, HD cells' average and peak firing rates decreased significantly while under anesthesia. With recovery from isoflurane, in the seconds immediately before the rats' initial movement, all HD cells showed a significant increase in firing rate, with most HD cells quickly recovering their directional tuning following the rat's initial movement. If the cue card was present in its original location during recovery, the HD cell returned to its baseline session preferred firing direction (PFD). In contrast, however, if the rat recovered in a cylinder with the cue card removed, the HD cell adopted a random PFD. Discussion: These results clearly show the loss of the directional signal in the HD cell network in cells recorded from the ADN while the rat is unconscious under anesthesia. During recovery (from isoflurane), however, the HD cell network is able to quickly re-establish a stable state, as seen in 1) the return of directional firing, and 2) the return of HD cell firing to their baseline session PFDs. Importantly, the activity of these cells under anesthesia is also consistent with expected network properties of a ring attractor network model, where a winner-takes-all increase in firing rates across all cells ultimately

stabilizes in a bump of activity in one location in the network, which is subsequently controlled by learned relationships with external landmarks.

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Poster

408. Hippocampal/Entorhinal Physiology II

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Program #/Poster #: 408.16

Topic: H.09. Spatial Navigation

Support: NIH Grant 5R01NS104193-04

Title: Optogenetic inhibition of mammillo-tegmental feedback terminals with halorhodopsin does not change head direction selectivity in freely moving rats

Authors: *J. A. GRAHAM[1], A. C. GUNDLACH[1], J. S. TAUBE[2];
[1]Psychological & Brain Sci., Dartmouth, Hanover, NH; [2]Psychological & Brain Sci., Darmouth Col., Hanover, NH

Abstract: An accurate sense of direction is required for successful navigation. In mammals, head direction (HD) cells respond selectively to a single direction anywhere in the environment. Computational models have used a 'Ring Attractor' construct to describe the dynamics of the HD signal and predict the required input and underlying connectivity needed to produce it (excited HD cells inhibit cells with preferred firing directions not currently being sampled). Lesion and recording studies suggest that the putative ring attractor lies within or across two reciprocally connected nuclei - the dorsal tegmental nucleus (DTN) and lateral mammillary nucleus (LMN). Current models suggest that selective inhibition of the feedback pathway from LMN to DTN would completely disrupt HD selectivity in DTN. This feedback projection could also be used to fine-tune HD representations and we would expect changes in tuning width, preference, and stability following disruption to this pathway. To test how the LMN to DTN pathway contributes to the generation of the HD signal, we injected anterograde virus (AAV5-hsyn-eNpHR3.0)(n=2 rats) or control virus (n=1 rat) bilaterally in LMN and implanted an optogenetic fiber above the terminals in DTN. HD cells in the anterior thalamus were recorded while rats freely foraged in an open arena and inactivated the feedback LMN to DTN pathway. Results from 20 cells in rats with eNpHR3.0 and 14 cells from control virus-injected rats suggest that optogenetic inhibition of LMN to DTN terminals has no effect on thalamic HD cells' peak firing rate , preferred firing direction, or tuning width in both light or dark conditions. Future work using alternative terminal inhibition strategies, and recording in DTN during terminal inhibition, will be used to confirm the extent and validity of these findings. If inhibition via these alternative strategies does not produce changes in HD cell responses or navigation behavior of the animal, then these findings would suggest that models of mammalian HD attractor dynamics

require rethinking to de-emphasize the role of the LMN to DTN projections in generating a stable HD signal.

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Poster

408. Hippocampal/Entorhinal Physiology II

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Topic: H.09. Spatial Navigation

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Title: Present and future: dual information processing modes in the hippocampal-medial entorhinal circuitry

Authors: P. CHAUDHURI-VAYALAMBRONE[1], M. E. RULE[2], M. BAUZA[3], S. BURTON[4], T. O'LEARY[1], ***J. KRUPIC**[1];
[2]Engin., [1]Univ. of Cambridge, Cambridge, United Kingdom; [3]SWC, [4]Univ. Col. London, London, United Kingdom

Abstract: Grid cells and place cells are key building blocks of the hippocampal formation's cognitive map. The firing patterns of these cells are usually viewed as encoding an animal's current location; however, previous studies have shown that they can also encode location in the recent past and near future. The relationship between these cells' spatiotemporal coding patterns is unclear. To address this, we analysed the firing patterns of co-recorded medial entorhinal grid cells and CA1 place cells in freely foraging rats. Both cell types mainly encode space prospectively, firing ~150ms before the rat entered a cell's preferred location. Grid cells' time shifts are proportional to their scale; similarly, CA1 place cells' time shifts increase with firing field size. In place cells, firing at different phases of the theta cycle was associated with different time shift lengths. This supports previous hypotheses that the cycle may be used to organise the encoding and retrieval of memories. We speculate that communication between CA1 and mEC may be involved in anticipating future trajectories, while CA1-CA3 communication may be used to compare past and present locations.

Disclosures: **P. Chaudhuri-Vayalambrone:** None. **M.E. Rule:** None. **M. Bauza:** None. **S. Burton:** None. **T. O'Leary:** None. **J. Krupic:** None.

Poster

409. Schizophrenia: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 409.01

Topic: H.13. Schizophrenia

Support: JSPS KAKENHI 17KK0074
JSPS KAKENHI 22K03200

Title: Low-dose ketamine disrupts associative blocking in mice

Authors: *R. SUZUKI, Y. KOSAKI;
Psychology, Waseda Univ., Tokyo, Japan

Abstract: Ketamine is an NMDA antagonist which, administered at sub-anaesthetic dose, induces a psychotic-like state in humans and non-human animals. In animal literature, it has been reported that ketamine facilitates mediated conditioning in rats; that is, an associatively activated but absent stimulus enters into association with an unconditioned stimulus (US; e.g., McDannald et al., 2011; Koh, Ahrens, & Gallagher, 2018; Fleming et al., 2022). A recent study also demonstrated that ketamine induces an abnormally strong activation of stimulus representation in response to a presentation of its associate, which is referred to as "conditioned hallucination" (Schmack et al., 2021). Thus, animals under ketamine tend to perceive a stimulus that is absent but only associatively activated, or 'retrieved'. Here, we further explored if the same tentative mechanism above would also disrupt Kamin's blocking in mice. Blocking is said to occur when conditioning of a novel conditioned stimulus (CS) is blocked by a concurrent presence of another CS which had previously been trained as a good predictor of the US. According to Wagner's SOP model (1981), this effect can be explained as the pre-trained CS (blocking cue) activates the US representation into A2 state, which is a 'memory' state for retrieved cue in Wagner's terminology, and hence ineffective for supporting new excitatory conditioning. Here we predicted that ketamine would disrupt the blocking effect by aberrantly activating the US representation into A1 state, which corresponds to the state of perception and focused processing. C57BL/6J mice were trained in a standard appetitive blocking design; during Phase 1, light or clicker CS (counterbalanced) signalled the sucrose solution US in Group Blocking. For mice in Group Control, tone CS was used to signal the US. In Phase 2, a light-clicker compound CS signalled the US. Half the animals in each group received 16 mg/kg ketamine (*i.p.*) before each session in Phase 2 and in the final test where the blocked cue alone was presented. The result showed that the blocking effect, *i.e.*, less CR to the blocked cue in Group Blocking compared to Group Control, was weakened in the mice treated with ketamine. The results offer further evidence for hallucination-like aberrant perception of absent stimuli under ketamine.

Disclosures: **R. Suzuki:** None. **Y. Kosaki:** None.

Poster

409. Schizophrenia: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 409.02

Topic: H.13. Schizophrenia

Title: Selectively enhanced in vivo firing activity of dopamine neurons in the medial substantia nigra in a 22q11.2 mouse model of schizophrenia

Authors: *S. BIKAS, J. ROEPER, A. DIAMANTOPOULOU;
Inst. of Neurophysiol., Frankfurt am Main, Germany

Abstract: Striatal dopamine (DA) elevation has been the hallmark of dopamine (DA) dysregulation in schizophrenia (SCZ), with more recent imaging studies establishing nigrostriatal pathways being primarily affected. However, its underlying mechanisms are yet to be resolved. To study DA dysregulation relevant to human disease, we made use of the *Df(16)A*$^{+/-}$ mouse model of the 22q11.2 Deletion Syndrome. The hemizygous microdeletions of the 22q11.2 locus carry a risk of approximately 25-30% for developing SCZ. It is the highest genetic risk factor for the development of SCZ accounting for 1-2% of sporadic SCZ cases. Previous work on the *Df(16)A*$^{+/-}$ mouse model revealed schizophrenia-like associated abnormalities on cellular, neurocircuitry, cognitive and behavioral levels . However, the function of the DA system has not yet been studied. Here, we set out to make the first steps towards an electrical characterization of DA dysfunction in the *Df(16)A*$^{+/-}$ mouse model. We established chronic *in vivo* single-unit extracellular recordings of pharmacologically identified DA neurons in the medial substantia nigra (mSN) and ventral tegmental area (VTA) during open field exploration in awake freely moving male and female *Df(16)A*$^{+/-}$ mice and wild type littermates. For these DA mSN neurons in *Df(16)A*$^{+/-}$ mice, which project to either dorsal or ventral striatum, we detected persistent electrophysiological hyperactivity in contrast to controls. This was characterized in male mice by increased mean firing frequencies (1.5-fold; $p = 0.0002$, n = 53, N = 7 for WT and n = 97, N = 7 for in *Df(16)A*$^{+/-}$) and elevated bursting activity (1.7-fold; $p < 0.0001$, n = 53, N = 7 for WT and n = 97, N = 7 for in *Df(16)A*$^{+/-}$). By considering the sexes separately, we found also in female *Df(16)A*$^{+/-}$ compared to controls significantly increased mean firing frequencies (1.6-fold; $p < 0.0001$, n = 95; N = 6 for WT and n = 95; N = 6 for in *Df(16)A*$^{+/-}$) and enhanced bursting activity (1.4-fold; $p < 0.0001$, n = 95; N = 7 for WT and n = 95; N = 6 for in *Df(16)A*$^{+/-}$). In contrast to mSN DA function, electrophysiological recordings of DA neurons in the VTA, the source of the mesolimbic dopamine system revealed no statistical differences between *Df(16)A*$^{+/-}$ and controls. In summary, our present findings provide strong evidence for a mSN genotype-specific increased DA function in the *Df(16)A*$^{+/-}$ mice of both sexes, but not in the VTA. These results might suggest that excess presynaptic dopaminergic transmission specifically in the dorsal striatum, as seen in SCZ, could be at least partially mediated by increased firing of mSN DA neurons.

Disclosures: **S. Bikas:** None. **J. Roeper:** None. **A. Diamantopoulou:** None.

Poster

409. Schizophrenia: Animal Models

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Program #/Poster #: 409.03

Topic: H.13. Schizophrenia

Support: NSF Grant HRD-1612530
NSF Grant . DGE-1747503

Title: Increasing Endocannabinoid 2-AG Levels Reverses Amphetamine-Induced Prepulse Inhibition Deficits

Authors: *A. K. LAMPKIN[1], C. ZUNIGA[2], J. GOTTLIEB[2], B. BALDO[2], V. BAKSHI[2]; [1]Mol. and Cell. Pharamacology, [2]Dept. of Psychiatry, Univ. of Wisconsin-Madison, Madison, WI

Abstract: There is much interest in the cannabinoid system as a potential therapeutic target for multiple psychiatric illnesses. The active component of marijuana, THC, has been studied for its ability to modulate multiple behavioral functions relevant to psychiatric illness. The role of endogenous cannabinoid ligands (AEA and 2-AG), termed endocannabinoids (eCB), is less understood. Here we increased brain levels of 2-AG by administering JZL184, a potent inhibitor of monoacylglycerol lipase (MAGL) (the primary breakdown enzyme for 2-AG) and assessed several behaviors including prepulse inhibition (PPI) of startle, anxiety-like states through the elevated plus maze (EPM) and ingestive, motor, and motivated approach behaviors through a behavioral observation paradigm in both male and female rats. To determine if 2-AG had modulatory effects on PPI, we first tested if JZL184 alone could alter PPI. When no deviations from basal PPI levels were seen, we tested if JZL184 would reverse deficient PPI. We administered amphetamine (AMPH), a psychotomimetic agent known to disrupt PPI, either with or without JZL184. When JZL184 was co-administered with AMPH, males exhibited a reversal in the AMPH-induced PPI deficit; however, no reversal effect was seen in the females. In contrast to PPI, no measures in the EPM, ingestive, motor, and motivated approach behavioral paradigms were affected by the administration of JZL184. This set of findings indicates that the PPI-restorative effects of JZL184 were not due to non-specific alterations on mood, motor, or motivated behaviors. Thus, JZL184 shows targeted restoration of disrupted sensorimotor gating (as assessed by PPI). Together these results demonstrate that eCBs, specifically 2-AG, can modulate cognitive processes related to psychiatric disorders such as schizophrenia and post-traumatic stress disorder, where startle abnormalities like disrupted PPI are seen. To our knowledge, we are the first to show reversal in AMPH-induced PPI deficits using JZL184. Previous findings show that cannabinoid receptors are localized on norepinephrine-producing neurons and that 2-AG potently stimulates these receptors; moreover, stimulation of these cannabinoid receptors inhibits norepinephrine release. Given that AMPH is known to disrupt PPI

at least in part by increasing norepinephrine release, one potential mechanism by which JZL184 reverses AMPH-induced PPI deficits could be the blockade of AMPH-induced norepinephrine release through enhanced levels of 2-AG.

Disclosures: A.K. Lampkin: None. **C. Zuniga:** None. **J. Gottlieb:** None. **B. Baldo:** None. **V. Bakshi:** None.

Poster

409. Schizophrenia: Animal Models

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Topic: H.13. Schizophrenia

Support: NIH grant P50 MH119569-01A1
NIH grant R01 MH123661-01

Title: Modeling of psychosis-like state representation deficits in mice.

Authors: *E. KNEP, G. ROJAS, N. GRISSOM;
Psychology, Univ. of Minnesota, Minneapolis, MN

Abstract: State-dependent conditional learning tasks in human subjects, such as the AX-CPT and DPX tasks, have demonstrated that schizophrenia and psychosis are associated with deficits in state representation. State representation processes are important for distinguishing and remembering different states of the world and applying appropriate conditional rules based on state. While an analogous task has been successfully used in non-human primates, the costs and limitations of molecular techniques make a rodent analog task a high-priority target in understanding mechanisms of state representation. We are developing a mouse analog of the dot pattern expectancy task, accessing state representation mechanisms through the combined usage of auditory and visual stimuli to reflect the current state in an operant conditional learning task. We find evidence indicating modality-dependent differences in the contextualization of these stimuli, with animals demonstrating improved ability to successfully associate presented stimuli with directional choice when presented with visual discriminatory stimuli as opposed to auditory. We also find notable differences in apparent image retention dependent on type of visual format, with “standard” objects with discrete edges (airplane/spider) associated with faster acquisition of task rules than the use of visual gradients. Furthermore, the complexity of the task requires a large number of training sessions, necessitating strict training regimens and progression criteria. In combination, these results suggest a specific line of approach for multi-step conditional learning tasks in mice, as well as help demonstrate the feasibility of the task itself.

Disclosures: E. Knep: None. **G. Rojas:** None. **N. Grissom:** None.

Poster

409. Schizophrenia: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 409.05

Topic: H.13. Schizophrenia

Title: Validating the use of PCP as a model of schizophrenia in rodents

Authors: P. LOVINGER, T. AMARLKHAGVA, G. DUENAS, R. CHANDRA, ***M. BOWMAN**;
Charles River Labs., South San Francisco, CA

Abstract: Schizophrenia is a complex disorder characterized by both positive and negative symptoms along with decline of cognitive performance. In humans, phencyclidine (PCP) use can produce a range of symptoms that closely resemble those seen in patients with schizophrenia. Therefore, PCP has been widely used in rodent models of schizophrenia whereby acute and subchronic PCP administration can cause hyperlocomotion, social withdrawal, and cognitive impairments. The objective of this study is to validate the effect of subchronic dosing of PCP or vehicle to induce a schizophrenia-like phenotype in rodents as determined by hyperlocomotion and deficits in object recognition memory. To this end, mice received a single injection of 10 mg/kg of PCP or vehicle on days 1-5 and 8-12. On day 1, mice underwent locomotor activity after initial treatment with PCP or vehicle. After a four-day washout period, mice underwent locomotor activity on day 16 followed by novel object recognition over days 17-19. On behavioral testing days, mice received saline, risperidone, or galantamine 30 minutes prior to testing as a potential rescue to the PCP-induced schizophrenia model. Mice treated with acute and subchronic PCP had an increase in locomotor activity compared to mice treated with saline. Also, treatment with risperidone reduced the increase in locomotor activity after subchronic treatment with PCP. In the novel object recognition task, saline treated mice spent more time exploring the novel object compared to the familiar object. Mice treated with subchronic PCP showed no difference in time spent with either object suggesting a deficit in object recognition memory. The results indicate that subchronic treatment with PCP is a valid model for inducing schizophrenia-like phenotype in a rodent model.

Disclosures: P. Lovinger: None. **T. Amarlkhagva:** None. **G. Duenas:** None. **R. Chandra:** None. **M. Bowman:** None.

Poster

409. Schizophrenia: Animal Models

Location: SDCC Halls B-H

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Program #/Poster #: 409.06

Topic: H.13. Schizophrenia

Title: Phenylcyclohexyl piperidine (PCP) evokes sex-dependent anxiety-related behaviours and altered sociability in mice.

Authors: *K. JASIONOWSKA[1], J. PRATT[2], B. MORRIS[1];
[1]Univ. of Glasgow, Univ. of Glasgow, Glasgow, United Kingdom; [2]Univ. of Strathclyde, Glasgow, United Kingdom

Abstract: PCP evokes a psychosis episode which is similar to experiences of patients with schizophrenia. As a result, PCP has been used in rodents to create an animal model of schizophrenia that can allow detailed investigation into behavioural, neurological and biochemical changes during psychosis. However, schizophrenia is also characterised by a series of negative and secondary symptoms such as anxiety or depression. Furthermore, in humans, there are clear sex differences in patients with schizophrenia, most clearly in these negative and secondary characteristics. Despite this, there is little research into the potential effects of PCP on negative symptoms and differences between sexes. Here, we utilise a series of behavioural tests aimed to assess, locomotor activity, anxiety and sociability in male and female mice with an acute, low-dose of PCP. In 2 hours in the open-field after PCP, there was no change in locomotor activity, however females exhibited a significant increase in the anxiety-related behaviour of thigmotaxis where male animals showed decreased thigmotaxis. In the elevated-plus-maze females also showed increased anxiety related behaviours with more time spent in the closed arms of the maze where males indicated decreased anxiety. However, during 48 hours of recorded home-cage behavioural tracking, there was an initial increase in locomotor activity in the first hour after PCP, contrasting to what was found in the open field. After 4 hours this reversed, and PCP caused a significant decrease in locomotor activity. Additionally, separation from cage mates increased in males only in the first two hours after PCP indicating reduced sociability in males but not females. However, 7-9 hours later, both males and females indicated decreased separation, suggesting increased sociability. At this time there were no changes in locomotor activity so this does not suggest changed social interaction was due to sleeping behaviours, but highlights a potential increase in socialisation between cage-mates in males and females. Overall, females indicated that PCP increased anxiety related behaviours where males showed no change or decreased anxiety. PCP also caused altered sociability in both males and females however, only males indicated decreased sociability immediately after injection. These results have implications for the understanding of sex specific changes in the PCP model for schizophrenia as well as the representation of negative symptoms in the PCP model. These results also mimic the differences found in humans with schizophrenia where females show more anxiety related negative symptoms and males show more difficulties in sociability.

Disclosures: K. Jasionowska: None. **J. Pratt:** None. **B. Morris:** None.

Poster

409. Schizophrenia: Animal Models

Location: SDCC Halls B-H

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Program #/Poster #: 409.07

Topic: H.13. Schizophrenia

Title: Investigating hippocampal synaptic deficits in the sub-chronic phencyclidine rat model for schizophrenia

Authors: *N. SUN[1], M. HARTE[2], J. GIGG[1];
[1]Div. of Neurosci. and Exptl. Psychology, [2]Div. of Pharm. and Optometry, Univ. of Manchester, Manchester, United Kingdom

Abstract: Background: Schizophrenia is a complex neuropsychiatric disorder characterised by three main symptom domains, namely positive, negative, and cognitive deficits. Recently, the cognitive impairments associated with schizophrenia (CIAS) have been considered the main factor contributing to case fatality. Up to now, however, no effective treatment for CIAS has been developed due to a lack of knowledge of the underlying mechanisms. N-methyl-D-aspartate (NMDA) receptor antagonist rodent models are commonly used to investigate the neuronal mechanisms of CIAS. Our project investigated the effect of NMDA antagonism on hippocampal synaptic physiology in the sub-chronic phencyclidine (scPCP) rat model for schizophrenia.**Methods:** Thirty female lister-hooded rats were injected with phencyclidine (scPCP; 2 mg/kg) or saline (scVehicle) twice daily for 7 days, followed by a 7-day washout. The novel object recognition task was conducted to ensure successful model induction. Synaptic function in dorsal hippocampus CA1 was then assessed by acute electrophysiology under urethane anaesthesia *in vivo*. CA3-evoked field excitatory postsynaptic potential (fEPSP) in CA1 was analysed to investigate changes in synaptic connectivity, short- and long-term synaptic plasticity. **Results:** The scPCP group showed the expected novel object recognition deficit. In turn, scPCP rats showed weaker baseline CA1 synaptic connectivity (input-output function) compared to controls, whilst short-term plasticity (paired-pulse facilitation) in CA1 was similar between groups. Following high-frequency stimulation, long-term potentiation (LTP) of CA1 fEPSP was induced in both groups; however, the fEPSP slope was significantly lower in the scPCP group in the first 5 minutes after HFS. Both slope and amplitude gradually plateaued to the same level 25 minutes after HFS. After subsequent low-frequency stimulation, both groups depotentiated to the same extent and responses returned to near baseline.**Conclusion:** In summary, our data indicate that: (a) dorsal CA1 synaptic excitability in scPCP rats is lower compared to controls; (b) CA1 LTP is weaker in scPCP rats; (c) scPCP and controls rats exhibit similar depotentiation of CA1 fEPSP slope and amplitude after LTP induction. This overall lowering of CA1 excitability and LTP induction in scPC rats may contribute to declarative memory aspects of CIAS.

Disclosures: **N. Sun:** None. **M. Harte:** None. **J. Gigg:** None.

Poster

409. Schizophrenia: Animal Models

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Title: Deep-neuronal-network analysis reveals selective behavioral alterations in a ketamine-induced pharmacological model of schizophrenia in mice.

Authors: *A. O. CUELLAR SANTOYO[1], T. B. MARES BARBOSA[1], O. RANGEL-PÉREZ[2], A. G. HOWE[3], V. M. RUIZ-RDRÍGUEZ[1], A. E. MIRELES-NAVARRO[1], K. HERNÁNDEZ-BALDERAS[1], C. M. PAREDES-POPOCA[1], A. PATRÓN-SOBERANO[1], A. M. ESTRADA-SÁNCHEZ[1];
[1]División de Biología Mol., [2]Ctr. Nacional de Supercómputo, Inst. Potosino de Investigación Científica y Tecnológica, San Luis Potosi, Mexico; [3]Dept. of Psychology, Univ. of California Los Angeles, Los Angeles, CA 90095, USA., Los Angeles, CA

Abstract: Abstract Schizophrenia is a complex mental disorder characterized by sudden changes in behavior, alterations in perception, distorted thinking, communication problems, motor disturbances, and cognitive alterations. Although a wide variety of factors might contribute to the development of schizophrenia, reduced activity of N-methyl-D-aspartate (NMDA) glutamatergic receptors seems to be a common factor. Therefore, NMDA antagonists such as ketamine are used to mimic the schizophrenia phenotype. In this work, we describe the behavioral changes induced by the intraperitoneal administration of ketamine (30 mg/kg) for 14 consecutive days compared to control mice that received 0.9% sterile saline solution. Twenty-four hours after the last ketamine or saline administration, mice performed behavioral tasks that evaluated movement, short-term memory, executive function, anxiety, and social interaction. We use the DeepLabCut program that uses deep neural networks to analyze mice performance on the differential behavioral tasks. Our results showed that ketamine-treated mice exhibited a significant decrease in novel object exploration during the novel object recognition task, indicating an impairment in short-term memory. Ketamine treatment also affects mice's social interaction, as shown by a low frequency of interaction between subjects and a short duration trend in each interaction interval. Finally, mice treated with ketamine showed similar motor performance, anxiety levels, and executive function compared to control mice. The results indicate that ketamine administration alters short-term memory and social interaction, resembling schizophrenia-like behaviors. Our data also suggest that the hippocampus and perhaps the amygdala and prefrontal cortex are vulnerable structures during ketamine treatment.

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Poster

409. Schizophrenia: Animal Models

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Topic: H.13. Schizophrenia

Support: NIH Grant 1R01MH123479-01A1

Title: Antipsychotic intervention reduces hippocampal hyperactivity in a mouse model of psychosis

Authors: *F. DYBOWSKI, D. SCOTT, C. A. TAMMINGA;
Psychiatry, UT Southwestern Med. Ctr., Dallas, TX

Abstract: Hippocampal hyperactivity in CA3 and CA1 is a biomarker indicating early schizophrenia. We first studied human postmortem hippocampal subfields and developed a data-driven model of hippocampal hyperactivity. Then, we back-translated this into a mouse model, first using a dentate gyrus (DG)-specific GluN1 KO, then a granule cell-specific inhibitory DREADD, in order to study the mouse mechanisms closely. Now, we have identified the molecular and behavioral characteristics of a model in animals putatively parallel to hippocampal hyperactivity in human schizophrenia brain study. As a reverse-translation model, it is a candidate for testing antipsychotic drug activity.
A subchronic DG inhibition only during adolescence engenders molecular and behavioral phenotypes of psychosis which persist weeks after Compound 21 (DREADD ligand) withdrawal. The molecular phenotype is manifested by an increase in immediate early gene (ie. cFos) protein expression in CA3 and CA1 hippocampal subfields. The behavioral phenotype consists of increased freezing in cued and contextual fear conditioning and a defect in social memory. Haloperidol (0.1mg/kg) and levetiracetam (5mg/kg) were administered either acutely (ip injection) or subchronically (p.o. for 72 hours). Both haloperidol and levetiracetam reversed hippocampal hyperactivity in CA3 and CA1 subfields, as measured by cFos-positive cell density. Haloperidol and levetiracetam also caused a normalization of the aberrant social memory phenotype. However, there was no effect on freezing in fear conditioning.
These promising findings validate this model and indicate that it could be important for the development of new, effective and safe medications that could improve clinical outcomes, increase patient adherence and advance quality of life for people with psychosis.

Disclosures: F. Dybowski: None. **D. Scott:** None. **C.A. Tamminga:** None.

Poster

409. Schizophrenia: Animal Models

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Program #/Poster #: 409.10

Topic: H.13. Schizophrenia

Title: Striatal baseline D2R may predict Olanzapine Side Effects in Mice

Authors: S. SRIRAMOJI[1], M. MAHBOOB[1], *M. BOCARSLY[2];
[1]Pharmacol. Physiol. and Neurosci., [2]Rutgers NJMS, Newark, NJ

Abstract: Antipsychotics are a class of drug prescribed to patients that suffer from conditions such as schizophrenia, bipolar disorder, depression and other personality disorders. They decrease neuroinflammation in the brain caused by these conditions but, tend to come with weight gain as a side effect .Although there is great variability among patients, other side effects include anxiety and depression. These side effects, along with the others, make adherence for some patients difficult. In this study Olanzapine, a commonly used antipsychotic that has known actions at the dopamine D2 receptors (D2R) is being studied in relation to its effect on obesity, anxiety and depression. Olanzapine administration in mice enables weight gain in the presence of a high fat diet. The striatum has the highest level of D2R in the brain and is therefore targeted by Olanzapine. When the D2Rs on the medium spiny neurons that project from the striatum are selectively removed, this transgenic mouse model showed less weight gain over an 8-week period compared to controls. Interestingly, Mice treated with Olanzapine regardless of D2R levels show higher degrees of depressive like behavior compared to vehicle treated controls. Furthermore, Olanzapine treated mice with lower levels of D2R had decreased anxiety-like phenotype compared to the controls. With examination of blood serum, western blot and IHC models from brain tissue, we will look for elevated inflammatory markers in the blood as well as increased neuroinflammation in the mice treated with Olanzapine. Blood serum markers indicative of general inflammtion will include TNF-alpha, IL-1b and IL-12. We will look at IL-1b also in the brain along with NF-kBeta and NLRp3 which are markers of both inflammasome presence and general neuroinflammation. In addition, with our behavioral data supporting depressive phenotypes in the Olanzapine treated mice, we expect to see higher levels of glial and astrocyte activation via S100B and GFAP analysis than controls. Olanzapine may draw out differences in depressive and anxiety phenotypes based on baseline levels of D2R in the striatum. Baseline D2R and the introduction of Olanzapine can directly influence neuroinflammation and inflammatory pathway activation in patients. With this understanding, the lack of knowledge regarding a potential patient and their interactions with Olanzapine could be predicted based on their levels of baseline D2Rs in the striatum.

Disclosures: S. Sriramoji: None. **M. Mahboob:** None. **M. Bocarsly:** None.

Poster

409. Schizophrenia: Animal Models

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Program #/Poster #: 409.11

Topic: H.13. Schizophrenia

Title: Characterization Of SUVN-7105012, A Potent And Selective Dual 5-HT1a Agonist And 5-HT2a Receptor Antagonist That Exhibits Antipsychotic And Antidepressant Like Effects

Authors: *R. ABRAHAM, S. PETLU, R. SUBRAMANIAN, V. GRANDHI, P. JAYARAJAN, J. THENTU, V. BENADE, R. BADANGE, K. BOJJA, A. MOHAMMED, A. SHINDE, R. NIROGI;
Suven Life Sci., Suven Life Sci., Hyderabad, India

Abstract: Treatment of psychosis has come a long way fromthe initial targeting of dopaminergic receptors to the selective targeting ofserotonergic (5-HT) receptors. SUVN-7105012 is one such new chemical entity that targets the 5-HT1A and5-HT2A receptors. SUVN-7105012 has been evaluated for its in-vitro affinityusing radioligand binding assay at recombinant human 5-HT and dopaminergic receptors. The pharmacokinetics ofSUVN-7105012 were assessed in Wistar rats (n=3/group). Receptor occupancy ofSUVN-7105012 towards the serotonergic (5-HT1A and 5-HT2A) and dopaminergic (D2)receptors were evaluated in Wistar rats using non-radiolabeled tracers(n=4/group). Assessment of SUVN-7105012 for its antipsychotic like effects inWistar rats were carried out in the open field test using MK-801 challenge(n=8/ group). Here, olanzapine was used as a positive control in the open fieldtest. SUVN-7105012 was also assessed for its antidepressant like properties ina mice forced swim test (n=8/group) with imipramine as a positive control. Thedata from the open field, and forced swim tests were compared using one-wayANOVA followed by Dunnett's test. SUVN-7105012 was assessed for its potentialto induce motor impediment in Wistar rats using the catalepsy test (n=6). Basedon the pharmacokinetics of SUVN-7105012, catalepsy was measured at hourlyintervals and upto 4h post dosing. Haloperidol was used as a positive controlin the catalepsy assay. The data from the catalepsy assay were compared usingtwo- way ANOVA followed by Dunnett's test. SUVN-7105012 has affinity to 5-HT2Aand 5-HT1A receptor with minimal affinity for dopamine D2 receptors.SUVN-7105012 was found to have receptor occupancy towards 5-HT2A and 5-HT1Areceptors in rats. SUVN-7105012 was found to be a dual serotonin modulator,5-HT2A receptor antagonist and 5-HT1A receptor agonist. SUVN-7105012 is orally bioavailable and hasbrain penetration properties in rats. SUVN-7105012 attenuated MK-801 inducedhyperlocomotor activity in the open field and reduced the duration ofimmobility in the forced swim test. SUVN-7105012 did not induce catalepsy atthe tested doses. In conclusion, SUVN-7105012 was found to be a dual serotonin5-HT2A receptor and a 5-HT1A receptor ligand that exhibited antipsychotic andantidepressant like effects. SUVN-7105012 was found to be devoid of motorimpediments as well.

Disclosures: **R. Abraham:** A. Employment/Salary (full or part-time):; Suven Life Sciences. **S. Petlu:** A. Employment/Salary (full or part-time):; Suven Life Sciences. **R. Subramanian:** A. Employment/Salary (full or part-time):; Suven Life Sciences. **V. Grandhi:** A. Employment/Salary (full or part-time):; Suven Life Sciences. **P. Jayarajan:** A. Employment/Salary (full or part-time):; Suven Life Sciences. **J. Thentu:** A. Employment/Salary (full or part-time):; Suven Life Sciences. **V. Benade:** A. Employment/Salary (full or part-time):; Suven Life Sciences. **R. Badange:** A. Employment/Salary (full or part-time):; Suven Life Sciences. **K. Bojja:** A. Employment/Salary (full or part-time):; Suven Life Sciences. **A.**

Mohammed: A. Employment/Salary (full or part-time):; Suven Life Sciences. **A. Shinde:** A. Employment/Salary (full or part-time):; Suven Life Sciences. **R. Nirogi:** A. Employment/Salary (full or part-time):; Suven Life Sciences.

Poster

410. Novel Genetic Tools for Understanding Brain Function

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 410.01

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: R01-MH119826
R37-MH080046
F32-MH119687
F31-MH124283

Title: Large-donor CRISPR for whole-CDS replacement of cell adhesion molecule LRRTM2

Authors: *S. L. POLLITT[1], A. D. LEVY[1], M. ANDERSON[2], T. A. BLANPIED[1];
[1]Dept. of Physiol., [2]Program In Neurosci., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: A flurry of recent studies has demonstrated that the cell adhesion molecule Leucine-Rich Repeat Transmembrane protein 2 (LRRTM2) plays an important role in synaptogenesis, synaptic and subsynaptic AMPA receptor retention, long-term synaptic plasticity, and maintenance of the transsynaptic nanocolumn. These findings emphasize that the biosynthesis, trafficking, and subcellular dynamics of LRRTM2 may play a critical part in regulating numerous neuronal functions. However, our understanding of endogenous LRRTM2 biology has been restricted due to the necessary reliance on overexpression and knockdown- or knockout-replacement. This is in part because, unfortunately, the *LRRTM2* gene lacks suitable guide sites for N-terminal insertions via well-established methods for CRISPR editing, and sites for N-terminal tagging and mutations of interest are far apart. Thus, molecular manipulation of endogenous LRRTM2 has remained unavailable. Recently, a novel CRISPR technique Targeted Knock-in using Two Guides (TKIT) has allowed for the N-terminal tagging of proteins without introducing frame-shifts by replacing an entire exon of the targeted gene. We have adapted this high-efficiency technique to replace the entire mature coding sequence (CDS) of LRRTM2, which is conveniently localized to a single large (4.3kb) exon (minus the first 4 bases of the signal peptide). This strategy offers total control of the endogenous LRRTM2 gene, and for instance allowed us to add an N-terminal tag to the protein while simultaneously introducing mutations of interest at distant locations within the gene. Further demonstrating the power of this whole-CDS replacement approach, we also engineered a knock-in dependent fluorescent marker to aid in screening and identification of knock-in neurons. Using this tool in primary cultures of rat hippocampus, we have demonstrated that LRRTM2 is largely synaptic in localization, most highly expressed in parvalbumin-positive interneurons, and is surprisingly less strongly expressed in pyramidal cells. Our whole-CDS replacement strategy offers a potential avenue for

strategic control of a large variety of genes controlling synaptic architecture, and may for instance be particularly effective for examining differential expression and regulation of LRRTM family members.

Disclosures: S.L. Pollitt: None. **A.D. Levy:** None. **M. Anderson:** None. **T.A. Blanpied:** None.

Poster

410. Novel Genetic Tools for Understanding Brain Function

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Program #/Poster #: 410.02

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: Rett Syndrome Research Trust Grant 20190460 (to M.E., M.F.)
NIH Pioneer DP1OD025535 (to V.G.)
Merkin Translational Research Grant (to M.E., V.G., A.H., M.F.)
The Donna and Benjamin M. Rosen Bioengineering Center
Howard Hughes Medical Institute (to M.E.)

Title: A miRNA-Based System for Dosage-Independent Control of MeCP2 Expression in Murine Models of Rett Syndrome

Authors: *A. HORI[1], M. FLYNN[1], V. GRADINARU[2], M. ELOWITZ[1];
[2]Biol. and Biol. Engin., [1]Caltech, Pasadena, CA

Abstract: Viral gene delivery vectors enable efficient gene delivery to many tissues, but the dosage in individual cells is nonuniform. These cell-to-cell differences in vector dosage pose a problem for effectors that require specific concentrations to achieve proper cell function. For example, Rett Syndrome (RTT) is an X-linked neurodevelopmental disease caused by mutations in the MeCP2 gene. Gene therapy development for RTT is hindered by cells' extreme sensitivity to variations in MeCP2 gene dosage; both over- and under-expression within a single order of magnitude cause deleterious neurodevelopmental effects. In humans, loss of MeCP2 renders male fetuses non-viable. In patients with multiple X chromosomes, single copy loss, or milder mutations which preserve some MeCP2 function, cause RTT, while gene duplication causes MeCP2 Duplication Syndrome (MDS). Thus, virally-delivered MeCP2 replacement therapies must buffer expression levels to compensate for differences in viral dose delivered to individual cells. To address this challenge, we have implemented a miRNA-based incoherent feed-forward loop (IFFL) control circuit to regulate delivery of a therapeutic MeCP2 transgene (US Patent No. US20210171582A1, 2020). This IFFL approach co-expresses a miRNA and a target mRNA containing a customized number of miRNA target sites. With this system, MeCP2 expression per gene copy declines with increasing gene dosage, compensating for variations in viral dosage spanning multiple orders of magnitude. We found that the MeCP2 IFFL circuit maintained its behavior in vivo when delivered to wild-type C57BL/6J mice by either systemic or direct injection of AAV.CAP-B22 (Goertsen et al. 2022), a next-generation AAV vector that efficiently

transduces the murine CNS, and observed no apparent toxicity from the synthetic circuit components. Our findings demonstrate the potential of engineered biological circuits to enable gene therapy for dosage-sensitive disorders like RTT. Ongoing work aims to evaluate the effect of regulated MeCP2 expression on CNS toxicity, neural physiology, and behavior in two mouse models of Rett Syndrome expressing different baseline amounts of functional MeCP2.

Disclosures: **A. Hori:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); C.I.T. filed IP for methods with A.H. as inventor. **M. Flynn:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); C.I.T. filed IP for methods with M.F. as inventor. **V. Gradinaru:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); C.I.T. filed IP for methods with V.G. as inventor. **M. Elowitz:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); C.I.T. filed IP for methods with M.E. as inventor.

Poster

410. Novel Genetic Tools for Understanding Brain Function

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 410.03

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: JSPS KAKENHI Grant 17K10846
JSPS KAKENHI Grant 22H03441
Taiju Life Social Welfare Foundation
Japan Brain Foundation

Title: Comparison of gene transduction efficiency into gerbil hippocampus using adeno-associated virus vector, 2, 5, and rh10

Authors: *Y. SEHARA[1], Y. HAYASHI[1], R. WATANO[1], K. OHBA[1], K. SHIMAZAKI[2], K. KAWAI[2], H. MIZUKAMI[1];
[1]Div. of Genet. Therapeut., [2]Dept. of Neurosurg., Jichi Med. Univ., Shimotsuke, Japan

Abstract: [Objective] Adeno-associated virus (AAV) vectors are promising tools for gene delivery for its safety and efficiency. It is considered to depend on the amino acid sequence of the AAV surface (capsid) which cell type is predominantly transduced. In this study, we focused on the transduction efficiency into neural stem cells of gerbil hippocampus using three different natural serotypes, AAV2, 5, and rh10. [Methods] Four-week-old male gerbils were injected with 1.5×10^{10} viral genomes of AAV2, 5, or rh10 carrying green fluorescent protein (GFP) gene, targeting right dentate gyrus (n = 3, each). One week later, the animals were euthanized, and the brains were taken for histological analysis. [Results] To reveal the transduction efficiency for neural stem cells, we counted double positive cells for Sox2, a marker for neural stem cells, and

GFP of the subgranular zone of dentate gyrus. AAV5 showed the largest number of double positive cells for GFP and Sox2 compared to the AAV2 and rh10 (AAV2: 0.0 ± 0.0, AAV5: 24.4 ± 1.6, AAVrh10: 15.4 ± 3.5, $p < 0.001$ and $p < 0.05$ for AAV5 compared to the control and AAVrh10, each). On the contrary, AAVrh10 showed the largest number of double positive cells for GFP and NeuN, a marker for mature neurons, compared to AAV2 and 5 groups (AAV2: 6.4 ± 3.3, AAV5: 13.6 ± 5.9, AAVrh10: 143.4 ± 20.0, $p < 0.001$ for AAVrh10 compared to the control). [Conclusion] In this study, we found that AAV5 showed the highest transduction efficiency to the neural stem cells in the dentate gyrus. On the contrary, AAVrh10 showed the highest transduction efficiency for mature neurons. It is important to choose the best AAV serotype which is suitable for the purpose of the study.

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Poster

410. Novel Genetic Tools for Understanding Brain Function

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Program #/Poster #: 410.04

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: Howard Hughes Medical Institute

Title: SuRe: a recombination tool to create complex transgenic animals for neuroscience experiments

Authors: *J. LUO[1], C. HUANG[2], J. LI[2], S. WOO[3], M. J. SCHNITZER[1,2,4,5];
[1]Howard Hughes Med. Inst., Stanford, CA; [2]Dept. of Biol., [3]Dept. of Electrical Engin., [4]Dept. of Applied Physics, [5]CNC Program, Stanford Univ., Stanford, CA

Abstract: To achieve highly specific expression patterns or to combine the use of multiple reporters and effectors, it is often desirable to create polytransgenic animals with multiple transgenic elements. Traditional approaches involve docking these transgenic elements on different chromosomes or integrating them at different loci far apart on the same chromosome and then recombining them. To avoid influencing essential genes, researchers generally integrate transgenic elements at a limited number of well-characterized genetic loci. Hence, traditional approaches for recombination are poorly suited to generate animals with more transgenic elements than the number of available docking sites.
To generate an organism with *N* transgenic elements, *N*-1 steps of combination or recombination are required. Furthermore, in diploid organisms, in the *k*'th recombination step the strain containing *k*+1 transgenes needs to be selected from 2^{k+1} possible combinations. To overcome these limitations, we developed a CRISPR/Cas9-based system in *Drosophila melanogaster* that we termed the 'Super Recombinator' (SuRe), as it allows the recombination of multiple genes at the same locus.

When recombining two transgenes, *A* and *B*, at the same locus, the SuRe system first inserts a pair of reciprocal adaptors, one upstream of transgene *A* and the other downstream of transgene *B*. This adaptor pair then facilitates the recombination of the two transgenes with high efficiency. The recombination product is a single large transgenic tandem that can be further recombined in an iterative manner through additional rounds of applying SuRe. This approach significantly reduces the turnover time for introducing *N* transgenes at the same locus from *N*-1 to $\log_2 N$ steps. Moreover, this approach requires selections from notably fewer possible genotypes per step and does not involve embryo injection. Its recombination efficiency is significantly higher than that of natural recombination.
Using deep sequencing, we confirmed that the SuRe system can recombine a ~100 kbp transgene. To demonstrate the use of SuRe in neuroscience, we applied it to create a transgenic fly strain expressing multiple fluorescent voltage indicators in different neuron-types. Using this strain, we were able to simultaneously monitor the spiking activity of multiple neurons in the mushroom body in the adult fly brain. Overall, the SuRe system enables the integration of multiple transgenic elements at one locus and opens new possibilities for designing transgenic animals that meet the increasing demands of sophisticated behavioral, imaging, and optogenetic experiments in neuroscience.

Disclosures: **J. Luo:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent application WO2022082225. **C. Huang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent application WO2022082225. **J. Li:** None. **S. Woo:** None. **M.J. Schnitzer:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent application WO2022082225.

Poster

410. Novel Genetic Tools for Understanding Brain Function

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Program #/Poster #: 410.05

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIHR21MH126400
NIGMSR35GM11931

Title: Parallel screening of development enhancer identifies relevant disease variants in-vivo in mouse brain

Authors: *C. S. ARDEKANI, J. T. LAMBERT, L. SU-FEHER, T. L. WARREN, A. C. SANTOS, S. A. LOZANO, E. CASTILLO PALACIOS, M. B. CORONA, J.-M. M. HANNERS, A. S. NORD;
Univ. Of California Davis, Davis, CA

Abstract: Spatial and temporal regulation of gene expression are crucial for proper brain development. Enhancers are cis-regulating non-coding regions of DNA that allow for precise gene regulation. Additionally, genome wide association studies (GWAS) have linked mutations in these non-coding regulatory regions to genetic risk of neurological disorders such as schizophrenia and epilepsy. Though predicting enhancer identity in the genome has developed, proper screening and large-scale function of enhancers *in vivo* remains a challenge. To identify active enhancers in the developing brain, we adapted an enhancer reporter assay, the self-transcribing active regulatory region sequencing (STARR-seq). To assemble a massively parallel reporter assay (MPRA) library we selected candidate sequences identified by *de novo* mutations and epigenetic marks. This approach enables us to rapidly screen libraries of identified DNA sequences for enhancer activity *in vivo*, allowing us to identify sequence variants that are crucial for proper enhancer activity. We delivered the MPRA library into neonatal mouse brain via intracranial injections at postnatal day 0 using recombinant adeno-associated virus (rAAV). A week later we collected tissues, sequence MPRA-associated RNA and DNA, and used immunohistochemistry to assess enhancer activity. We validated the activity of multiple intronic enhancers within *CACNA1C*, which are associated with schizophrenia risk. Preliminary cell line data and other published studies suggest that different mutant variants of *CACNA1C* enhancers resulted in different levels of enhancer activity. In ongoing work, we synthesized a follow up MPRA library for deeper comparison reference and disease alleles to validate and visualize CACNA1C variant functionality and assess cell-type specificity of these enhancers in the brain. Overall, our work functional examination in vivo is not only critical for identifying active enhancers during windows of neurodevelopment, but also provides important insight into what mutations contribute to genetic risk of neuropathology.

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Poster

410. Novel Genetic Tools for Understanding Brain Function

Location: SDCC Halls B-H

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Program #/Poster #: 410.06

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH NHGRI Grant R01HG009283

Title: Mapping the regulation of synaptogenic molecules with optical pooled screens

Authors: *A. LE[1,2], T. BIEDERER[3], P. C. BLAINEY[1,2,4];
[1]Dept. of Biol. Engin., MIT, Cambridge, MA; [2]Broad Inst. of MIT and Harvard, Cambridge, MA; [3]Dept. of Neurol., Yale Sch. of Med., New Haven, CT; [4]Koch Inst. for Integrative Cancer Res. at MIT, Cambridge, MA

Abstract: Synapse formation, or synaptogenesis, plays a critical role in shaping the connectivity of the nervous system. Dysregulation of this process has been implicated in neurodevelopmental and neurodegenerative diseases, including autism, schizophrenia, and Alzheimer's disease. Understanding the mechanisms underlying synapse formation has therefore remained an longstanding goal in neurobiology. However, current approaches lack the throughput required to systematically map the regulatory networks driving synaptogenesis. In this study, we integrate an artificial synapse formation co-culture assay with *in situ* sequencing of genetic perturbations to build a scalable, high-throughput optical pooled screening platform for identifying regulators of synaptogenic cell adhesion molecules. We performed a CRISPR knockout screen targeting 644 genes in HEK293 cells expressing Neuroligin-1 (NLGN1) and co-cultured with rat hippocampal neurons. We then measured the formation of artificial synapses between HEK cells and neurons in over 1.8 million cells via immunofluorescence of synaptic markers and an automated image analysis pipeline. This screen identified novel positive and negative regulators of NLGN1 and implicated diverse cellular processes in synapse formation, such as cytoskeletal dynamics and signal transduction. Taken together, our work establishes a pooled screening approach that enables large-scale, systematic studies for elucidating and uncovering molecular interactions driving synaptogenesis.

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Poster

410. Novel Genetic Tools for Understanding Brain Function

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 410.07

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Visualization of perineuronal nets for in vivo imaging applications

Authors: *F. CALUGI[1,2], L. LUPORI[2], S. CORNUTI[2], M. CALVELLO[2], M. COSTA[3], E. GHIRARDINI[3], E. PUTIGNANO[3], J. C. KWOK[4], L. BARONCELLI[3], P. TOGNINI[5], T. PIZZORUSSO[2,3];
[1]Dept. of Neuroscience, Psychology, Drug research and Child Hlth. NEUROFARBA, Univ. of Florence, Florence, Italy; [2]Bio@SNS lab, Scuola Normale Superiore, Pisa, Italy; [3]Inst. of Neurosci., Natl. Res. Council, Pisa, Italy; [4]Sch. of Biomed. Sci., Univ. of Leeds, Leeds, United

Kingdom; [5]Dept. of Translational Res. and New Technologies in Med. and Surgery, Univ. of Pisa, Pisa, Italy

Abstract: Perineuronal nets (PNNs) are extracellular matrix structures associated mostly with fast-spiking parvalbumin interneurons in the cortex. PNNs mature along with brain circuits post-natal refinements, having an inhibitory role in plasticity and stabilizing synaptic connections. PNNs are also involved in long-term memory retention, probably acting as a physical framework for memory storage or stabilizing the neuronal network of the engram. Moreover, PNNs might confer peculiar electrophysiological properties to parvalbumin interneurons, which may be essential in sustaining their role as balancers of neuronal network activity state. Indeed, alterations in PNNs have been described in psychiatric diseases and neurodevelopmental disorders characterized by an imbalance between excitation and inhibition, such as schizophrenia and fragile X syndrome. Despite the prominent role of PNNs in physiological and pathological conditions, there is no means to study their impact on neuronal activity in vivo. Here we aim to develop a fluorescent reporter to visualize PNNs and simultaneously perform functional studies from PNN-associated neurons. For this purpose, we developed an adeno-associated virus (AAV) to deliver a PNNs component, the Hapln1 protein, fused to a fluorophore suitable for two-color calcium imaging. By injecting the AAV directly into the cortex of adult mice, we were able to visualize PNNs in ex vivo conditions. To maximize the contrast between PNNs and the surrounding diffuse extracellular matrix and minimize the possible off-targets, we developed a neuron-specific construct and a conditional construct to make Hapln1-mRuby2 expressed only by parvalbumin interneurons. Our results indicate that limiting the expression of our construct only to parvalbumin interneurons could be a promising strategy for the visualization of PNNs in vivo.
In summary, our reporter has the potential to be a seminal tool to unravel PNNs functions in vivo, allowing the scientific community to perform anatomical, functional, and longitudinal studies either in physiological or pathological conditions.

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Poster

410. Novel Genetic Tools for Understanding Brain Function

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Program #/Poster #: 410.08

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: 1R01MH116203

Title: Imaging presynaptic release of neuropeptides in vivo

Authors: *D. KIM[1], S. HAN[2];
[1]PBL-H, The Salk Inst. for Biol. Studies, La Jolla, CA; [2]PBL-H, Salk Inst. For Biol. Studies, La Jolla, CA

Abstract: Imaging presynaptic release of neuropeptides *in vivo* Neuropeptides are one of core molecules for neuronal communication and they are involved in critical brain functions. However, attempts to study neuropeptides in circuit-level have been restricted, possibly due to lack of methods for detecting its release at presynaptic terminals in behaving animals. Here we genetically engineered the sensor that detects neuropeptide release using large dense core vesicle (LDCV) targeting protein and a superecliptic pHluorin as a fluorescence reporter. In the assays with cultured cell line and acute brain slices, our results show that the sensor is pH- and calcium-dependent and displays increased activity in response to varying intensities of electrical stimulation. We also tested the efficacy of the LDCV release sensor in awake behaving mice using fiber photometry technique in mice expressing it in the neuropeptidergic neurons. Our preliminary results show that the sensor reliably detect presynaptic release of neuropeptides in awake behaving mice in response to multiple sensory and emotional stimuli. We propose that the LDCV sensor as a readout of neuropeptide release is a useful tool for real-time measurement of presynaptic neuropeptide release and thus for understanding neuropeptidergic signaling.

Disclosures: D. Kim: None. **S. Han:** None.

Poster

410. Novel Genetic Tools for Understanding Brain Function

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Program #/Poster #: 410.09

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH grant DP2 MH122398

Title: Neural somatic genome editing in the brain for personalized patient models

Authors: *C. D. ROBERTSON[1], P. H. IFFLAND[2], P. N. MCKEON[1], D. C. O. VIERA[3], P. DAVIS[4], R. RICHARDSON[1], E. JAŠAREVIC[5], M. STEYERT[1], I. DICK[3], B. N. MATHUR[1], P. CRINO[2], T. BALE[1], A. POULOPOULOS[1];
[1]Dept. of Pharmacol., [2]Dept. of Neurol., [3]Dept. of Physiol., Univ. of Maryland Sch. of Med., Baltimore, MD; [4]Boston Children's Hosp., Harvard Med. Sch., Boston, MA; [5]Dept. of Computat. and Systems Biol., Magee-Women's Res. Institute, Univ. of Pittsburgh Sch. of Med., Pittsburgh, MD

Abstract: There are more than 75,000 known disease-associated genetic variants in humans, but the vast majority of these remain unstudied or underexplored. The cost, both financial and temporal, to investigate these variants using transgenic animal models is infeasible, while more reductionist *in vitro* models may omit tissue-level interactions critical to disease pathology. These limitations render our understanding of rare genetic variants incomplete. Recent advances

in CRISPR/Cas9 genome editing technologies have resulted in increased editing precision that could supplant the need for these traditional transgenic models. Here, we propose the application of precision genome editing agents in wildtype mice to model individual patient genetic variants and create personalized patient models of neurogenetic disease. We use *in utero* electroporation (IUE) of prime editor, a hybrid genome editing agent combining Cas9 with reverse transcriptase, to introduce point mutations derived from individual epilepsy patients to rapidly generate personalized models in outbred wildtype mice. Prime editing guide RNAs (pegRNAs) and secondary nicking guides (PE3(b) guides) were designed using PegAssist.app and cloned in-house. These reagents were delivered via IUE to mouse embryos at E14.5. Adult mice were screened for disease phenotypes using electrophysiology and electroencephalography (EEG). Using a mutation in GluN2A (A243V) found in patients with Rolandic epilepsy, we test our accelerated patient modeling pipeline. Mice generated in this fashion displayed aberrant currents recorded by patch electrophysiology in slice and demonstrated spontaneous seizures and epileptiform activity when recorded via EEG. This approach provides the first direct evidence for a causal role of the rare GluN2A A243V variant in disease pathology and is a proof-of-concept for acute genome editing for disease modeling. Within 7 weeks, reagents were developed and delivered to wildtype mice to reprogram their genome and recapitulate patient pathology. These animals can be used to elucidate disease etiology and screen for optimal treatment selection on an individual basis. With further development this approach could represent a paradigm shift in the study of genetic disease, creating a workflow between bedside and benchtop research with the aspiration of making animal models for personalized medicine accessible to any patient who would benefit.

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Poster

410. Novel Genetic Tools for Understanding Brain Function

Location: SDCC Halls B-H

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Program #/Poster #: 410.10

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Efficient transduction of large numbers of neurons in rat brains by systemic delivery of AAV capsids

Authors: *V. M. PLATTNER[1], X. CHEN[2], V. GRADINARU[3], A. AKRAMI[1];
[1]Sainsbury Wellcome Ctr., UCL, London, United Kingdom; [2]BBE, [3]Biol. and Biol. Engin., Caltech, Pasadena, CA

Abstract: Transgenic model organisms, especially rodents, are routinely used in neuroscience. In these animals, specific cell types are genetically modified, allowing them to be easily manipulated (optically or pharmacologically) or imaged in awake and behaving animals under

different experimental conditions. Transgenic mouse strains are commercially available but engineering such rats is still challenging despite the great demand for them in research due to their size, social behaviour, and cognitive abilities. Local injection of viral vectors packaged with genes that are controlled by specific promoters can be an alternative solution to using transgenic rat strains. The injected vectors, however, have limited spread in the tissue, and the location and the expression level cannot be controlled precisely. Moreover, the invasive procedure can potentially trigger inflammation. This makes it difficult to do systematic dissection of neural circuits, across animals, for manipulation or imaging purposes. In this study we investigated several neuron-specific, engineered, blood-brain-barrier crossing AAV capsids to compare their efficacy in transducing neurons in rat brains. Capsids packaged with genome encoding fluorescent reporters were injected systemically (IV tail injections) and the animals were perfused after a 4-6 weeks incubation period. Automated two-photon serial sectioning and imaging was used to process and image the brains. The pre-processed data was fed into a deep learning algorithm (cellfinder developed as part of the BrainGlobe project) to count and identify the location of labeled cells across the entire brain. Large numbers of labeled neurons were found across the cortex, hippocampus, cerebellum thalamus and striatum. Our results show that systemic injection of engineered AAV capsids is an effective way of transfecting large numbers of neurons. The number and density of labeled cells can precisely be controlled by adjusting the amount of injected capsids. By injecting a high enough concentration of capsids, strong and dense labeling can be achieved for optogenetic and imaging experiments.

Disclosures: **V.M. Plattner:** None. **X. Chen:** None. **V. Gradinaru:** None. **A. Akrami:** None.

Poster

410. Novel Genetic Tools for Understanding Brain Function

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 410.11

Title: WITHDRAWN

Poster

410. Novel Genetic Tools for Understanding Brain Function

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 410.12

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: 5UG3MH120102

Title: Engineering Viral Vectors for Acoustically Targeted Gene Delivery in Brain

Authors: *H. R. LI[1], J. HEATH[1], J. TRIPPETT[3], J. O. SZABLOWSKI[4], M. G. SHAPIRO[2]; [1]Biol. and Biol. Engin., [2]Chem. and Chem. Engin., Caltech, Pasadena, CA; [3]Bioengineering, Rice Univ., Houston, TX; [4]Bioengineering, Rice Neuroengineering Initiative, Houston, TX

Abstract: *Objectives:* We aim to apply high-throughput *in vivo* selection to engineer novel AAV vectors specifically for local neuronal transduction at site of focused ultrasound blood-brain-barrier opening (FUS-BBBO).
Methods: We employ an in vivo viral evolution method: a library of AAVs with mutated capsids based on AAV9(Fig. 1a) is injected intravenously into hSyn1-Cre mouse and delivered via FUS-BBBO to one hemisphere. When a particular AAV variant transduces Cre-expressing neurons, its viral genome is modified, becomes detectable by a Cre-dependent PCR and Next-generation Sequencing (NGS)(Fig. 1b). Repeated rounds of selection for vectors uniquely appear in the targeted hemisphere led to desired novel AAV vectors for objectives.
Results: Histological analysis revealed higher efficiency of transduction in the brain for all final 5 viral variants (AAV.FUS.1-5)(Fig. 2a,b; up to 130% improvement over AAV9). Each serotype transduced the liver less strongly (Fig. 2c, d; up to 6.8-fold reduction compared to AAV9). The top AAV.FUS variant (AAV.FUS.3) showed 12.1-fold improvement in overall tissue specificity (Fig. 2e). All candidates show improved neuronal tropism as well: AAV.FUS.3 has a 69.8% likelihood of transducing a neuron, compared to 44.7% for AAV9(Fig. 2g, h). Our screen yielded AAV.FUS.3, the first viral vector expressly engineered to work in conjunction with ultrasound-mediated gene delivery to the brain. Overall, this study shows that the molecular engineering of AAV capsids such as AAV.FUS.3 can lead to improved ultrasound-mediated gene delivery to the brain.
Conclusion: Overall, this study shows that the molecular engineering of AAV capsids can lead to improved noninvasive, site-specific ultrasound-mediated gene delivery to the brain. Our screen yielded AAV.FUS.3, the first viral vector expressly engineered to work in conjunction with a specific physical delivery method.

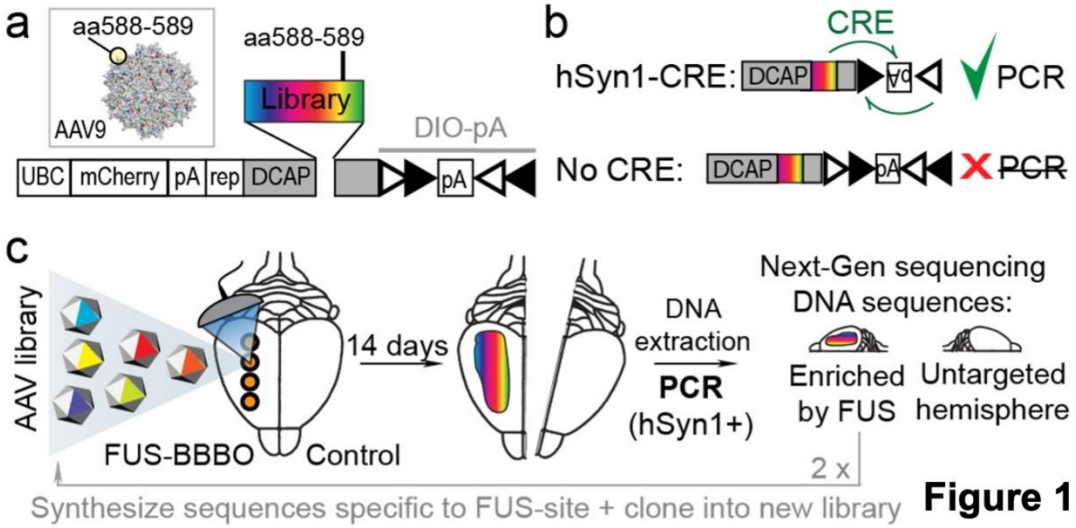

Figure 1

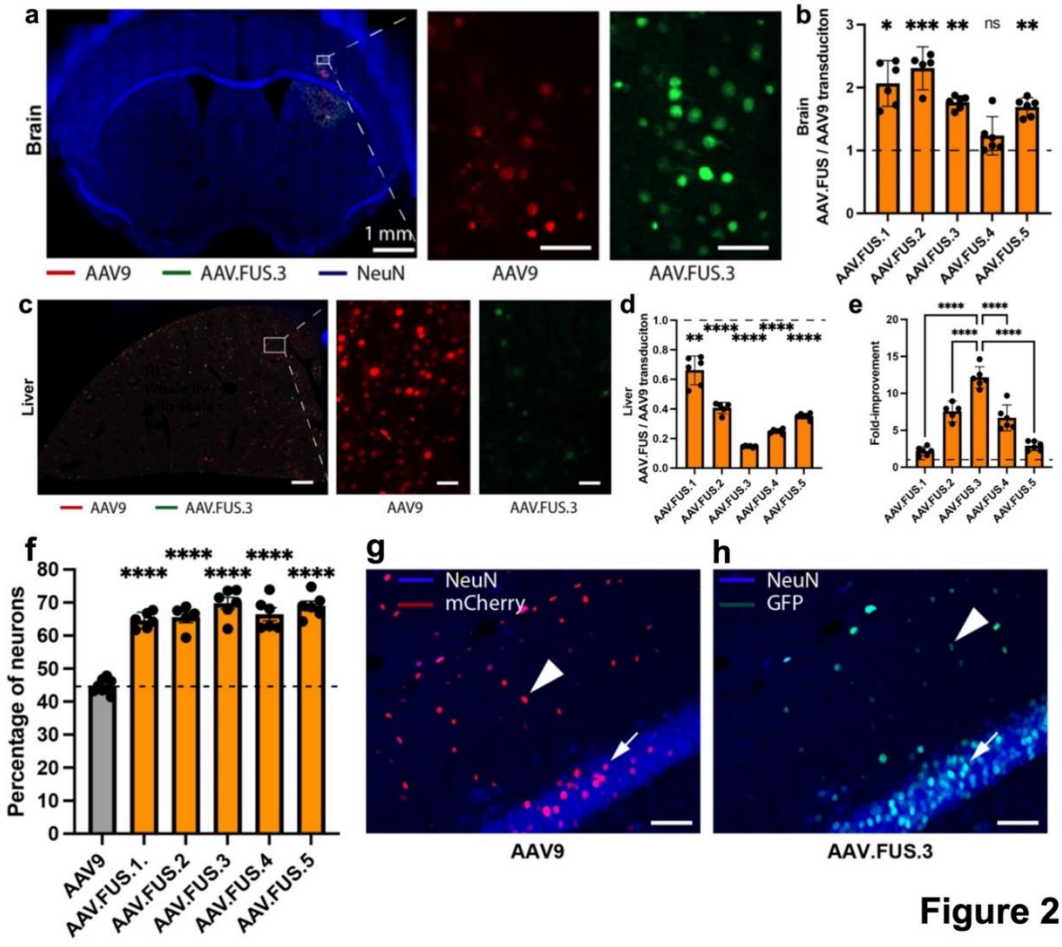

Figure 2

Disclosures: **H.R. Li:** None. **J. Heath:** None. **J. Trippett:** None. **J.O. Szablowski:** None. **M.G. Shapiro:** None.

Poster

410. Novel Genetic Tools for Understanding Brain Function

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 410.13

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant GM132500

Title: High-throughput genotyping for genetic analysis in outbred zebrafish

Authors: R. CHENG[1], K.-M. NGUYEN[1], A. CHITRE[1], J. XU[2], R. CASANAVE[2], S. GUO[2], A. A. PALMER[1], ***O. POLESSKAYA**[1];
[1]Univ. of California - San Diego, San Diego, CA; [2]Univ. of California - San Francisco, San Francisco, CA

Abstract: Zebrafish is a model organism that is used for the analysis of behavior. In particular, outbred populations of zebrafish, such as EKW, are well-suited for genome-wide association studies (GWAS) because they provide good mapping resolution and have extensive phenotypic and genetic diversity. However, zebrafish have not previously been used for GWAS, in part because the cost of dense genotyping of large numbers has been an obstacle. We present a cost-effective and high-throughput genotyping method that we have developed in support of a GWAS in EKW zebrafish to study the genetic underpinning of complex traits related to light-dark preference, a behavior reflecting exploration and anti-predation balance. We genotyped 5,759 larvae that were behaviorally characterized. DNA was extracted from larva in a high-throughput manner to prepare libraries with a low-cost library preparation method. Samples were barcoded to enable the sequencing of 960 samples in a single Illumina NovaSeq lane, which provided an average of 0.5x coverage per individual. That coverage is insufficient for directly calling SNPs, but can be used for imputation. We deeply sequenced a subset of 95 F0 individuals to use as an imputation reference panel for this population. We demonstrated that these 95 F0s represent haplotypes that exist in the population well enough to call genotypes, even in individuals whose parents were not genotyped. Variant calling and imputation to the reference were performed using STITCH, and then further imputation was done using BEAGLE. We called more than 19 million SNPs with 98.3% accuracy, as measured by comparing genotypes to a “truth set” of deeply sequenced individuals. This work served as a foundation for GWAS to elucidate the genetic underpinning of individual variations in exploration and anti-predation. The low-cost high-throughput genotyping enables large-scale genetic analysis in this model organism.

Disclosures: **R. Cheng:** None. **K. Nguyen:** None. **A. Chitre:** None. **J. Xu:** None. **R. Casanave:** None. **S. Guo:** None. **A.A. Palmer:** None. **O. Polesskaya:** None.

Poster

410. Novel Genetic Tools for Understanding Brain Function

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 410.14

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NRF-2021R1I1A1A01061343

Title: Enhancing the Survival of Human Mesenchymal Stem Cells via CRISPR/Cas9-based genome editing

Authors: *N. LEE[1,2,3,4,5], H. KIM[1,2,3,4,5], D. NA[2,3,5];
[1]Sungkyunkwan Univ., Seoul, Korea, Republic of; [2]Neurol., Samsung Med. Ctr., Seoul, Korea, Republic of; [3]Cell and Gene Therapy Inst., Seoul, Korea, Republic of; [4]Neurosci. Ctr., Seoul, Korea, Republic of; [5]Alzheimer's Dis. Convergence Res. Ctr., Seoul, Korea, Republic of

Abstract: Human mesenchymal stem cells (MSCs) have many advantages that make them a useful source for cell-based therapy. Since MSCs secrete diverse neurotrophic factors, they have been shown promising efficacy in treating neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and Amyotrophic lateral sclerosis. However, transplantation of MSCs in hostile disease models leads to reduced survival and therapeutic efficacy. The majority of transplanted MSCs are only short-lived, which may require repeated administration. To overcome this, genetic modification is an effective method to up-regulate the cellular functions of MSCs. It is important to find the appropriate target gene to improve the survival of MSCs by genetic modification. In this study, we focused on apoptosis signaling and hypothesized that the survival of MSCs was enhanced by repressing apoptosis associated genes. For this, we employed clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9-based non-viral gene editing approach to reduce apoptosis related genes. First, we designed single guide RNAs (sgRNAs) that contain a targeting sequence to apoptosis signaling receptors, mediators, and transcription factors. Using Wharton's jelly-derived human MSCs, CRISPR/Cas9 components were transfected in the ribonucleoprotein (RNP) complex. The insertion and deletion (Indel) analysis showed that all candidates were successfully edited, and their expressions were down-regulated. Since there were no changes in the expression of surface markers and the potential of tri-lineage differentiation, the characterization of MSCs was maintained. To test the viability of genetically modified MSCs *in vitro*, they were cultured in an environment of oxidative stress, inflammation, and amyloid-beta. Although most candidates had no effects on cell viability, some genetically modified MSCs had a slight increase in viability under stressful conditions. This result suggested the possibility of increasing the survival of MSCs *in vivo*, and it can be expected that the therapeutic efficacy will be improved.

Disclosures: **N. Lee:** None. **H. Kim:** None. **D. Na:** None.

Poster

410. Novel Genetic Tools for Understanding Brain Function

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Program #/Poster #: 410.15

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Director's New Innovator DP2NS087949
PECASE
NIH BRAIN 453 R01MH117069
NIH Pioneer DP1OD025535
SPARC 1OT2OD024899
Vallee Foundation
Moore Foundation

Title: Brain endothelial-specific cell targeting in rodent and broad CNS gene delivery from rodent to primate with engineered systemic AAVs

Authors: *X. CHEN[1], D. SIVADASAN BINDU[2], D. A. WOLFE[1], M. ZHANG[1], D. GOERTSEN[1], T. F. MILES[1], E. SULLIVAN[1], S. RAVINDRA KUMAR[1], S.-F. HUANG[3], C. M. AROKIARAJ[1], V. PLATTNER[4], L. J. CAMPOS[5], V. NGO[6], X. DING[1], B. P. LEVI[7], E. S. LEIN[7], A. AKRAMI[4], A. KELLER[3,8], C. MILLER[6], J. T. TING[7,9], A. S. FOX[5], C. EROGLU[2], V. GRADINARU[1];
[1]BBE, Caltech, Pasadena, CA; [2]Dept. of Cell Biol., Duke Univ. Med. Ctr., Durham, NC; [3]Dept. of Neurosurgery, Clin. Neurosci. Center, Zürich Univ. Hosp., Univ. of Zürich, Zürich, Switzerland; [4]Sainsbury Wellcome Ctr., Univ. Col. London, London, United Kingdom; [5]Dept. of Psychology and California Natl. Primate Res. Ctr., Univ. of California, Davis, Davis, CA; [6]Cortical Systems and Behavior Lab., Univ. of California San Diego, La Jolla, CA; [7]Allen Inst. for Brain Sci., Seattle, WA; [8]Neurosci. Ctr. Zürich, Univ. of Zürich and ETH Zürich, Zürich, Switzerland; [9]Dept. of Physiol. and Biophysics, Univ. of Washington, Seattle, WA

Abstract: Delivering genes to and across the brain vasculature efficiently and specifically across species remains a critical challenge for addressing neurological diseases. Through a combination of directed evolution and semi-rational engineering, we identified a family of novel vectors, including AAV-X1 and AAV-X1.1, which target brain endothelial cells specifically and efficiently following systemic delivery in mice with a ubiquitous promoter. We characterized these novel AAVs across rodent models (genetically diverse mouse strains and rats), non-human primates (marmosets and rhesus macaques), and ex vivo human brain slices, demonstrating brain endothelial-specific cell targeting in rodent and broad CNS targeting in primates. To illustrate the utility of AAV-X1 for CNS delivery of neuroactive proteins, we transformed mouse brain endothelial cells into a biofactory for producing the synaptogenic protein Sparc-like protein 1 (Sparcl1)/Hevin. Hevin/Sparcl1 is an astrocyte-secreted protein that controls formation of vesicular glutamate transporter 2 (VGluT2)-containing synapses such as thalamocortical synapses. AAV-X1-mediated ectopic expression of Sparcl1/Hevin in brain endothelial cells was sufficient to rescue the thalamocortical synaptic loss phenotype of Sparcl1/Hevin knockout mice. We also demonstrated the transferability of AAV-X1's properties from the AAV9 serotype to AAV1, enabling repeated AAV administration to increase CNS transduction. In general, we provide novel engineered systemic AAVs for brain endothelial-specific cell targeting in rodent and broad CNS targeting from rodent to primate with potential for re-administration.

Disclosures: **X. Chen:** None. **D. Sivadasan Bindu:** None. **D.A. Wolfe:** None. **M. Zhang:** None. **D. Goertsen:** None. **T.F. Miles:** None. **E. Sullivan:** None. **S. Ravindra Kumar:** None. **S. Huang:** None. **C.M. Arokiaraj:** None. **V. Plattner:** None. **L.J. Campos:** None. **V. Ngo:** None. **X. Ding:** None. **B.P. Levi:** None. **E.S. Lein:** None. **A. Akrami:** None. **A. Keller:** None. **C. Miller:** None. **J.T. Ting:** None. **A.S. Fox:** None. **C. Eroglu:** None. **V. Gradinaru:** None.

Poster

410. Novel Genetic Tools for Understanding Brain Function

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Program #/Poster #: 410.16

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH/NINDS Grant NS091546

Title: A CRISPR/Cas9 toolbox for tissue-specific gene knockout at the Drosophila neuromuscular junction.

Authors: ***E. LOXTERKAMP**[1], C. CHIEN[1], S. PERRY[1], S. RELLE[1], X. CHEN[2], B. WANG[2], C. HAN[2], D. K. DICKMAN[1];
[1]Neurobio., USC, Los Angeles, CA; [2]Weill Inst. for Cell and Mol. Biology, Dept. of Mol. Biol. and Genet., Cornell Univ., Ithaca, NY

Abstract: The *Drosophila* larval neuromuscular junction (NMJ) has been established as a powerful system to study synaptic development, growth, function, and plasticity. Studies in this system have relied largely on germline mutations, which have revealed important insights into synaptic biology. However, genetic studies in this system are limited, where essential genes cannot be investigated, and interpretations are obscured by pleiotropic gene functions in many tissues. RNA interference can target genes for reduced expression in specific cell types, but this method rarely achieves complete gene loss of function. Recent innovations in somatic mutagenesis using CRISPR/Cas9 approaches offer a powerful alternative with the potential to completely eliminate gene function in a tissue-specific manner. Here, we sought to generate an "NMJ toolkit" for rapid and efficient somatic CRISPR/Cas9 mutagenesis targeting the three principal cell types at the NMJ: motor neurons, muscle, and glia. First, we have systematically converted a variety of NMJ GAL4 driver lines into corresponding Cas9 lines. We have successfully converted GAL4 to Cas9 that express in all neurons, all motor neurons, a subset of motor neurons, all muscles, subsets of muscles, and peripheral glia. This has enabled the targeted expression of Cas9 in all principle NMJ cell types. Next, we have engineered guide RNAs (gRNAs) in optimized multiplexed vectors for somatic mutagenesis targeting a series of pre- and post-synaptic genes encoding key proteins involved in synaptic communication. Using these transgenes, we have systematically tested the efficiency of the Cas9 lines to reliably induce gene knock out, with some approaching over 95% reduction in protein levels and exhibiting the expected mutant phenotypes. Finally, we have optimized these reagents to test whether

additional copies of Cas9 or gRNA transgenes lead to more efficient gene knock out and determine whether simultaneous knock out of up to 8 genes can be achieved in a single cross targeting a single tissue. Ultimately, this genetic toolkit will unlock new approaches to study essential and combinatorial gene function in specific tissues at the NMJ.

Disclosures: E. Loxterkamp: None. **C. Chien:** None. **S. Perry:** None. **S. Relle:** None. **X. Chen:** None. **B. Wang:** None. **C. Han:** None. **D.K. Dickman:** None.

Poster

410. Novel Genetic Tools for Understanding Brain Function

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Program #/Poster #: 410.17

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: CIHR_400528
NIH R01 AG068563A
NIH R01 R01DA053301-01A1
CIHR 438531
CIHR 470425
SKGJ-MED-021
R01MH085953

Title: Rare CNVs show late convergence on phenotypic profiles

Authors: *J. KOPAL[1], K. KUMAR[2], K. SALTOUN[1], C. MODENATO[3], C. MOREAU[4], S. MARTIN-BREVET[3], G. HUGUET[2], M. JEAN-LOUIS[2], C.-O. MARTIN[2], Z. SACI[2], N. YOUNIS[2], A. MAILLARD[3], B. RODRIGUEZ-HERREROS[3], A. PAIN[3], S. RICHETIN[3], L. KUSHAN[5], A. SILVA[6], M. B. VAN DEN BREE[7], D. LINDEN[6], M. J. OWEN[7], J. HALL[7], S. LIPPE[2], B. DRAGANSKI[3], I. SØNDERBY[8], O. A. ANDREASSEN[8], D. C. GLAHN[9], P. THOMSPON[10], C. BEARDEN[5], S. JACQUEMONT[2], D. BZDOK[1];
[1]McGill Univ., Montreal, QC, Canada; [2]Ctr. de recherche CHU Sainte-Justine and Univ. of Montréal, Montreal, QC, Canada; [3]Ctr. Hospitalier Universitaire Vaudois and Univ. of Lausanne, Lausanne, Switzerland; [4]Inst. Pasteur, Paris, France; [5]UCLA, Los Angeles, CA; [6]Maastricht Univ., Maastricht, Netherlands; [7]Cardiff Univ., Cardiff, United Kingdom; [8]Oslo Univ. Hosp. and Univ. of Oslo, Oslo, Norway; [9]Boston Children's Hosp. and Harvard Med. Sch., Boston, MA; [10]Keck Sch. of Med. of USC, Marina del Rey, CA

Abstract: Copy number variations (CNVs) are rare genomic deletions and duplications that can exert profound effects on brain and behavior. Previous reports of pleiotropy in CNVs imply that they converge on shared mechanisms at some level of pathway cascades, from genes to large-scale neural circuits to the phenome. However, studies to date have primarily examined single CNV loci in small clinical cohorts. It remains unknown how distinct CNVs escalate the risk for the same developmental and psychiatric disorders. Here, we quantitatively dissect the impact on

brain organization and behavioral differentiation across eight key CNVs. In 534 clinical CNV carriers from multiple sites, we explored CNV-specific brain morphology patterns. We extensively annotated these CNV-associated patterns with deep phenotyping assays through the UK Biobank resource. Although the eight CNVs cause disparate brain changes, they are tied to similar phenotypic profiles across ~1000 lifestyle indicators. Our population-level investigation established brain structural divergences and phenotypical convergences of CNVs, with direct relevance to major brain disorders.

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Poster

410. Novel Genetic Tools for Understanding Brain Function

Location: SDCC Halls B-H

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Program #/Poster #: 410.18

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH
University of Kentucky
ACS Division of Analytical Chemistry
Graduate Student Congress, University of Kentucky

Title: Er-gcamp6f: an endoplasmic reticulum targeted calcium probe to study calcium signaling in astrocytic soma and processes

Authors: *S. P. ARYAL[1], M. XIA[2], P. I. ORTINSKI[3], C. I. RICHARDS[1];
[1]Dept. of Chem., [2]Dept. of Neurosci., [3]Univ. of Kentucky, Univ. of Kentucky, Lexington, KY

Abstract: Astrocyte calcium signaling is very important to understand normal brain function as well as brain activity during neuropathological conditions and substance use disorder. Endoplasmic reticulum (ER) is the major source of intracellular Ca^{2+} and small calcium exchange takes place between the ER and nearby cell organelles. Despite the importance of understanding calcium activity in astrocytes, one major challenge is currently available tools target either the plasma membrane (PM) or the lumen of the ER. These tools are unable to adequately measure minute Ca^{2+} exchange occurring between the ER and nearby organelles. We used genetic and molecular biology tools to develop a genetic probe to target cytosolic side of the ER to image Ca^{2+} activity in astrocytic soma and processes. This probe was characterized by different fluorescence microscopy techniques. Structured Illumination microscopy was used to

verify the specific location of the sensor in the ER as well as localization of the probe in astrocytic cell soma and processes. Total Internal Reflection Fluorescence Microscopy (TIRFM) was used in conjunction with ER-GCaMP6f to measure low-amplitude Ca^{2+} events which are otherwise undetected by epifluorescence microscopy. Our pharmacological studies demonstrated this probe measures Ca^{2+} activity differently than the currently used plasma membrane targeted or cytosolic sensors. These studies also demonstrated that ER-GCaMP6f can measure IP3R and RyR mediated Ca^{2+} in astrocytes. We generated AAV viral vectors to pack the probe and deliver stereotactically in rodents. Sprague Dawley rats showed robust expression of the ER-GCaMP6f and Ca^{2+} activity was characterized to study effect of cocaine self-administration in ER Ca^{2+} activity. In conclusion our sensor reports calcium signaling in close proximity to the endoplasmic reticulum in astrocytic soma, processes and *in vivo*.

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Poster

410. Novel Genetic Tools for Understanding Brain Function

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Program #/Poster #: 410.19

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

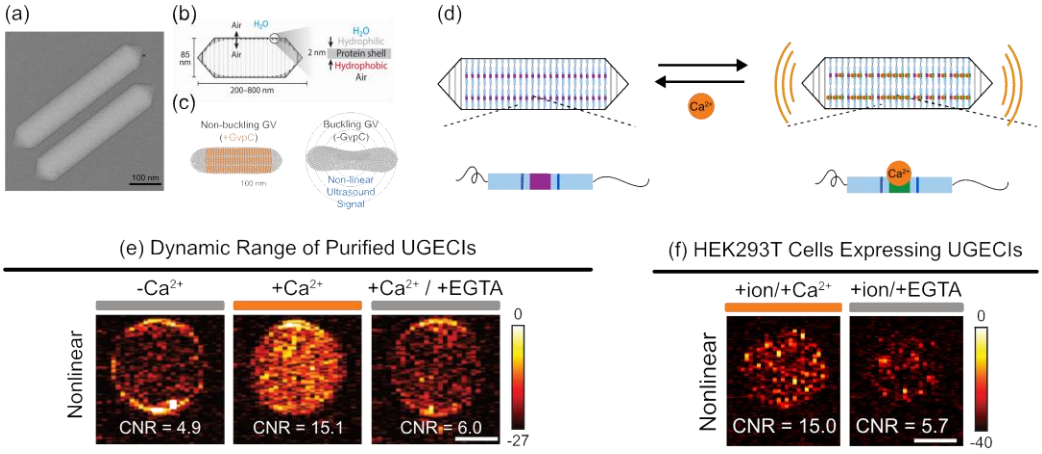
Support: NIH Grant 1R01NS120828-01

Title: Ultrasonic Genetically Encoded Calcium Indicators

Authors: *Z. JIN, A. LAKSHMANAN, T. A. TRAN, R. ZHANG, M. DUAN, R. C. HURT, D. MALOUNDA, M. G. SHAPIRO;
Caltech, Pasadena, CA

Abstract: Imaging technologies enabling noninvasive observation of specific neural signals represent a “holy grail” of tools for neuroscience. While widely used neuroimaging approaches based on fluorescent reporter genes and biosensors have enabled major neuroscience discoveries, they fall far short of providing whole-brain recordings of neural activity due to the limited tissue penetration of light. In contrast, ultrasound-based imaging techniques overcome this limitation and enable imaging of deep brain regions with high spatiotemporal resolution. To connect ultrasound to specific neural signals, we take advantage of gas vesicles (GVs), microbially derived gas-filled protein nanostructures, which our group introduced as the first genetically encodable acoustic reporters (Fig.1a-b). Based on GVs, we are developing the first ultrasonic genetically encoded calcium indicators (UGECIs). We engineered the GV surface protein GvpC, which controls GVs' acoustic properties by stiffening their protein shell (Fig.1c). We demonstrated that incorporating calcium-sensitive motifs in the GvpC sequence enables changes of its binding with the GV shell upon sensing calcium, enabling GVs to become softer and thereby produce stronger ultrasound contrast (Fig.1d). In purified format, UGECIs showed ~ 3-fold enhancement in ultrasound contrast in response to sub-micromolar Ca2+, and the contrast

was reversed by addition of calcium chelator EGTA (Fig.1e). Furthermore, UGECIs were expressed transiently in mammalian cells and these cells showed calcium-dependent ultrasound contrast with similar dynamic range (Fig.1f). These results provide the first demonstration of genetically encoded calcium indicators for ultrasound as a crucial step towards whole-brain imaging of molecular neural activity.



Disclosures: **Z. Jin:** None. **A. Lakshmanan:** None. **T.A. Tran:** None. **R. Zhang:** None. **M. Duan:** None. **R.C. Hurt:** None. **D. Malounda:** None. **M.G. Shapiro:** None.

Poster

410. Novel Genetic Tools for Understanding Brain Function

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Program #/Poster #: 410.20

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

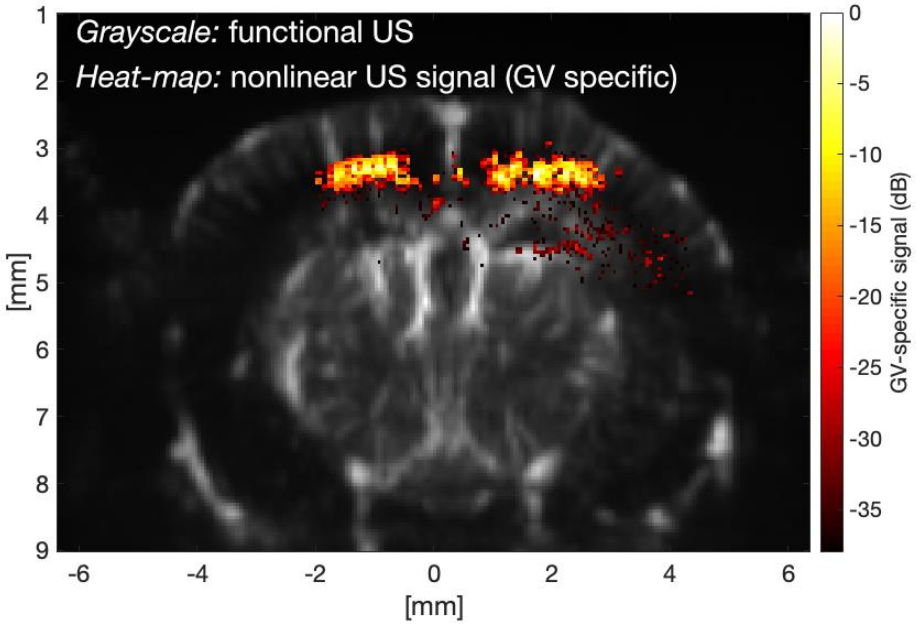
Support: NIH Grant RO1

Title: Ultrasound imaging of in situ gene expression in the brain

Authors: *S. SHIVAEI[1], C. RABUT[2], E. CRIADO HIDALGO[2], M. ABEDI[2], J. WEKSELBLATT[2], R. J. ZHANG[2], P. BARTUREN-LARREA[2], M. G. SHAPIRO[2];
[1]Bioengineering, [2]Caltech, Pasadena, CA

Abstract: One of the major gaps in neurotechnology is the lack of a method that enables whole-brain imaging of neural activity over long periods of time. However, tracking neural activity in an intact brain requires noninvasive imaging tools that can penetrate deep inside the tissue. Commonly used light-based imaging techniques, while providing high spatiotemporal resolution, suffer from poor penetration into an intact brain. Ultrasound is a widely used biomedical technology that enables imaging of cells deep inside the body without a significant sacrifice of spatiotemporal resolution. Recent work has established gas vesicles (GVs) as genetically encodable ultrasound reporters. These microbially derived gas-filled protein nanostructures

enable deep tissue imaging at a cellular level, pushing the field beyond conventional organ-scale imaging. However, the use of these proteins in mammalian cells has been limited by the need for tedious clonal expansions of sorted cell lines. Here, we develop a modular viral delivery platform that efficiently delivers GVs into mammalian cells and tissues. Our viral vector architectures successfully co-express more than 10 genes to produce strong ultrasound contrast in a variety of cell types including primary neurons. We further demonstrate that these vectors can be used to record intracellular activity by placing the expression of GVs under the control of several immediate early gene (IEG) promoters and designing the basal level of expression to be 'non-leaky'. Intracranial injection of our designed viral vectors in 8-week old mice produced strong ultrasound contrast in the hippocampus that was confirmed by histology (see figure). In ongoing work, we integrate our IEG-based neural activity sensors to image *in situ* neural activity in response to a variety of sensory stimuli, enabling noninvasive imaging of IEG expression in the same animal over long periods of time. Our viral GV delivery platform will serve as a critical tool for whole-brain imaging of gene expression in live intact brains.



Disclosures: **S. Shivaei:** None. **C. Rabut:** None. **E. Criado Hidalgo:** None. **M. Abedi:** None. **J. Wekselblatt:** None. **R.J. Zhang:** None. **P. Barturen-Larrea:** None. **M.G. Shapiro:** None.

Poster

410. Novel Genetic Tools for Understanding Brain Function

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 410.21

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant R01GM109984
NIH Grant R01GM121944
NIH Grant U01NS113294
NIH Grant U01NS099709
NSF Grant NeuroNex 1707352
NIH Grant R01EB032854

Title: New fluorescent proteins for long-term imaging of living cells

Authors: G. G. LAMBERT, ***N. C. SHANER**;
Neurosciences, UCSD, La Jolla, CA

Abstract: Fluorescent proteins (FPs) have been a part of the biological imaging toolbox for nearly 30 years, and while light microscope technology continues to advance to allow imaging of living systems at increasingly higher time and spatial resolution, the FPs used in this imaging have not advanced at the same pace. At the same time, the majority of biological imaging is still performed on “traditional” widefield and laser scanning confocal microscopes, while FP development efforts have largely focused on generating probes for advanced techniques such as single-molecule localization microscopy (SMLM). Properties critical to long-term imaging of living cells, such as photostability and behavior in fusions with other proteins have been slow to improve. To address the shortfall in development of improved FPs for both general and advanced uses, we have engineered green- and yellow-emitting monomeric variants of the ultra-bright dimeric FP AausFP1 and a red-emitting monomeric derivative of mCherry with much higher brightness than its parent. All of these new FP variants appear to be very inert when fused to other proteins and are highly photostable under both widefield and scanning laser illumination. We anticipate that these FPs will enable more sensitive detection of signal with lower illumination power, allowing researchers to observe cells expressing lower amounts of fusion protein for more biologically authentic behavior.

Disclosures: G.G. Lambert: None. **N.C. Shaner:** None.

Poster

410. Novel Genetic Tools for Understanding Brain Function

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 410.22

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NSF NeuroNex 1707352

Title: The role of higher-order thalamic inputs in generating oscillatory dynamics in sensory neocortex: Integrated electrophysiological, interluminescence and fluorescence studies

Authors: ***J. MURPHY**[1], E. M. KLEIN[1], E. L. CRESPO[2], M. PRAKASH[3], S. R. JONES[1], U. HOCHGESCHWENDER[3], C. I. MOORE[1];

[1]Neurosci., Brown Univ., Providence, RI; [2]Biochemistry, Cell. and Mol. Biol., [3]Neurosci., Central Michigan Univ., Mount Pleasant, MI

Abstract: Oscillatory brain dynamics are correlated with many different cognitive functions. Additionally, many disorders of the nervous system are correlated with alterations in these dynamics. Understanding which brain structures, and which neuron subtypes, coordinate these dynamics holds the potential to link the rich non-invasive correlation-based data on oscillatory brain dynamics with concrete mechanisms. Further, correlational oscillatory biomarkers of nervous system disorders can be evaluated through the lens of concrete pathology. However, a detailed, mechanistic understanding of oscillatory brain dynamics remains incomplete.
Prior research has implicated thalamocortical interactions as a source of oscillatory dynamics, and a parallel body of research has implicated 'higher-order' thalamic nuclei, such as the pulvinar in humans and primates and the posterior medial nucleus (POm) in rodents, in contextual modulation of cortical sensory processing.
We present here preliminary evidence that bursting in POm precedes oscillatory 'Beta Events', discrete triphasic signals (15-29 Hz). The anatomical projection pattern of POm to primary somatosensory neocortex (SI) aligns well with our detailed computational model predictions as to the laminar origins of beta-events. Further, burst spike firing, common in thalamus, is an ideal driver in this model. POm also targets a diverse array of areas, all of which express Beta Events, and prior studies have shown enhanced beta-band power with optogenetic POm drive.
We recently introduced a bioluminescent 'all-optical synapse', in which light emitting bioluminescent molecules are released from presynaptic terminals and activate opsins expressed on virally targeted postsynaptic partners. We demonstrated that Cre-mediated expression of the excitatory opsin ChR2(C128S) in parvalbumin positive cells in SI postsynaptic to virally targeted bioluminescent thalamocortical excitatory cells was sufficient to drive increases in gamma band (30-100 Hz) oscillations in SI. We present new data here in which POm is exclusively targeted as the bioluminescent presynaptic element in VGAT-ChR2 transgenic mice in which all GABAergic neurons express the excitatory opsin. This arrangement produced a distinct increase in delta oscillations (1-5 Hz) as recorded in SI, suggesting that excitatory POm projections to different neural subpopulations in cortex are able to drive different oscillatory motifs in cortex. Further, initial studies combining 1-Photon imaging of POm axon activity with electrophysiology, and multi-site recordings, support the view that POm activity precedes Beta Events.

Disclosures: J. Murphy: None. **E.M. Klein:** None. **E.L. Crespo:** None. **M. Prakash:** None. **S.R. Jones:** None. **U. Hochgeschwender:** None. **C.I. Moore:** None.

Poster

410. Novel Genetic Tools for Understanding Brain Function

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Program #/Poster #: 410.23

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: Sloan Fellowship Grant FG-2021-16332
University of Rochester start-up funding

Title: Non-invasive injections of coelenterazine elicit proportional neocortical bioluminescence responses

Authors: *E. MURPHY, M. GOMEZ-RAMIREZ;
Brain and Cognitive Sci., Univ. of Rochester, Rochester, NY

Abstract: BioLuminescent OptoGenetics ("BL-OG") is a chemogenetic method that enables optogenetic reactions without the use of an external light device. In BL-OG, a photo-enzyme (e.g., slow-burn Gaussia Luciferase; *sbGLuc*) is tethered to an optogenetic element (e.g., Volvox-Channelrhodopsin-1; *VCHR1*) that is activated via bioluminescent light. Bioluminescence is generated by injecting a chemical substrate (*luciferin*, e.g., Coelenterazine; *CTZ*) that is catalyzed by the photo-enzyme neighboring the opsin. In a previous study, we showed proportional increases in neural firing and bioluminescence in response to higher doses of CTZ. However, these BL-OG effects were observed during invasive injections of the luciferin directly into the brain. Thus, to demonstrate that BL-OG is a viable method for modulating neural activity non-invasively, it is key to determine dose response curves when the substrate is injected systemically. Here, we injected different dosages of h-CTZ via the tail vein of mice expressing the BL-OG construct LMO7, a novel and more photo-efficient BRET-based molecule that tethers the bioluminescent and fluorescent protein NCS2 to VCHR1. Bioluminescence was imaged using an EMCCD camera while animals ran on a wheel, and were injected with six different concentration dosages of h-CTZ. Data reveal systematic increases in bioluminescence in response to larger dosages of h-CTZ. These bioluminescent responses were observed through a thinned-skull preparation (i.e., without the need of implanting a cranial window), highlighting the photo efficiency of the LMO7 construct. Our study furthers our understanding of the BL-OG technique by providing a map between BL-OG effects and systemic injections of h-CTZ via the tail vein, and shows that thin skull technique can be used as a less invasive window for bioluminescence imaging studies.

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Poster

410. Novel Genetic Tools for Understanding Brain Function

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Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NSF Grant NeuroNex 1707352
NIH Grant U01NS099709

Title: Expanding the bioluminescent optogenetics (BL-OG) platform with improved opsins

Authors: J. SIMKINS[1], N. GORANTLA[1], *E. IKEFUAMA[1], G. G. LAMBERT[2], M. TREE[1], K. RISELAY[1], E. L. CRESPO[1], M. PRAKASH[1], N. C. SHANER[2], U. HOCHGESCHWENDER[1];
[1]Central Michigan Univ., Mount Pleasant, MI; [2]Univ. of California San Diego, San Diego, CA

Abstract: Efforts are ongoing in improving light-sensing molecules for use in optogenetic applications. Recent developments are opsins with increased light sensitivity, red-shifted activation wavelength, as well as GPCR-based opsins. These advances are based on discoveries of new natural opsins, targeted mutation of available opsins, or molecular evolution, including machine-learning-guided engineering. We took advantage of the progress in this field to expand our bioluminescent optogenetics (BL-OG) platform. Here, light-sensing molecules are activated by light emitted from a luciferase enzyme when it oxidizes a small molecule luciferin. The luciferase is usually fused to the opsin in a luminopsin, LMO. BL-OG enables both direct activation of the opsin using an LED for temporally precise control and chemogenetic manipulation of neuronal populations distributed throughout the brain via systemic administration of the luciferin, using the same genetic construct in the same animal. To expand the utility of BL-OG, we generated a series of luciferase-opsin fusion proteins that employ recently improved opsins. The fusion proteins were expressed in primary cultured neurons and assessed for their effects on activating and silencing neurons in multi-electrode arrays (MEAs) and in patch recordings. New luminopsin constructs had robust expression and showed efficient activation of opsins by increasing and decreasing spiking of cultured neurons.

Disclosures: J. Simkins: None. **N. Gorantla:** None. **E. Ikefuama:** None. **G.G. Lambert:** None. **M. Tree:** None. **K. Riselay:** None. **E.L. Crespo:** None. **M. Prakash:** None. **N.C. Shaner:** None. **U. Hochgeschwender:** None.

Poster

410. Novel Genetic Tools for Understanding Brain Function

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 410.25

Topic: B.04. Synaptic Transmission

Support: NIH Grant DA045284
Whitehall Foundation

Title: Decoding mesolimbic dopamine transmission across the olfactory tubercle and its role in reward and aversion through neurochemical sensing and chemogenetic techniques

Authors: *R. BHIMANI[1], C. E. BASS[2], J. PARK[3];
[1]Univ. At Buffalo, Buffalo, NY; [2]955 Main Street, Univ. At Buffalo SUNY, Buffalo, NY; [3]Univ. at Buffalo, buffalo, NY

Abstract: Increasing evidence has highlighted that the olfactory tubercle (OT), a limbic structure and part of the ventral striatum, distinctly processes sensory information related to reward and

aversion, which is encoded by the densest dopamine (DA) innervation in the brain coming from the ventral tegmental area (VTA). Furthermore, VTA-DA signaling across OT subregions (e.g. medial and lateral) is heterogeneously regulated and may play a critical role in the reinforcing effects of many drugs of abuse. The OT, only a few hundred microns thick in rodents, is positioned within close proximity to many other DA-rich brain areas (e.g. nucleus accumbens, caudate putamen), limiting the ability to selectively target as well as monitor DA transmission. Herein, we used a novel combination of cell type specific chemogenetics to restrict designer receptors exclusively activated by designer drugs (DREADDs) to VTA-DA neurons as well as *in vivo* fast-scan cyclic voltammetry at carbon-fiber microelectrodes to determine the effects of chemogenetic modulation of VTA-DA transmission in the OT of both anesthetized and awake-behaving rats. We further demonstrate how inhibition of VTA-DA neurons is correlated with methamphetamine-induced reward and its associated DA signaling in the OT. These results highlight that chemogenetic inhibition of VTA-DA neurons attenuates methamphetamine-induced DA transmission in the OT and its rewarding effects. These findings will provide a novel understanding of VTA-DA in the OT and how it is involved in processing rewarding and aversive stimuli.

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

Poster

411. Light and Electron Microscopy Techniques

Location: SDCC Halls B-H

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Program #/Poster #: 411.01

Topic: I.03. Anatomical Methods

Support: NRTS 2199 from the American Academy of Neurology
K08NS114165-01A1 from the National Institute of Neurological Disorders and Stroke

Title: Methods for high sensitivity intrinsic optical signal imaging through flexible, low cost adaptations of an upright microscope

Authors: B. CAMPOS, B. VASQUEZ, A. CAO, ***W. ZEIGER**;
Neurol., Univ. of California - Los Angeles, Los Angeles, CA

Abstract: Intrinsic optical signal imaging (IOSI) is a staple technique in modern neuroscience. Pioneered over thirty years ago, IOSI allows macroscopic mapping of neuronal activity throughout the cortex. The technique has been used to study sensory processing, experience-dependent plasticity and other fundamental processes of the brain. More practically, IOSI is often used as an adjunctive procedure to localize cortical areas for subsequent targeting by other imaging or physiology techniques, such as two-photon fluorescence microscopy or silicon probe recordings. Despite the ubiquity of IOSI in neuroscience there are few commercially available IOSI systems. As a result, investigators have typically resorted to building their own imaging

systems. Most designs still rely on a tandem-lens macroscope configuration coupled to a high-sensitivity CCD camera. Building such a custom IOSI imaging system from scratch presents challenges for investigators without significant experience in optics or microscopy design. Over the years, simplified systems built on existing microscope platforms have been proposed. Still, these often require additional custom-built hardware. Since IOSI relies on accurate measurement of tiny changes in light reflectance from the surface of the cortex, using custom-built systems and hardware can introduce problems with illumination or signal detection, further complicating do-it-yourself approaches. Here we present a straightforward set of adaptations that can be applied to any upright microscope, using readily available, inexpensive, commercial parts for illumination, optics, and signal detection, that enables high sensitivity IOSI. Using these adaptations, we are able to readily map sensory-evoked signals across the somatosensory and visual cortex, including single-whisker barrel cortical activity maps in mice. We show that these IOSI maps are highly reproducible across animals and can be used to study plasticity mechanisms in the somatosensory cortex. We also provide open-source applications to control illumination and analyze raw data to generate activity maps. We anticipate these resources will be particularly useful for neuroscience investigators from broad technical backgrounds looking to add IOSI capabilities to an existing microscope on a budget.

Disclosures: **B. Campos:** None. **B. Vasquez:** None. **A. Cao:** None. **W. Zeiger:** None.

Poster

411. Light and Electron Microscopy Techniques

Location: SDCC Halls B-H

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Program #/Poster #: 411.02

Topic: I.03. Anatomical Methods

Support: HFSP Fellowship LT000687/2021-L
SNF Grant 192617
SNF Grant 180316

Title: Mollusc-inspired multi-immersion microscope objectives for imaging cleared tissue

Authors: *F. F. VOIGT[1,2], A. REUSS[3], T. NAERT[4], S. HILDEBRAND[5], M. SCHAETTIN[6], A. HOTZ[6], A. BAHL[7], S. C. NEUHAUSS[6], A. ROEBROECK[8], E. STOECKLI[6], S. LIENKAMP[4], A. AGUZZI[9], F. HELMCHEN[10];
[1]Harvard Univ., Harvard Univ., Cambridge, MA; [2]Brain Res. Institute, Univ. of Zurich, Zurich, Switzerland; [3]Univ. Hosp. Zurich, Zurich, Switzerland; [4]Inst. of Anatomy, Univ. of Zurich, Zurich, Switzerland; [5]Maastricht Univ., Maastricht Univ., Maastricht, Netherlands; [6]Univ. of Zurich, Zurich, Switzerland; [7]Univ. of Konstanz, Konstanz, Germany; [8]Univ. Maastricht, Maastricht, Netherlands; [9]Inst. Neuropathol, Inst. Neuropathol, Zurich, Switzerland; [10]Univ. of Zurich, Brain Res. Inst. / Univ. of Zurich, Zurich, Switzerland

Abstract: In the 1960's, the physiologist M. F. Land discovered that the eye of the great scallop (*Pecten maximus*) has a very uncommon structure: It utilizes the combination of an aspherical lens and a spherical mirror for image formation. In astronomy, a similar optical system combining a large field of view (FOV) and excellent light collection power was invented in the 1930s by Bernhard Schmidt and is known today as the "Schmidt telescope".
When revisiting the underlying design principles, we noted that it is possible to use these ideas to design a novel class of cost-efficient microscope objectives that combine medium to high numerical aperture (NA), large FOV (>1 mm) and long working distance (>1 cm). Most importantly, such an objective can be made "immersion-independent": It delivers a diffraction-limited image both in air and in any homogeneous liquid immersion medium. This is of special interest for high-resolution imaging in cleared samples which typically requires highly expensive microscope objectives with complex optical design. Notably, despite their high cost, most commercial options with NA>0.6 only support a narrow range of immersion media.
In contrast, using our approach we were able to build a prototype multiphoton microscope objective that contains only two optical elements and achieves a NA of 0.69 in air (diffraction-limited FOV-diameter: 1.6 mm). In water, the higher index of refraction increases the NA to 0.92 (FOV: 1.4 mm) and in typical organic solvents such as ethyl cinnamate (ECI, n=1.56), the NA reaches 1.08 (FOV: 1.1 mm). In our design, the product NA*FOV is a constant and any increase in refractive index thus leads to a reduction in FOV. The working distance (11 mm) is independent of the medium.
We demonstrate the versatility of our microscope objective by imaging *Xenopus* tadpoles and chicken embryos cleared with BABB, mouse brains processed with iDISCO, and human cortex samples cleared with MASH and imaged in ECI. In addition, we showcase that the same objective can also be used for live imaging in aqueous media, for example to record brain activity in larval zebrafish using the calcium indicator GCaMP6s. We believe that our novel design approach forms a highly promising template for designing both cheaper microscope objectives for mass production and extremely capable next-generation "mesolenses" that allow high-NA imaging over cm-scale FOVs.

Disclosures: **F.F. Voigt:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); University of Zurich. **A. Reuss:** None. **T. Naert:** None. **S. Hildebrand:** None. **M. Schaettin:** None. **A. Hotz:** None. **A. Bahl:** None. **S.C. Neuhauss:** None. **A. Roebroeck:** None. **E. Stoeckli:** None. **S. Lienkamp:** None. **A. Aguzzi:** None. **F. Helmchen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); University of Zurich.

Poster

411. Light and Electron Microscopy Techniques

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Program #/Poster #: 411.03

Topic: I.03. Anatomical Methods

Support: NIH Grant 1RF1MH123402
NIH Grant 1RF1MH124611

Title: A parallelly distributed microscope and software system for scalable high-throughput multispectral 3D imaging

Authors: H. JIANG[1,2], L. WALKER[5], Y. LI[6], M. MCGLOTHLIN[6], M.-C. CHENG[6], M. CUI[3,2,4], ***D. CAI**[6,5,7];
[1]Sch. of Electrical and Computer Engineering, Bindley Biosci. Ctr., [2]Bindley Biosci. Ctr., [3]Sch. of Electrical and Computer Engin., [4]Dept. of Biol., Purdue Univ., West Lafayette, IN; [5]Biophysics, [6]Cell and Developmental Biol., [7]Michigan Neurosci. Inst., Univ. of Michigan, Ann Arbor, MI

Abstract: High throughput microscopes have recently been successful in achieving whole-organism-scale imaging. Current microscopes, however, require making compromises between achieving the optimal resolution, imaging depth, multispectral capability, and data throughput due to limitations in optical design and data stream handling. To bridge this gap, we created a parallel-line scanning confocal microscope (plSCM), which achieves 1.1 Gigavoxels/second throughput in 3 simultaneous spectral channels at a resolution of 220nm x 250nm x 600nm throughout millimeters of imaging depth. This speed is achieved by leveraging recent advances in CMOS camera technology to perform hundreds of thousands of confocal line scans each second. To handle such a massive imaging data stream, we have engineered a scalable network-distributed image acquisition framework (SNDiF), a software package which facilitates continuous capture, real-time processing, and cluster storage of petabyte-size single image datasets. With proper networking configuration, SNDiF can be deployed to allow direct data streaming and analysis in a supercomputer environment, offloading the cost and resource limitation of image processing. The applications of our parallelly distributed imaging system include, but not limited to high-speed dual channel neuron activity live imaging and super-resolution 3D imaging of over millimeter thick mouse brain sections. Putting together, our parallelly distributed microscope and software system presents a general solution for high throughput imaging.

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Poster

411. Light and Electron Microscopy Techniques

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Program #/Poster #: 411.04

Topic: I.03. Anatomical Methods

Support: EU H2020 grant agreement no. 945539 (Human Brain Project, SGA3)
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Italian Ministry of University and Research (MUR) in the framework of Eurobioimaging (ESFRI research infrastructure) - Advanced Light Microscopy Italian Node
The General Hospital Corporation Center of the National Institutes of Health (NIH) under award number 1U01MH117023-01

Title: High-throughput light-sheet microscopy imaging: a custom hardware setup and a software stack for large-scale brain tissue reconstruction

Authors: *G. MAZZAMUTO[1,2,3], L. SILVESTRI[1,2,3], G. SANCATALDO[2,5], V. GAVRYUSEV[2,3], M. SCARDIGLI[2,3], I. COSTANTINI[2,4], F. S. PAVONE[1,2,3];
[1]Natl. Inst. of Optics (INO), Natl. Res. Council (CNR), Sesto Fiorentino, Italy; [2]European Lab. for Non-Linear Spectroscopy (LENS), Sesto Fiorentino, Italy; [3]Dept. of Physics and Astronomy, Univ. of Florence, Sesto Fiorentino, Italy; [4]Dept. of Biol., Univ. of Florence, Florence, Italy; [5]Dept. of Physics and Chem. - Emilio Segrè, Univ. of Palermo, Palermo, Italy

Abstract: We describe the latest developments of our pipeline for high-throughput Light-Sheet Fluorescence Microscopy (LSFM) of large neural tissue samples. Our setups include a dual view, inverted, dual color LSFM for large tissue slabs (up to 0.5mm in thickness, several cm in lateral size) and a single view, dual color LSFM for whole organs (e.g. whole mouse brains). On the hardware side, the instruments feature a custom sample holder, an autofocus system and the capability of acquiring two channels simultaneously. On the processing side, we have developed a dedicated software stack to be able to tackle the challenges related to the management of large datasets (in the range of several terabytes each), from data acquisition in the lab to image post-processing. For dual-channel acquisition, the system must be able to sustain a data rate of 1GB/s. To this aim, we have developed an open source set of hardware libraries (QtLab) and a dedicated data acquisition software (SPIMlab), written in C++, that are able to control and synchronize multiple cameras and to automate the acquisition of big samples. Concerning image processing, among other tools we have developed an open source Python package for stitching TB-sized datasets named ZetaStitcher. Concerning data curation and dissemination, we describe our strategy for data management and sharing through public repositories such as DANDI and EBRAINS. We also demonstrate how this efficient pipeline allows us to acquire and reconstruct large datasets in the framework of several scientific collaborations, and showcase examples of our most recent acquisitions (e.g. large portions of the human Broca's area).

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Poster

411. Light and Electron Microscopy Techniques

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Program #/Poster #: 411.05

Topic: I.03. Anatomical Methods

Support: DFG Grant SFB1158

Title: In vivo two-photon imaging of the awake mouse brain using a novel, customizable, 3D-printed holding system

Authors: *M. WEINREICH[1], T. KUNER[2], J. KNABBE[2,3], H. MONYER[1];
[1]Clin. Neurobio., German Cancer Res. Ctr. and Univ. of Heidelberg, Heidelberg, Germany; [2]Inst. of Functional Neuroanatomy, Heidelberg, Germany; [3]Dept. of Gen. Psychiatry, Ctr. for Psychosocial Med. Univ. Hosp. Heidelberg, Heidelberg, Germany

Abstract: *In vivo* 2 photon imaging of the anesthetized and awake mouse brain has developed into a versatile tool for the analysis of single organelles up to large networks consisting of hundreds of neurons in the living brain. In commercially available head fixation systems, usually metal bars are glued to the mouse skull and clamped in a compatible imaging holder apparatus during the imaging process. Here, we present a novel lightweight and fully customizable head holding system consisting of a head stage and a running disc, which is easily produced at low cost using a commercial 3D printer. Additionally, we present a user-friendly software tool for adaption of the system to individual experimental needs. The head-fixation provides a remarkably high stability even during animal movement, which is partially superior to commercially available systems. Furthermore, the system offers the possibility to influence the running speed of the mouse and switch between resting, freely and externally induced moving states. Employing our newly developed system, we performed calcium imaging in the visual cortex of awake mice while presenting visual gratings during resting and moving states. We identified non-overlapping cell populations that were active either during stimulus presentation or movement of the mouse. In addition to functional imaging, our system allowed us to perform longitudinal high-resolution structural imaging of small-scale structures in the awake mouse brain. We imaged EGFP-tagged Connexin36 (Cx36-EGFP) expressing mice across several hours as well as over the time course of several months. Notably, we observed a striking positional stability of Cx36-EGFP punctae over the whole time-course of the experiments. Furthermore, 2-photon fluorescence recovery after photobleaching (2P-FRAP) of individual punctae revealed an estimated turnover half-life of 3-6 weeks. In summary, we present a novel, highly stable head-fixation and running system for brain imaging of awake mice that enabled us to analyse differences in neuronal activity between resting and moving states and investigate the stability and plasticity of electrical synapses in the mammalian brain for the first time.

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Poster

411. Light and Electron Microscopy Techniques

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Program #/Poster #: 411.06

Topic: I.03. Anatomical Methods

Support: NRF grant 2022R1A2C3007818

Title: Quantitative and volumetric analysis of mouse hippocampal region using optical coherence microscopy and tissue clearing technique

Authors: *M. KIM[1], E. LEE[1], S. LEE[1], K. PARK[1], Y. AHN[1], E. MIN[2];
[1]UNIST, Ulsan, Korea, Republic of; [2]Max Planck Inst. for Biol. Cybernetics, Tubingen, Germany

Abstract: The hippocampus is a complex structure of the limbic system, which plays a vital role in regulating learning and memory encoding, emotion, and spatial navigation. The observation of the architecture of the hippocampus is particularly important because its morphological abnormalities are directly associated with a number of neurological illnesses such as Alzheimer's disease, epilepsy, and schizophrenia. Therefore, the volumetric quantification of the hippocampal region in mouse is required not only for understanding the brain structure but also for identifying various neurological diseases. Recently, various optical imaging methods have been introduced to create volumetric anatomy data of ex vivo tissues using physical tissue sectioning or optical clearing. Even though these new approaches present the distinguished volumetric anatomy on various scales, they are still not suitable for use in statistical studies with multiple brains.Here, we introduce a novel label-free and quantitative imaging modality based on serial optical coherence microscopy (OCM) and tissue clearing technique. We developed the home-built spectral-domain OCM system using 840 nm light source, and its axial and lateral resolution has shown as 1.3 μm and 5.2 μm, respectively. For imaging the entire hippocampal region, we conducted the serial block-face imaging technique, which repeatedly cut out and images the brain tissue, which is cleared by OptiMuS clearing solution for deeper imaging. OptiMuS is an aqueous-based single-step solution with fast clearing and high retention ability in volume. After acquiring a high-resolution brain image, we manually segmented cornu ammonis (CA) and dentate gyrus (DG) regions based on the Allen brain atlas map using AMIRA software. In our preliminary experiment, we were successfully able to obtain 3D hippocampal images within ten sliced tissues without any labeling or contrast agents. Our results also showed that the new imaging platform could accurately measure regional volumes such as CA and DG while providing statistical information in multiple hippocampal tissues. Our technique would be widely utilized in various neuronal studies, including the effects of environmental enrichment.

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Poster

411. Light and Electron Microscopy Techniques

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Program #/Poster #: 411.07

Topic: I.03. Anatomical Methods

Support: NRF Grant 2021R1A4A1031644

Title: Comprehensive visualization of the mouse spinal cord using serial optical coherence microscopy and deep learning techniques

Authors: *S. LEE, E. LEE, H. YANG, Y. AHN, K. PARK, W. JUNG;
Biomed. Engin., Ulsan Natl. Inst. of Sci. and Technol., Ulsan, Korea, Republic of

Abstract: The morphological observation of the central nervous system (CNS) of mouse models is crucial for identifying the function of the CNS and understanding the neuronal diseases. Although the spinal cord (SC) is the major communication pathway between the brain and the peripheral nervous system, there is lack of effort to visualize and quantify the SC compared to actively investigated the case of the brain. There are also inherent challenges for optical imaging modalities to monitor the volumetric SC, because its long and tubular structure as well as the location surrounded by three layers of meninges. In addition, white matter consists of highly scattered lipids which restrict the light penetration. Therefore, new imaging modality and protocols are essentially required to provide comprehensive visualization of SC. In this study, we suggest a novel imaging approach which enables us to offer high throughput quantitative information as well as native 3D context. Our technique is based on optical coherence microscopy (OCM) and deep learning techniques. OCM utilizes the endogenous back-scattering signal, and it does not require chemical labeling, staining, or external contrast agents. OCM is also suitable for deep tissue imaging because OCM uses near-infrared light that reaches deeper into biological tissues than visible light can reach. In order to collect volumetric and informative anatomy, we built a unique imaging protocol with four steps. First, SC was embedded in agarose gel, sequentially sectioned with 50μm thickness using a vibratome, and imaged by OCM. Second, the process of imaging and sectioning is repeated until the thoracic part of the spinal cord image set is completed. It was then trained by a convolutional neural network (CNN) which is targeted to distinguish the region of the gray and white matters in SC. The performance of the trained network was evaluated and adjusted at volumetric data which was not included in the training dataset. Finally, the virtual segmentation algorithm was completed which has the capability to connect and visualize the connectivity of long SC. In preliminary research, a large field of SC anatomy was successfully constructed through our new imaging approach while delineating a detailed spinal nerve. We also quantitatively concluded the distribution of white and gray matters of the SC in a lengthy direction. Based on experimental results, we confirmed that the recovery process after partial spinal cord injury or multiple sclerosis disability could be monitored with comprehensive means.

Disclosures: **S. Lee:** None. **E. Lee:** None. **H. Yang:** None. **Y. Ahn:** None. **K. Park:** None. **W. Jung:** None.

Poster

411. Light and Electron Microscopy Techniques

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 411.08

Topic: I.03. Anatomical Methods

Support: NIMH grant MH108053-01

Title: Registering experimental data from serial section reconstructions and cleared brains to standardized mouse and rat reference atlases

Authors: *N. O'CONNOR[1], B. S. EASTWOOD[1], P. J. ANGSTMAN[1], A. D. LEDUC[1], N. D. LIESE[1], C. S. GERFEN[1], C. R. GERFEN[2], J. R. GLASER[1];
[1]MBF Biosci., Williston, VT; [2]LNP NIMH, Bethesda, MD

Abstract: Molecular neuroanatomical methods have expanded the ability to map connections activity of neuron subtypes and circuits in behavioral and pathologic models. Analyses of connectivity and neuronal activity across whole mouse and rat brains registered to a reference atlas reveal details about the functional organization of brain circuits related to behavior and pathologies that are comparable across animals, experiments, and laboratories. Here we present recent advances to our work in semi-automatically aligning and reconstructing images of mouse brain sections labeled with histochemical techniques into whole-brain 3D image volumes, subsequently registering those brain image volumes to the the Allen Mouse Brain Common Coordinate Framework (CCF), and then classifying cellular populations within brain regions using these registration results.Specifically, we have extended these methods to register sections with positional accuracy within the CCF. This advancement results in a compiled section brain volume that is closer in shape to the atlas model brain. We have also extended these tools for registering intact brain volumes imaged via light sheet microscopy, rather than physical sections. Intact mouse brain volumes imaged from cleared brain tissues by light sheet microscopy methods can be registered to the Allen CCF using linear and nonlinear registration methods.Additionally, we have created an open specification for including digital atlases in our NeuroInfo registration technologies. This specification is documented and available online. The specification was used to include the Waxholm Rat Brain Atlas in NeuroInfo, enabling the registration of rat brain volumes to a standardized reference space.We present whole brain analysis of connectivity using trans-synaptic rabies labeling of neurons. For analysis, coronal brain sections were reconstructed into a 3D volume, labeled neurons were automatically marked using a neural network, and brain sections and detected neurons were registered to the CCF. The number of labeled neurons in each of the 2500 brain structures in the CCF were calculated, allowing for comparative quantitative analysis between mice. Similar results are presented for rat brain volumes expressing cFos. We also present the results of registering mouse brain volumes from light sheet imaging to the CCF.

Disclosures: **N. O'Connor:** A. Employment/Salary (full or part-time):; MBF Bioscience. **B.S. Eastwood:** A. Employment/Salary (full or part-time):; MBF Bioscience. **P.J. Angstman:** A. Employment/Salary (full or part-time):; MBF Bioscience. **A.D. LeDuc:** A. Employment/Salary (full or part-time):; MBF Bioscience. **N.D. Liese:** A. Employment/Salary (full or part-time):; MBF Bioscience. **C.S. Gerfen:** A. Employment/Salary (full or part-time):; MBF Bioscience. **C.R. Gerfen:** None. **J.R. Glaser:** A. Employment/Salary (full or part-time):; MBF Bioscience.

Poster

411. Light and Electron Microscopy Techniques

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Program #/Poster #: 411.09

Topic: I.03. Anatomical Methods

Support: NRF grant 2022R1A2C3007818

Title: Volumetric digital histopathology of brain tissue using quantitative phase imaging and clearing technique using quantitative phase imaging and clearing technique

Authors: *M. CHOI, E. LEE, I. PARK, N. AIMAKOV, M. KIM, W. JUNG;
Ulsan Natl. Inst. of Sci. and Technol., Ulsan, Korea, Republic of

Abstract: The histological optical imaging is a gold standard method to observe the biological tissues, which follows routine process such as dissection, embedding, sectioning, staining, visualization and interpretation of specimens. This technique has a long history of development, and is used ubiquitously in pathology, despite being highly time and labour-intensive. Advanced optical imaging techniques developed over the last decade have enabled to provide high sensitivity, high resolution and non-invasive biological information. In particular, new optical imaging contrast rather than chemical staining has been presented to be utilized in histopathology while showing the strong potential. However, acquiring fast, high throughput, large volume tissue anatomy remains a difficult challenge. In this study, we propose novel protocol using staining-free digital histopathology combined with quantitative phase imaging (QPI) and tissue clearing. QPI has been evaluated to have outstanding performance to image transparent cells and tissue without staining. It reconstructs phase distribution of specimens using asymmetric illumination and regularized deconvolution method. Typically QPI has been utilized to image brain tissue within 20 micrometers, but it could be enhanced when it is combined with clearing technique. In order extend the imaging depth, we utilized near infrared QPI and OptiMuS solution which is uniquely developed to minimize size deformation and fast clearing. In validation work, one drop of OptiMuS solution was treated on the mouse brain sample slide glass and it made the tissue transparent in a few seconds. Whole process of clearing was monitored every 5 seconds followed by time series QPI images. Through preliminary experiment, we confirmed that our approach shows that the morphology of brain tissue between QPI and the stained histological section has a strong correlation. QPI images have even better contrast for the fiber bundles in the region of the corpus callosum (cc), caudoputamen (CP), and cerebral peduncle (cpd) due to phase and light scattering. QPI imaging with cleared brain presented 3D myelin fibers over 50 micrometer while identifying the distribution and orientation of each fiber without a contrast agent or any labeling process. Our system has been utilized to reconstruct single brain slices, but can be applied to multiple slices for the volumetric visualization of the brain. Thus, a new platform of QPI and clearing method is a very promising tool for use in neuroscience research, and is particularly well suited for systematic studies of brain anatomy for the understanding of mouse models of various cognitive disorders.

Disclosures: **M. Choi:** None. **E. Lee:** None. **I. Park:** None. **N. Aimakov:** None. **M. Kim:** None. **W. Jung:** None.

Poster

411. Light and Electron Microscopy Techniques

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Program #/Poster #: 411.10

Topic: I.03. Anatomical Methods

Title: Investigation of oral-brain neuro-axis by development of the optimized tissue clearing protocol in jaw bones

Authors: ***Y. YUN**[1], S. KANG[2], J.-Y. PARK[2], H. CHOI[1];
[1]Dept. of Anat. and Cell Biol., Seoul Natl. Univ. Col. of Med., Seoul, Korea, Republic of; [2]Dept. of Oral and Maxillofacial Surgery, Seoul Natl. Univ. Sch. of Dent., Seoul, Korea, Republic of

Abstract: With the growing demand for dental work such as tooth extraction and implant surgery, trigeminal nerve injuries are increasingly common. Although conventional neurohistological analysis was attempted to provide microscopic images of the nerves in dental pulp, three-dimensional (3D) visualization of the whole maxilla and mandible in an intact tooth has remained a technically challenging task. In particular, the neural structure of the peripheral nerve branch that passes through the alveolar bone where the teeth are planted is not known. In this work, we established a simple and rapid method that combines decalcification, tissue clearing, immunohistochemistry, confocal microscopy, light-sheet fluorescence microscopy, and quantitative analysis of full-thickness bowel for 3D imaging at high resolutions of the maxilla and mandible in mice. Three-dimensional image reconstruction and statistical methods were used to describe the neuronal network and provide novel insights into neuronal morphology. The results in the present study confirmed the fact that the time required for the complete decalcification process was at least 40% EDTA for 2 weeks. The overall structure details, as well as staining characteristics, were optimal after electrophoretic tissue clearing mode for 16 hours and then further immersed in SDS buffer at 60℃ for 14 hours. We achieved 3D macroscale-level visualization of the mouse mandible and maxilla, and visualized peripheral nerve branch that passes through the alveolar bone in the whole dental pulp. Full-thickness mandible images, viewed with a 10x objective lens, were as large as 8 x 3 x 1 mm^3. Quantitative data for maxilla and mandible showed relatively different aspects. In conclusion, the established methods could provide a comprehensive 3D visualization of nerve fibers of soft tissues surrounded by hard tissues. This method could be used to enable diverse research methods on neural-immune interaction by providing 3D visualization of various immune cells in intact mouse maxilla and mandible.

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Poster

411. Light and Electron Microscopy Techniques

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Topic: I.03. Anatomical Methods

Support: NIH Grant U01 MH117023
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EuroBioImaging Italian Infrastructure
Fondazione CR Firenze

Title: 3d molecular phenotyping of human brain samples with light-sheet fluorescence microscopy

Authors: L. PESCE[1], M. SCARDIGLI[2], V. GAVRYUSEV[3], A. LAURINO[5], G. MAZZAMUTO[6], N. BRADY[2], G. SANCATALDO[7], ***L. SILVESTRI**[8], C. DESTRIEUX[9], P. R. HOF[10], I. COSTANTINI[3], F. S. PAVONE[4];
[1]Scuola Normale Superiore, Pisa, Italy; [2]European Lab. for Non-linear Spectroscopy, Florence, Italy; [3]Univ. of Florence, Florence, Italy; [4]Univ. of Florence, Sesto Fiorentino (FI), Italy; [5]Univ. of Siena, Siena, Italy; [6]INO - CNR, LENS, INO - CNR, LENS, Sesto Fiorentino, Italy; [7]Univ. of Palermo, Palermo, Italy; [8]European Lab. For Non-Linear Spectroscopy, European Lab. For Non-Linear Spectroscopy, Sesto Fiorentino, Italy; [9]Univ. of Tours, Tours, France; [10]Icahn Sch. of Med. At Mount Sinai, New York, NY

Abstract: The combination of optical tissue transparency with immunofluorescence allows the mole- cular characterization of biological tissues in 3D. However, adult human organs are particularly challenging to become transparent because of the autofluorescence contributions of aged tissues. To meet this challenge, we optimized SHORT (SWITCH—H2O2—antigen Retrieval—TDE), a procedure based on standard histological treatments in combination with a refined clearing procedure to clear and label portions of the human brain. 3D histological characterization with multiple molecules is performed on cleared samples with a combination of multi-colors and multi-rounds labeling. By performing fast 3D imaging of the samples with a custom-made inverted light-sheet fluorescence microscope (LSFM), we reveal fine details of intact human brain slabs at subcellular resolution. Overall, we proposed a scalable and versatile technology that in combination with LSFM allows mapping the cellular and mole- cular architecture of the human brain, paving the way to reconstruct the entire organ.

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Poster

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Program #/Poster #: 411.12

Topic: I.03. Anatomical Methods

Support: U01MH114824
U19 MH114821

Title: High-throughput airy beam light-sheet tomography of brain-wide imaging at single-cell resolution

Authors: *X. QI[1], Z. WU[2], A. NARASIMHAN[1], R. MUÑOZ-CASTAÑEDA[1], J. PALMER[1], C. ELOWSKY[1], R. DREWES[1], J. MIZRACHI[1], P. OSTEN[1];
[1]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [2]Cell, Developmental & Regenerative Biol., Icahn SOM, Mount Sinai, New York, NY

Abstract: Fluorescence light-sheet microscopy has become popular for studying large cleared biological samples with cellular resolution. However, it's still lacking methods capable of handling centimeter-sized cleared samples with subcellular resolution in three-dimensional with high throughput. Here, we describe an automated method, combined with a new optical sectioning and mechanical sectioning, named confocal airy beam light-sheet tomography. It employs a confocal airy beam scanned light-sheet replacing the non-confocal Gaussian beam to extend the depth of field to generate a uniform and low noise light-sheet. We demonstrated whole mouse brain imaging with 0.26 μm× 0.26 μm× 1 μm within 58 hours. This method provides high-throughput automated whole-brain imaging at single-cell resolution.

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Poster

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Topic: I.03. Anatomical Methods

Support: NIH Grant 2R01MH113257-06

Title: Assessment of rhesus macaque primary visual area borders using 26 markers currently available in MacBrain Resource

Authors: *M. ELLIOTT, K. WORTHINGTON, L. GREENE, F. KAMARA, T. SPADORY, P. RAKIC, A. DUQUE;
Yale Univ., New Haven, CT

Abstract: Cytoarchitectonic regional and areal differences of cortical organization were well defined by Brodmann (1909) and continue to be refined by modern techniques. Precise brain mapping is necessary for proper interpretation of functional imaging, comparison of normal physiological and lesion data, guide for specific pharmacological targets, and other venues that pave the way to understanding the relationship between brain neuronal networks and mental processes. The goal of the present study was to assess the degree of difficulty in distinguishing the border between visual areas V1 and V2 of the macaque monkey, at the level of calcarine sulcus, by using publicly available zoomable images of coronal sections histo- and immuno-histochemically stained for 26 different markers (https://medicine.yale.edu/neuroscience/macbrain/collections/). A scale from 1 to 5 was devised to grade observations (1: not visible to 5: very clear). The observations were carried out by independent observers in 9 brains of different ages. Not all markers were available in all brains. All sections were cut in a freezing microtome at 50 μm, and sections within each series were 1mm apart. The markers were: serotonin (5-HT), acetylcholinesterase (AChE), calbindin (CB), cholecystokinin (CCK), choline acetyltransferase (ChAT), calretinin (CR), gamma-aminobutyric acid (GABA), GABA receptor b (GABA-B), ionized Ca^{2+} binding adaptor 1 (Iba1), latexin (LTX), myelin basic protein (MBP), neuronal nuclear antigen (NeuN), neurogranin (NRGN), Nissl substance (Nissl), nitric oxide synthase (NOS), neuropeptide Y (NPY), nuclear receptor related 1 (Nurr1), oligodendrocyte transcription factor (Olig2), parvalbumin (PV), S100 protein (S100), special AT-rich sequence-binding protein 2 (SATB2), neurofilament protein (SMI-32), somatostatin (SOM), substance P (SP), T-box brain 1 transcription factor (TBR1), and tyrosine hydroxylase (TH). Our results indicate that most neuronal markers, within a gradient, are useful border indicators, but their intensity, in some cases, may be age dependent (e.g., for TH and SMI-32); beyond typical Nissl, strongly stained borders appeared under 5-HT, AChE, MBP, and PV; glia stains for Olig2 and Iba1 are not good border indicators. These results expand on V1/V2 classical border characterization and corroborate the usefulness of MacBrain Resource high quality data sets, which by virtue of providing serially stained sections spanning the whole rostro-caudal extent, provide internal controls and aid in understanding biochemical differences between and among cortical and subcortical regions at different ages.

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Poster

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Support: MOEA Grant 111-EC-17-A-19-S6-009
MOST Grant 110-2221-E-002-048-MY3
MOST Grant 108-2321-B-002-001

Title: Realtime and non-invasive biopsy of intra-epidermal free nerve endings by a novel multiple modality nonlinear microscopy: A translational approach from mouse to human

Authors: P.-J. WU[1], S.-T. HSIEH[2], C.-C. CHAO[3], Y.-H. LIAO[3], C.-T. YEN[1], W.-Y. LIN[3], W.-Z. SUN[3], ***C.-K. SUN**[1];
[1]Natl. Taiwan Univ., Taipei, Taiwan; [2]Natl. Taiwan Univ. Med. Coll, Natl. Taiwan Univ. Med. Coll, Taipei, Taiwan; [3]Natl. Taiwan Univ. Hosp., Taipei, Taiwan

Abstract: Diabetic peripheral neuropathy (DPN) is a frequent complication of diabetes. Biopsy is the only way to provide free intraepidermal nerve ending (FINE) structure information and is critical for differential diagnosis of PN, which is with a wide array of etiologies. However, its invasive nature is unfavorable for patients with diabetic coagulation abnormalities. Moreover, the limitation of current *in vivo* optical imaging tools requires a clear contrast through myelin sheath and large enough fiber diameters. Thus, no available technology can replace skin biopsy in quantifying thin
unmyelinated fibers of FINE ranging from 0.2-1μm. To observe unmyelinated FINE *in vivo*, we develop the multi-modality microscopy which combined the third harmonic generation (THG) and two photon fluorescence (2PF) channels. To attest its clinical capability, we injected nerve-specific stain, methylene blue (MB), intradermally to produce the far red emission with a two photon excitation at 1260 nm. We further combined this 2PF of MB with epi THG detection at 420 nm, to compensate the injection and temporal dependent MB emission. To confirm if the fiber like signals were generated from nerves, in wild type mice (N=5), we first injected MB into the mice toe tips and *in vivo* studied the correlation between the 2PF and THG signals. Studies were approved by IACUC, NTU. Submicron resolution 3D slide-free imaging of unmyelinated FINE can be observed, further assisted by the comparison with the transgenetic mice model and a longitudinal spared nerve injury model study. Moreover, 3 volunteers were recruited to test if the proposed method can be further translated to *in vivo* slide-free human FINE 3D imaging. The clinical study protocol was approved by NTUH IRB, 201904083MINA. This clinical study not only confirmed the capability of the proposed methodology, but further suggested that that unstained THG alone, through specific imaging processing, can provide the FINE structure contrast in human epidermis for DPN diagnosis. The strong MB 2PF provides sharp dermal nerve fiber contrast even in the deep layers. Our data not only supports the fact that the combined THG/2PF approach could allow a complete picture of small nerve fibers in the human skin, while the label-free THG channel is capable to provide the unmyelinated FINE imaging. This novel imaging system clearly provide a real time and non-invasive tool to quantify FINE structures in clinical settings. The serial and follow-up morphological evidence over the same lesion site can powerfully expand the limitation of conventional nerve conduction test in both diagnosis and treatment of peripheral nerve dysfunction.

Disclosures: **P. Wu:** A. Employment/Salary (full or part-time):; NTU/Full. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; MOEA/MOST. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent inventor, stock owner. Other; holds an equity position in a company that produces a product or service related to the work being reported. **S. Hsieh:** A. Employment/Salary (full or part-time):; NTU/full; NTUH/part-time. **C. Chao:** A. Employment/Salary (full or part-time):;

NTU/full; NTUH/part-time. **Y. Liao:** A. Employment/Salary (full or part-time):; NTU/full; NTUH/part-time. **C. Yen:** 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.; MOST. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent inventor. **W. Lin:** A. Employment/Salary (full or part-time):; NTUH/full. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent inventor. **W. Sun:** A. Employment/Salary (full or part-time):; NTU/full; NTUH/part-time. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent inventor. **C. Sun:** A. Employment/Salary (full or part-time):; NTU/full. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; MOEA/MOST. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent inventor/Stock Owner. Other; holds an equity position in a company that produces a product or service related to the work being reported.

Poster

411. Light and Electron Microscopy Techniques

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 411.15

Topic: I.03. Anatomical Methods

Support: NIH/SPARC OT2OD026585
Dr. Miriam and Sheldon G. Adelson Medical Research Foundation

Title: Myelinated Preganglionic Parasympathetic Fibers and Unmyelinated Candidate Autonomic Fibers Cluster Together in Lumbosacral Ventral Roots of Rhesus Macaques

Authors: *N. P. BISCOLA[1], P. M. BARTMEYER[1], G. B. CHIAROTTO[2], Y. SIM[3], E. CELIK[1], L. A. HAVTON[4,5];
[1]Icahn Sch. of Med. at Mount Sinai, New York, NY; [2]Neurol., Icahn Sch. of Med. at Mount Sinai, NEW YORK, NY; [3]Neurol., [4]Dept. of Neurol. and Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY; [5]James J. Peters Veterans Affairs Med. Ctr., Bronx, NY

Abstract: In the conus medullaris (CM) of mammals, the sacral parasympathetic nucleus (SPN) provides preganglionic autonomic fibers to pelvic ganglia for autonomic control of bladder, bowel, and sexual function. The lumbosacral (LS) ventral roots (VRs) and spinal nerves are attractive and emerging targets for electrical stimulation to modulate autonomic function in a variety of neurological conditions, including spinal cord injury. One limitation for translational studies on neuromodulation is extensive inter-individual variability in the rostro-caudal distribution of autonomic fibers between LS VRs and lack of information on the spatial

distribution of different fiber types within individual VRs. We performed a light microscopy (LM) and transmission electron microscopy (TEM) study of plastic resin-embedded L6-S3 VRs in female rhesus macaques (n=6). Myelinated fibers were segmented and preganglionic parasympathetic fibers (PPFs) were demonstrated within the L7-S2 VRs with extensive variation with regards to the most dominant segmental level for PPFs between animals. Using a new non-binary approach for determining the 2-dimensional distribution of myelinated nerve fibers within the L6-S3 VRs, a markedly higher degree of fiber clustering was detected in VRs with a high proportion of PPFs. For individual sacral roots (S1-S3), a positive correlation appears for the number of small myelinated axons within the PPF size range and the number of unmyelinated axons. Two size ranges of unmyelinated fibers were detected in S1-S3 VRs. A small proportion of very small unmyelinated fibers were detected across all VRs. In addition, a population of unmyelinated fibers with a larger diameter was detected in VRs with a prominent proportion of myelinated PPFs. Unmyelinated fibers also tended to cluster with small myelinated fibers in the PPF size range. We conclude that PPFs include both myelinated and unmyelinated fibers, which form clusters within individual VRs. Neuromodulation strategies that target the CM and lumbosacral VRs in primates need to take into consideration an extensive variability in the rostro-caudal distribution of efferent PPF, patterns of PPF clustering within individual VRs, and the lack of myelination in a large subset of PPFs.

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Poster

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Topic: I.03. Anatomical Methods

Title: Investigation of function and structure of Corpus Callosum following a CRMP2 knockout in a Schizophrenia mouse model

Authors: *K. GRYCEL[1], J. MIDTGAARD[2], Z. XU[3], S. HASSELHOLT[1], J. R. NYENGAARD[1,4];
[1]Dept. of Clin. Med., Aarhus Univ., Aarhus N, Denmark; [2]Dept. of Neurosci., Univ. of Copenhagen, Copenhagen, Denmark; [3]Inst. of Genet. and Developmental Biol., Beijing, China; [4]Dept. of Pathology, Aarhus Univ. Hosp., Aarhus, Denmark

Abstract: Myelination is an essential process to keep signal transduction and communication within the brain at an optimal level. There is evidence for impaired myelination in several neuropsychiatric disorders including Schizophrenia (SZ) (Ohtani et al., 2015). Collapsin Response Mediator Protein 2 (CRMP2) is one of the SZ risk-genes (Hensley et al., 2011). This project focuses on Central Nervous System demyelination as a component of SZ. We used a CRMP2 conditional knock out mouse (cKO) model to investigate myelin of the largest white

matter structure, Corpus Callosum (CC). We looked at the function, structure and ultrastructure to elucidate which myelin parameters may be mostly affected. We investigated **function** of CC using multielectrode electrophysiology of compound action potentials(CAPs) (*n* = 4-9/group, P60-P90) in acute brain slices. Light microscopy was used to investigate **structure**, we measured the volume of CC and estimated the total number of oligodendrocytes (*n* = 6-7/group, P60-P90) with stereological methods. We examined the **ultrastructure** with 3D axonal and myelin reconstructions of image stacks acquired by Serial Block Face Scanning Electron Microscopy (SBEM),implementing an image-segmentation pipeline. Several parameters of Nodes of Ranvier (NR) (volume, length, diameter, axon diameter and myelin thickness) were manually quantified (*n* = 2/group, P60-P90). We saw indication of altered CAP signals and a reduction in CC volume, but not in the number of oligodendrocytes. Ultrastructural results suggest changes in NR structure in CRMP2-cKO animals. Morphometric analysis of full SBEM volumes are ongoing. Together our findings indicate that CRMP2 plays a role in white matter function in a SZ model. Both the function and structure of CC have been affected by the lack of CRMP2. Our project has implemented analysis methods to shed light on the role of myelin defects in SZ. The analytic pipelines can be used in future work on genetic models of neuropsychiatric disorders.
Hensley, K., Venkova, K., Christov, A., Gunning, W., & Park, J. (2011). Collapsin Response Mediator Protein-2 : An Emerging Pathologic Feature and Therapeutic Target for Neurodisease Indications. *MolecularNeurobiology, 43,* 180-191. https://doi.org/10.1007/s12035-011-8166-4Ohtani, T., Bouix, S., Lyall, A. E., Hosokawa, T., Saito, Y., Melonakos, E., Westin, C. F., Seidman, L. J.,Goldstein, J., Mesholam-Gately, R., Petryshen, T., Wojcik, J., & Kubicki, M. (2015). Abnormal whitematter connections between medial frontal regions predict symptoms in patients with first episodeschizophrenia. *Cortex*, *71*, 264-276. https://doi.org/10.1016/j.cortex.2015.05.028

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Poster

411. Light and Electron Microscopy Techniques

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 411.17

Topic: I.03. Anatomical Methods

Support: Wellcome Trust

Title: Morphometrics of the pyramidal tract axons in human and non-human primates

Authors: L. L. L. LASANUDIN, C. MCCARDLE, M. A. SAVAGE, ***A. KRASKOV**; Biosci. Inst., Newcastle Univ., Newcastle, United Kingdom

Abstract: The corticospinal tract (CST) is the major descending pathway. The CST is known to accomplish a number of different functions and this is probably reflected in the wide range of

fibre diameters, demonstrated by anatomical studies. While these studies have emphasized the huge preponderance of fine, myelinated axons with diameters of 0.5-3 µm, electrophysiological recordings are dominated by pyramidal tract neurons with big, fast conducting axons. These large diameter axons (>3µm) are not well sampled anatomically probably due to their spatial sparsity compared to the small diameter axons.
The present study focuses on evaluating the deep learning technique AxonDeepSeg (ADS)[1] for both automatic segmentation and morphometrics estimation of microscopy images of CST axons using a 3D convolutional neural net (CNN)[2]. This approach allows to generate larger datasets in a shorter amount of time compared to manual analysis.
We applied this technique (a) to previously acquired nonhuman primate (NHP) light microscopy (LM) images of medullary pyramid[3] and (b) to newly acquired human data of medullary pyramid using a modified protocol from the standard tissue preparation.
A CNN was trained on manually segmented images denoting axon and myelin regions of interest (n=7). Axon diameter was computed using the ellipse minor axis, while myelin thickness was obtained from half of the difference between the total fibre diameter and axon diameter. Axons touching the image borders were excluded.
Using ADS on the of NHP images (n=107) yielded a cohort of more than 130,000 axons compared with 1,600 in original study[3]. Although ADS under sampled small diameter axons, it uncovered far more large diameter axons (>3µm), 5141 vs 58 in original study.
The current model's underestimation of the small diameter axons from NHP LM images can potentially be improved by a better training set. Overestimation of the myelin thickness is partially caused by a separation of the myelin sheaths due to preparation of the tissue for LM and EM images and might require a different CNN with more classes.
The standard methods for preserving and storing human brain tissue (fresh freezing or formalin-fixed paraffin-embedded) did not maintain intact axon morphology suitable for automatic segmentation and morphometry analysis. The preliminary electron microscopy (TEM) imaging of human medullary pyramid tissue specifically prepared for EM from fresh tissue showed intact axon morphology suitable for automatic segmentation and morphometry.
Bibliography
1.Zaimi *et al. Sci. Rep.* (2018).
2.Gros *et al. JOSS* (2021).
3.Firmin *J. Neurophysiol.* (2014).

Disclosures: L.L.L. Lasanudin: None. **C. McCardle:** None. **M.A. Savage:** None. **A. Kraskov:** None.

Poster

411. Light and Electron Microscopy Techniques

Location: SDCC Halls B-H

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Program #/Poster #: 411.18

Topic: I.03. Anatomical Methods

Support: NIH/NIA R01AG043640
NIH/NIA R01AG075727
NIH/NIA R56NS112207
NIH/NIA R21NS102991

Title: Using quantitative birefringence microscopy to identify myelin defects in the aging primate brain

Authors: *R. E. ROBINSON[1], N. BLANKE[2], A. GRAY[2], F. MORTAZAVI[4], I. J. BIGIO[3], D. L. ROSENE[1];
[1]Anat. & Neurobio., [2]Biomed. Engin., [3]Biomed. Engineering, Electrical & Computer Engin., Boston Univ., Boston, MA; [4]Anat. and Neurobio., Sch. of Med., Boston, MA

Abstract: In normal aging, myelin's structural integrity is compromised, the axon is less able to efficiently transmit action potentials, and conduction may fail. With loss of conduction in the monkey cerebral cortex, cognition is impaired. Quantifying the deterioration of myelin across the lifespan and understanding the conditions that cause it to degrade are key to understanding the mechanisms of cognitive decline seen in normal aging. Quantitative birefringence microscopy (qBRM) is a technique that exploits the birefringent nature of myelin with minimal manipulation of fixed tissue sections. The images produced render myelin bright against a darkfield background with few-to-no additional visible structures. Additionally, the qBRM technique is highly amenable to being scaled up and automated for detailed analysis of myelin status at high resolution and across significant volumes of brain tissue.
To identify both normal and degraded myelin with qBRM, standard neurohistological methods that demonstrate myelin must be optimized for compatibility. This is especially important for immunohistochemical (IHC) labeling of myelin. Here we report our validation studies conducted using fixed, frozen 15µm-thick sections of brain tissue from aged rhesus monkeys to resolve these issues. The first step was to optimize the mounting of tissue sections on microscope slides to minimize myelin degradation and to match the refractive index (RI) of myelin lipids for reduction of scattering. These mounted sections were covered with 85% glycerol and are imaged in their entirety with qBRM at low resolution, and then at high resolution in predetermined regions of interest, including the corpus callosum. The coverslips were removed, and the tissue was then subjected to multiple established histological procedures to label myelin, including staining with Fluoromyelin, and IHC for myelin basic protein (MBP) and damaged MBP (dMBP). This allows our qBRM images of myelin to be imaged afterwards with confocal epifluorescence. Images from qBRM, Fluoromyelin, and IHC were aligned and compared to identify myelin degradation. This is a first step to developing a standardized protocol for cross-examining potential myelin defects that have been identified with qBRM. Once fully validated, this approach will be extremely useful for identifying myelin defects with high accuracy and precision, allowing for quick, inexpensive, non-damaging, and reliable results. The caveat is that thicker (e.g., 30µm) sections do not allow accurate qBRM identification of myelin defects in white matter.

Disclosures: R.E. Robinson: None. **N. Blanke:** None. **A. Gray:** None. **F. Mortazavi:** None. **I.J. Bigio:** None. **D.L. Rosene:** None.

Poster

411. Light and Electron Microscopy Techniques

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 411.19

Topic: I.03. Anatomical Methods

Title: Viral strategies for longitudinal in vivo spine imaging in genetic mouse models

Authors: *S. BENEZRA, A. GHOSHAL;
Biogen, Cambridge, MA

Abstract: Synapse dysfunction is a core phenotype of many neurodevelopmental and neurodegenerative disorders, and is often associated with aberrant loss or gain of dendritic spines. In mouse models of disease, these spine deficits can be readily detected *in vivo* by imaging fluorescently labeled dendrites using 2-photon microscopy. Sparse labeling of dendrites is required for accurate detection of spines *in vivo* and is typically achieved using a Thy1-GFP mouse line, but applying this method to genetic mouse models is challenging because it requires lengthy periods of breeding, often in sensitive lines that are already very difficult to breed. As an alternative, we explore viral approaches to achieving sparse and bright labeling of dendrites in cortex for longitudinal *in vivo* spine imaging. Our goal is to ultimately build a platform to accurately and efficiently detect dendritic spine abnormalities in genetic mouse models and longitudinally track changes in spine morphology in response to experimental treatments.

Disclosures: S. Benezra: None. **A. Ghoshal:** None.

Poster

412. High-Throughput Patch-Clamp Approaches

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 412.01

Topic: I.04. Physiological Methods

Support: R01DA029639
R01NS102727
UL1-TR002378

Title: Pilot demonstration of end-to-end directed evolution engineering of optimized red-shifted opsins using automated patch clamping

Authors: *M. W. BADAWY[1], M. STOCKSLAGER[1], A. YANG[3], D. PARK[3], B. YANG[1], M. C. YIP[1], E. S. BOYDEN[3,4,5], C. R. FOREST[1,2];
[1]Woodruff Sch. of Mechanical Engin., [2]Wallace H. Coulter Sch. of Biomed. Engin., Georgia

Inst. of Technol., Atlanta, GA; [3]MIT McGovern Inst. for Brain Res., [4]Dept. of Brain and Cognitive Sci., MIT, Cambridge, MA; [5]Howard Hughes Med. Inst., Cambridge, MA

Abstract: Optogenetics is a powerful toolset that enables the control of neuronal activities through the expression of light-activated ion channels and pumps in genetically targeted neurons. Channelrhodopsin-2 (ChR2), the most commonly used opsin, is excitable with blue light with a peak response at 470 nm. Although recent efforts have led to the discovery of the ChrimsonR light-gated ion channel, with a peak response at a wavelength of 590 nm, there is a persistent need to find even more red-shifted opsins with suppressed blue light responsivity that allows for the activation of two different cell populations at the same time, in addition to enabling deeper tissue penetration and less invasiveness. We present a novel directed evolution approach for opsin engineering, by introducing ChrimsonR mutants into HEK293FT cultured cells through plasmid transfection. After initial fluorescence screening, opsin-expressing cells are then screened using the gold-standard patch clamping technique via the PatcherBot, a high-throughput patch clamping robot capable of automated unattended patching for up to 3 hours, while being stimulated with red color LED light. The opsin response is then evaluated for kinetics and wavelength of responsivity. The best performing mutants are then picked up individually using a cell sorter robot, and placed in PCR tubes containing a lysis buffer to prepare them for mutant isolation and DNA sequencing. This approach enables the screening of hundreds of mutants within a few days. In this work, we present the first pilot end-to-end demonstration of this technology by screening approximately 50 different ChrimsonR mutations showing their light-stimulated responses coupled with the corresponding DNA sequences.

Disclosures: **M.W. Badawy:** None. **M. Stockslager:** None. **A. Yang:** None. **D. Park:** None. **B. Yang:** None. **M.C. Yip:** None. **E.S. Boyden:** None. **C.R. Forest:** None.

Poster

412. High-Throughput Patch-Clamp Approaches

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Program #/Poster #: 412.02

Topic: I.04. Physiological Methods

Support: NIH Grant R01NS102727
NIH Grant R01DA029639

Title: Automated dual-electrode patch clamp electrophysiology for efficiently studying local brain synaptic connectivity

Authors: *M. C. YIP[1], B. YANG[1], C. F. LEWALLEN[3], E. S. BOYDEN[4,5,6], C. R. FOREST[1,2]; [1]Woodruff Sch. of Mechanical Engin., [2]Wallace H. Coulter Sch. of Biomed. Engin., Georgia Inst. of Technol., Atlanta, GA; [3]Natl. Eye Inst., NIH, Bethesda, MD; [4]Dept. of Brain and Cognitive Sci., [5]McGovern Inst. for Brain Res., MIT, Cambridge, MA; [6]HHMI, Cambridge, MA

Abstract: Patch clamp recording remains the gold-standard technique for high-quality electrophysiological measurements of single cells in brain slices. Because of the many delicate steps required to perform this method, patch clamp recording is a high-skill, time-intensive process. We have previously developed a robotic system, the "PatcherBot", capable of performing unattended, multi-hour patch clamp experiments in brain slices, with a whole-cell success rate of 50% and throughput of 7 whole-cell recordings/hr. While this system is broadly useful across neuroscience (e.g., drug screening, cell typing, etc.), the fields of connectomics and synaptic physiology can benefit uniquely from multiple automated patch clamp electrodes operating on the same tissue slice in coordination. These "multipatching" experiments, traditionally performed with a single user operating multiple manipulators (up to 12) simultaneously, is an incredibly challenging experimental method to master. Specifically, careful positioning and translation of the electrodes must be performed to maximize the probability of patching synaptically connected neurons, and, simultaneously, avoid potential electrode collisions. Previously, we presented a route planning and collision avoidance algorithm that enables automated multipatching. Using this algorithmic approach of route planning and collision avoidance will enable a user to probe a greater number of possible connections in a single experiment. Here, we implement these theoretical algorithms, enabling dual-electrode coordinated synapse hunting, by stepping one over the other in turn and checking for the presence of a connection. This novel approach, one of "walking" across the tissue by keeping one electrode recording while another establishes a new whole-cell configuration, doubles the throughput of connections probed as compared to the ubiquitous practice of placing and removing multipatching electrodes simultaneously.This dual-patching PatcherBot can enable up to 20 whole-cell recordings of neurons a day leading to more than 40 probed connections in mouse brain tissue. The method also improves the efficiency of our classification of neurons to understand cortical circuit function and the dynamic synaptic relationship between pre- and postsynaptic cell types. The scalability and enhanced productivity, towards high-fidelity recordings of neuronal connectivity, increases ease of use for "meso-scale" studies on local brain circuits.

Disclosures: **M.C. Yip:** None. **B. Yang:** None. **C.F. Lewallen:** None. **E.S. Boyden:** None. **C.R. Forest:** None.

Poster

412. High-Throughput Patch-Clamp Approaches

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Program #/Poster #: 412.03

Topic: I.04. Physiological Methods

Support: R01NS102727
R01DA029639
UL1-TR002378

Title: Multimodal cell identification for automated patch clamp electrophysiology in brain tissue

Authors: *M. M. GONZALEZ[1], M. C. YIP[1], C. R. VALENTA[1], M. J. ROWAN[2], C. R. FOREST[1];
[1]Georgia Inst. of Technol., Atlanta, GA; [2]Emory Univ., Atlanta, GA

Abstract: One of the major questions in neuroscience is how neuronal subtypes in the brain play a role in brain function, especially in the context of neurodegenerative diseases. The gold standard electrophysiology technique used to study this is patch clamp: a Nobel-prize winning method in which a glass pipette electrode facilitates single cell electrical recordings from individual neurons. Patch clamp experiments are arduous and time consuming. Further, they often rely upon fluorescent labels to achieve cell type specificity, though some cell types, namely pyramidal cells, are identified by visual inspection. To this end, we recently developed an automated fluorescent-guided patch clamp electrophysiology rig which enables automatic identification and patching of specific cell-types based on fluorescence. Building upon this technology, here we developed a computational method for cell-type-specific identification of healthy pyramidal cells in wild-type mouse brain tissue. We trained a convolutional neural network based on both images of the cells in brain slices and corresponding electrophysiology recordings to identify (1) cell health and (2) cell type, achieving precision (70%) and F1 scores (70%) comparable to the state-of-the-art deep-learning cell identification methods. We validated the performance of this deep learning method in an automated patch clamp experiment and successfully recorded from 20 pyramidal neurons with quality of recording and intrinsic properties similar to those recorded manually. Further development of this method will enable automated, high throughput, unbiased studies of living cells embedded in intact tissue for studying brain function and how it goes awry in disease states.

Disclosures: M.M. Gonzalez: None. **M.C. Yip:** None. **C.R. Valenta:** None. **M.J. Rowan:** None. **C.R. Forest:** None.

Poster

412. High-Throughput Patch-Clamp Approaches

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Program #/Poster #: 412.04

Topic: I.04. Physiological Methods

Support: Work supported by Biogen

Title: Automated high throughput patch clamp studies of voltage gated ion channels inhiPSC derived neurons

Authors: D. NAGY[1], W. YU[2], N. OKUGAWA[3], K. L. LEE[3], S. J. ENGLE[3], *C. A. HINCKLEY[4];
[1]Sophion Bioscience, 400 Tradecenter Dr Suite 6900, Wooburn, MA; [2]Sophion Biosci. Inc, Sophion Biosci. Inc, Woburn, MA; [4]Biogen, [3]Biogen, Cambridge, MA

Abstract: Human induced pluripotent stem cell (hiPSC) derived neurons express native complements of human ion channels and their accessory proteins providing enhanced translatability from early *in vitro* studies to patient biology. Despite this promise, few studies have examined the suitability of hiPSC neurons for automated patch clamp studies. Here, we establish the feasibility of recording voltage gated channel activity from hiPSC derived excitatory neurons in 384 well format with the Sophion Qube automated patch clamp system. hiPSC derived neurons were generated by overexpression of the transcription factor NGN2 driven from a stably integrated cassette in the AAVS1 locus. We first optimized dissociation procedures with 2-3 weeks *in vitro* NGN2 neurons by assessing the percentage of cells with voltage gated Sodium (Na_v) and potassium (K_v) currents on the Qube system. Recordings following optimized dissociation found that ~30% of single cells had Nav currents >200 pA, leading to recordings of >100 cells in parallel. Minimal reduction of experimental throughput was observed with recordings following culture up to 4 weeks. Isolation of Nav currents with Cesium internal solution showed expected Na_v activation and inactivation curves with mean Na_v currents >1 nA. Exchange of intracellular solution from cesium to potassium- based reversed block of K_v channels and did not significantly impact recording success rate. In multi-cell recording configurations we attained success rates of ~80%, sufficient to examine dozens of experimental conditions simultaneously.
These results suggest that key hiPSC NGN2 neuronal properties, Na_v and K_v activity, are retained in conditions that support high throughput patch clamp studies. Furthermore, we show that the automated patch clamp drastically increases experimental throughput for hiPSC neuron neurophysiology. Future studies will examine properties of other hiPSC derived neuronal types and establish the diversity of ion channels amendable to automated recordings.

Disclosures: D. Nagy: A. Employment/Salary (full or part-time):; Sophion Bioscience. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sophion Bioscience. **W. Yu:** A. Employment/Salary (full or part-time):; Sophion Bioscience. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sophion Bioscience. **N. Okugawa:** A. Employment/Salary (full or part-time):; Biogen. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Biogen. **K.L. Lee:** A. Employment/Salary (full or part-time):; Biogen. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Biogen. **S.J. Engle:** A. Employment/Salary (full or part-time):; Biogen. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Biogen. **C.A. Hinckley:** A. Employment/Salary (full or part-time):; Biogen. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Biogen.

Poster

412. High-Throughput Patch-Clamp Approaches

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 412.05

Topic: I.04. Physiological Methods

Title: Ion channel currents endogenously expressed in Neuro2A cells recorded using automated patch clamp

Authors: A. OBERGRUSSBERGER[1], N. BECKER[1], A. HORVÁTH[1], F. X. SUREDA[2], M. ROTORDAM[1], R. HAEDO[3], G. OKEYO[3], *E. DRAGICEVIC[1], N. FERTIG[1], **A. BRUGGEMANN**[1];
[1]Nanion Technologies GmbH, Munich, Germany; [2]Pharmacol. Unit, Dept. of Basic Med. Sci., Univ. Rovira i Virgili, Reus, Spain; [3]Nanion Technologies Inc., Livingston, NJ

Abstract: Neuro2A cells are a mouse neuroblastoma cell line used extensively to investigate neuronal differentiation, axonal growth and cell signaling pathways. They are also used as an expression system for studying ion channels. Neuro2A cells have been shown to endogenously express Na_V channels, predominantly $Na_V1.7$ but also $Na_V1.2$, 1.3, and 1.4, mechanosensitive channels Piezo 1, purinergic receptors, and glutamate receptors. Using an automated patch clamp (APC), we recorded different ion channels endogenously expressed in Neuro2A cells. On medium and high throughput APC devices, we recorded voltage-gated sodium channels that were blocked by TTX, tetracaine and lidocaine, with an IC_{50} of 4.1 ± 0.8 nM (n = 8), 9.9 ± 1.9 µM (n = 8) and 713.6 ± 57.3 (n=14), respectively. The V_{half} of activation was -19.4 ± 0.6 mV (n = 8), although there was some variation between cells. We also recorded Piezo1-mediated responses in Neuro2A cells activated by Yoda1. In addition, we recorded a heat-activated response to an external solution heated to 42°C. This heat-activated response could be attributed to TRPV3 or TRPV4 but not TRPV1 as ligand-gated responses to 2-APB and GSK1016790A were observed but no response to the TRPV1 ligand, capsaicin. In conclusion, Neuro2A cells can be used on APC devices with success rates of 60-80% for >1 GOhm seals and are a suitable cell type for investigating endogenous Na_V currents, as well as Piezo1 and some TRP channels.

Disclosures: **A. Obergrussberger:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **N. Becker:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **A. Horváth:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **F.X. Sureda:** None. **M. Rotordam:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **R. Haedo:** A. Employment/Salary (full or part-time):; Nanion Technologies Inc. **G. Okeyo:** A. Employment/Salary (full or part-time):; Nanion Technologies Inc. **E. Dragicevic:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **N. Fertig:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **A. Bruggemann:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH.

Poster

412. High-Throughput Patch-Clamp Approaches

Location: SDCC Halls B-H

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Program #/Poster #: 412.06

Topic: I.04. Physiological Methods

Support: National Institutes of Health Grant NS105200
National Institutes of Health Grant NS093866

Title: Conduction Velocities of Axons Innervating Parvalbumin Interneurons in Primary Somatosensory Cortex

Authors: *K. S. SCHEUER[1], A. CANALES[1], X. ZHAO[2], M. JACKSON[1];
[1]Neurosci., Univ. of Wisconsin-Madison, Madison, WI; [2]Waisman Ctr., Madison, WI

Abstract: Timing differences impact many fundamental neural computations including coincidence detection and temporal integration. Axonal conduction velocity (CV) contributes to these timing differences and therefore affects a variety of core cortical functions such as oscillations and plasticity. Previous studies of CV have been hampered by low throughput, lack of cell type specificity, and limited spatial and temporal resolution. The present study used the genetically-encoded hybrid voltage sensor hVOS to measure CV by imaging voltage changes specifically in parvalbumin (PV) interneurons in cortical slices. PV interneurons have been implicated in psychiatric disorders including autism spectrum disorder and schizophrenia. These fast-spiking, inhibitory neurons play a critical role in controlling the temporal integration windows of their targets and in the generation of gamma oscillations (30-80 Hz oscillations with functions that include executive processing and perceptual binding). Despite the extensive studies of cortical circuitry, CV along axons targeting specific cell populations has received little attention. Thus, little is known about one of the key factors that controls the timing of PV interneuron activation. We developed a rigorous, reproducible method for semi-automatic detection of voltage changes in individual PV interneurons. This approach allowed us to measure responses in up to 75 PV interneurons simultaneously with sub-millisecond temporal resolution over multiple cortical layers. Following electrical stimulation in different locations, we used hVOS to report the arrival time of synaptic inputs to PV interneurons and determined CV along excitatory axons in mouse barrel cortex. Stimulation in L2-5 produced varying patterns of activity corresponding to both intra- and interlaminar spread. Using these patterns of spread, we estimated interlaminar CV for L2/3 to L4, L4 to L2/3, L5 to L2/3, L4 to L5, and L5 to L4 and intralaminar CV within L2/3 and L5. Values varied widely from 28 – 241 μm/msec (96 ± 52 μm/msec, mean ± SD). Interlaminar CV (111 ± 49 μm/msec) was approximately twice as fast as intralaminar CV (57 ± 41 μm/msec), and interlaminar L5 to L4 CV (154 ± 58 μm/msec) was about 2.7 times faster than L2/3 and L5 intralaminar CV. Thus, interlaminar computations can proceed on a faster time scale than intralaminar computations. These layer-specific differences in CV have important implications for how PV interneurons contribute to cortical processing.

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Poster

412. High-Throughput Patch-Clamp Approaches

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Program #/Poster #: 412.07

Topic: I.04. Physiological Methods

Support: NIH Grant 1R01NS114514

Title: Voltage error due to series resistance in patch clamp: not as bad as you think.

Authors: *M. L. GRAY[1], J. M. SANTIN[2];
[1]Biol., Univ. of North Carolina Greensboro, Greensboro, NC; [2]Biol., Univ. of North Carolina at Greensboro, Greensboro, NC

Abstract: Tight-seal whole-cell patch-clamp recording is a method of recording from cells using a pipette to rupture the cell membrane for electrochemical access to the cell via low-resistance electrodes. Patch-clamp series resistance is typically small compared to sharp electrode recordings; however, the use of one electrode to simultaneously measure voltage and inject current yields voltage errors that deviate from the command voltage (V_{Com}). This error is typically approximated using Ohm's law, and the expected voltage error is assumed to be the current multiplied by the effective series resistance following partial compensation ($Rs_{-effective}$). Thus, large currents have been assumed to have large voltage errors, thereby limiting the use of patch-clamp studies. We used dual patch recordings from two types of frog motor neurons (Hypoglossal (HG) and Vagus (VG); ~25-50 µm diameter) to perform a voltage clamp with one electrode while directly measuring the real voltage error with the other. This allowed us to directly measure the series resistance error and compare it to Ohm's law approximations. We compared this voltage error at three time points for outward K^+ currents: average current of the entire step, the transient inactivating current (TC) in the first 50 ms after a step, and the steady-state (SS), non-inactivating portion of this current occurring 800-900ms after a step. In all portions, and in each neuron type, we found that real membrane voltage was closer to the V_{Com} than predicted by Ohm's law, especially so for TCs. For example, at a V_{com} of 0 mV, the mean voltage for the TC in HG neurons (despite a current amplitude of 23.1 ± 9.0 nA) was -9.5 ± 5.5 mV vs the predicted voltage of -23.9 ± 9.1 mV. In contrast, the SS current had a mean voltage of -8.8 ± 4.9 mV and a predicted voltage of -16.5 ± 6.5 mV despite a current of 15.7 ± 5.8 nA. A similar pattern was shown for VG neurons. Both types of neurons showed better voltage control than predicted and greater deviations from Ohm's law in TC compared to SS currents. For HG neurons, V_{Com} was a far better predictor for TC voltage than the Ohm's law estimate at a V_{com} ranging from 0-20 mV, suggesting that Ohm's law does not provide an accurate estimate for voltage error with time-varying currents. These data suggest that in standard operating conditions, Ohm's law approximations overestimate the voltage error for TCs at all tested voltages; while it is only useful for approximating voltage errors of the SS currents measured above 10 mV steps, 800-900 ms after the step. This may also open the possibility for patch clamp studies in neurons that may be difficult to access with two-electrode voltage clamp.

Disclosures: M.L. Gray: None. **J.M. Santin:** None.

Poster

413. Computational Tools for Calcium Imaging Experiments

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 413.01

Topic: I.07. Data Analysis and Statistics

Support: Collaborative Research Center 1080, DFG Germany
Add-on Fellowship by Joachim Herz Stiftung
Starting grant by European Research Council

Title: Development of a novel semi-automated tool for analyzing neuronal imaging data

Authors: *S. WAGLE[1], M. EGGL[1], T. TCHUMATCHENKO[1,2];
[1]Inst. for Physiological Chemistry, Univ. Med. Ctr. Johannes Gutenberg Univ., Mainz, Germany;
[2]Inst. of Exptl. Epileptology and Cognition Research, Univ. of Bonn Med. Ctr., Bonn, Germany

Abstract: Synapses are the elementary unit of information storage and transfer in the brain. Their ability to dynamically strengthen and weaken connections in response to neural activity, also known as synaptic plasticity, has been shown to have a fundamental role in learning and memory. Synaptic plasticity is associated with changes in the structure of the synapse and requires specific mRNA and proteins in the spine. However, the exact mechanisms that drive this plasticity are still active areas of research. Thus, monitoring the changes in spine structure and the associated protein and mRNA compositions via visual inspection is often used to gain insight into the dynamics of memory formation and learning in the brain.
Recent imaging techniques of neurons allow for high precision tracking of spine structure changes on the order of minutes to days. In parallel, advanced molecular labeling coupled with contemporary imaging techniques have allowed more accurate protein and mRNA trafficking recordings in subcellular neuronal compartments (e.g., dendrites or spines) and thus provide a careful characterization of the kinetic parameters involved in subcellular trafficking mechanisms. Current methods in analyzing these datasets often rely on manual inspection, which may lead to difficulty achieving reproducibility and robustness. Therefore, advanced computational tools can significantly speed up the analysis of these datasets and introduce a measure of robustness that would benefit all studies attempting to study synaptic phenomena. However, these tools can often come with a high learning curve, particularly those based on packages requiring pre-existing programming knowledge, which can often deter experimentalists from using them. Therefore, we present a novel tool with a user-friendly graphical interface for automated analysis of neuronal imaging data to perform various statistical analyses on the images. Features of our tool include spine detection using deep learning, spine ROI generation, automated dendritic path tracing, and spine tracing in time-series data. We are sure that the computation framework we have developed will be a significant asset for robust and reproducible analysis of neuronal imaging data and we hope will be helpful to the broad scientific community.

Disclosures: S. Wagle: None. **M. Eggl:** None. **T. Tchumatchenko:** None.

Poster

413. Computational Tools for Calcium Imaging Experiments

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Program #/Poster #: 413.02

Topic: I.06. Computation, Modeling, and Simulation

Support: DARPA
NIH

Title: Fast and statistically robust cell extraction from large-scale neural calcium imaging datasets

Authors: *F. DINC[1], H. INAN[1], C. SCHMUCKERMAIR[1], T. TASCI[1], B. O. AHANONU[1], O. HERNANDEZ[1], J. LECOQ[2], O. ZOHAR[1], M. J. WAGNER[2], M. ERDOGDU[3], M. J. SCHNITZER[4];
[2]Stanford Univ., [1]Stanford Univ., Stanford, CA; [3]Univ. of Toronto, Toronto, ON, Canada; [4]Stanford Univ. Dept. of Biol., Stanford Univ. Dept. of Biol., Stanford, CA

Abstract: State-of-the-art fluorescence Ca^{2+} imaging studies that monitor large-scale neural dynamics can produce video datasets ~10 terabytes or more in total size, roughly comparable to ~10,000 Hollywood films. Processing such data volumes requires automated, general-purpose and fast computational methods for cell identification that are robust to a wide variety of noise sources. We present EXTRACT, an algorithm that is based on robust estimation theory and uses graphical processing units (GPUs) to extract neural dynamics in computing times up to 10-times faster than imaging durations. Owing to its agnosticism about the statistical distributions of noise that may be present in the Ca^{2+} imaging data, and its lack of assumptions about Ca^{2+} signaling waveforms or background fluorescence, EXTRACT can be broadly applied to data from a wide range of Ca^{2+} imaging formats, including one- or two-photon fluorescence Ca^{2+} imaging data from either head-fixed or freely behaving animals, without requiring any algorithmic changes. We validated EXTRACT on simulated and experimental data and processed 94 public datasets from the Allen Institute Brain Observatory in one day. EXTRACT outperforms prior state-of-the-art cell extraction algorithms regarding both speed and accuracy using a single graphical processing unit (GPU), and its capability to use multiple GPUs at once makes EXTRACT a scalable approach that can analyze the dynamics of tens of thousands of cells in close to real-time. Notably, runtime comparisons between EXTRACT and prior cell extraction algorithms show that EXTRACT processes two-photon Ca^{2+} imaging datasets about an order of magnitude faster than previous methods. Showcasing its superiority over past cell extraction methods at removing noise contaminants, neural activity traces from EXTRACT also allow more accurate decoding of animal behavior. Overall, EXTRACT provides neuroscientists with a powerful computational tool matched to the present challenges of neural Ca^{2+} imaging studies in behaving animals.

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Poster

413. Computational Tools for Calcium Imaging Experiments

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 413.03

Topic: I.07. Data Analysis and Statistics

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NIH New Innovator Program (1DP2-NS111505)
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Vallee Young Investigator Program

Title: Temporal unmixing of calcium traces for fluorescence imaging videos using targeted nonnegative matrix factorization

Authors: *Y. BAO[1], E. REDINGTON[1], A. AGARWAL[2], Y. GONG[1];
[1]1Department of Biomed. Engin., Duke Univ., Durham, NC; [2]North Carolina Sch. of Sci. and Mathematics, Durham, NC

Abstract: Fluorescence microscopy and genetically encoded calcium indicators help understand brain function through large-scale in vivo recordings in multiple animal models. Typical fluorescent calcium sensors respond to an action potential with a stereotypic transient, including a rapid rise and slow decay. However, the fluorescence signals are often contaminated with false transients from nearby neurons, other calcium sources, and backgorund fluctuations. We developed and characterized a novel method, Temporal Unmixing of Calcium Traces (TUnCaT), to quickly and accurately unmix the calcium signals of neighboring neurons and background, and thus remove false transients.
TUnCaT removed false transients using targeted background subtraction and targeted nonnegative matrix factorization (NMF). TUnCaT first calculated and removed false transients caused by background fluctuations specific to each neuron. TUnCaT then applied NMF to a targeted set of traces, including the neuron of interest, neighboring neurons, and the outside region containing axons and dendrites; this targeted NMF effectively and efficiently assigned false transients caused by neighboring calcium sources to their correct origins instead of to the neuron of interest.
We tested TUnCaT using cross-validations on multiple datasets, including experimental two-photon videos from Allen Brain Observatory, simulated two-photon videos using NAOMi, and experimental one-photon videos from our lab. TUnCaT effectively removed false transeints in all situations. We compared TUnCaT with existing unmixing algorithms, including FISSA, CNMF, and the demixing algorithm from the Allen SDK. TUnCaT was more accurate than existing algorithms, and its speed was faster than or comparable to existing algorithms. The superiority of TUnCaT generalized to a variety of conditions in simulation. Temporal downsampling further improved the speed of TUnCaT without sacrificing accuracy. We combined TUnCaT with our previously developed shallow U-Net neuron segmentation (SUNS), to both spatially segment neurons and calculate the unmixed traces of segmented neurons. Such a two-step pipeline offered better accuracy and speed than CaImAn, a widely used single-step spatiotemporal unmixing

algorithm. Our automated, flexible, fast, and accurate trace unmxing algorithm improves the state-of-the-art, and it can be widely used in analyzing various fluorescence calcium imaging videos.

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Poster

413. Computational Tools for Calcium Imaging Experiments

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Program #/Poster #: 413.04

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH BRAIN Initiative 1R01NS109994
T32 T32AG052909

Title: Robust algorithms for unlocking activity dynamics of densely labeled dendritic and somatic calcium imaging datasets

Authors: *J. J. MOORE[1,2], S. K. RASHID[1], N. J. CODRINGTON[1], D. B. CHKLOVSKII[2], J. BASU[1];
[1]NYU Sch. of Med., New York, NY; [2]Flatiron Inst., New York, NY

Abstract: Dendrites support non-linear processing in the form of dendritic spikes, which can enhance the computational capacity of individual neurons. These properties have primarily been studied using single neuron recordings in brain slices or with calcium imaging in sparsely labeled *in vivo* preparations. While this approach allows faithful tracking of the activity of individual dendrites, it provides limited insight into the population dynamics of large numbers of dendrites. From an experimental point of view, densely labeled datasets provide the opportunity to simultaneously record activity from thousands of dendrites, matching or exceeding the throughput of *in vivo* somatic calcium imaging. Existing calcium imaging pipelines are optimized for fields of view containing only cell soma, and have varying success at handling dendrites. Dense dendritic imaging poses unique challenges due to dendrites' varied morphology and thin diameter, hindering exploration into dendritic coding properties.To address these challenges, we developed an automated detection algorithm flexible enough to identify somatic and dendritic regions of interest (ROIs) in dense datasets. The algorithm utilizes non-parametric approaches with few assumptions to identify initial ROI estimates. It then employs sparse constrained non-negative matrix factorization to simultaneously refine the ROI estimates and extract their activity over time. Finally, it screens putative calcium transients with an additional goodness-of-fit measure to eliminate spurious activity from undetected ROIs. We name this approach "dendritic NMF," or "d-NMF." d-NMF is easy to use with few tunable parameters and runs quickly on standard computer workstations. We validated its performance on densely labeled dendritic recordings from area CA3 of mouse hippocampus exhibiting range of labeling densities, and found that it matched or exceeded human expert manual identification of dendritic,

somatic, and axonal ROIs. Performance matched or outpaced that of contemporary calcium analysis suites such as CaImAn and suite2p. Using these tools we demonstrate strong place selectivity in apical and basal dendrites of area CA3, demonstrating the prevalence of spatial information throughout the CA3 circuit. We present this set of analysis approaches as an open-access toolbox for the community, with code posted and maintained and Github. These tools will enable investigation of population-level dendritic dynamics, crucial to understanding the role of dendrites in neural computations *in vivo*.

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Poster

413. Computational Tools for Calcium Imaging Experiments

Location: SDCC Halls B-H

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Program #/Poster #: 413.05

Topic: I.06. Computation, Modeling, and Simulation

Title: Quantitatively comparing data across various neuronal selection and image-processing methods in in-vivo fluorescence microscopy and using results to help validate neuronal selection choices

Authors: T. MAHMOUDI[1], J. KANEM[1], W.-K. YOU[2], A. BANERJEE[2], R. CHRISTO[5], ***Y. SOUDAGAR**[1], S. P. MYSORE[2,3,4];
[1]Neurescence Inc., Toronto, ON, Canada; [2]Psychological and Brain Sci., [3]Neurosci., [4]Kavli Neurosci. Discovery Inst., Johns Hopkins Univ., Baltimore, MD; [5]Psychology, Univ. of Toronto, Toronto, ON, Canada

Abstract: Neuronal tracing is a critical step in the analysis of neuronal activity data obtained from fluorescence imaging methods such as calcium imaging. Methods to automate neuronal tracing have been introduced, but require extensive manual input and judgment from users to determine parameters, eliminate false positives and re-add false negatives. Moreover, in neuronal populations that are sparse, it is often more efficient to manually locate and select neurons. While both manual and automated methods are widely used, a systematic evaluation of parameters of the image preprocessing (underlying both approaches) that have the greatest impact on the variability of the fluorescence time series extracted from the traced neurons are currently lacking. Here, we address these issues by quantifying and comparing outcomes as a function of various parameters of tracing and image preprocessing applied to 1p calcium imaging data obtained from different mouse brain areas and using two different technologies - a multiscope and a miniscope. Specifically, we investigated combinations of the values of three parameters: (1) Shape of the contour - 2 shapes, (2) Constant signals removed ($\Delta f/f$) or not, (3) Spatial filtering: no filter, low filter setting, high filter setting. For each of the 12 parameter combinations, we traced neuronal outlines manually and compared the resulting fluorescence time series. To this end, we used pairwise correlation measurements among these time series

using cross-correlation and Pearson's correlation coefficient. This approach allowed us to estimate which parameters have the most influence over the neuronal time series data, and hence the greatest ability to introduce errors (and cause potential lack of reproducibility). Notably, we found that for neurons with high firing activity, the fluorescence time series obtained across the different combinations of parameters exhibited a high correlation (rho>0.9), whereas for neurons with high background fluctuations or for traced outlines that were mistakenly detected as neurons, correlations across the different parameter combination swas low (rho<0.5). Additionally, signals with different contour shapes tended to be highly correlated, indicating that the shape of the contour does not significantly affect neuronal traces. These results introduce a powerful new approach using the simple correlation to differentiate a 'real' neuron from a false positive. They also establish a systematic framework for validating neuronal tracing choices for the analysis of fluorescence time series data in neuroscience.

Disclosures: **T. Mahmoudi:** A. Employment/Salary (full or part-time):; Neurescence Inc. **J. Kanem:** A. Employment/Salary (full or part-time):; Neurescence Inc.. **W. You:** None. **A. Banerjee:** None. **R. Christo:** None. **Y. Soudagar:** A. Employment/Salary (full or part-time):; Neurescence Inc.. **S.P. Mysore:** None.

Poster

413. Computational Tools for Calcium Imaging Experiments

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Program #/Poster #: 413.06

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH R01 NS045193
NIH U19 NS104648
NIH F32 MH120887

Title: A biophysically-inspired decoder for burst activity and single spikes using jGCaMP8f

Authors: ***G. BROUSSARD**[1], F. J. URRA QUIROZ[2], G. DIANA[2], L. A. LYNCH[1], B. S. SERMET[2], D. A. DIGREGORIO[2], S. S.-H. WANG[1];
[1]Princeton Neurosci. Inst., Princeton, NJ; [2]Inst. Pasteur, Paris, France

Abstract: The recently developed calcium sensor jGCaMP8f has faster on-responses than previous variants - a 50% rise time of 2 ms in response to a single action potential, about 5x faster than GCaMP6f and 3x faster than jGCaMP7f. This speed makes it advantageous for monitoring single-spike activity in populations of neurons. However, it exhibits a residual spike-history-dependent slowing observed during periods of elevated neural activity. Here we examine the kinetic basis of this distortion. We performed cuvette stopped-flow fluorimetry and ex vivo axon bouton recordings to construct a detailed biophysical framework for spike encoding. We found that a kinetic model consisting of dual slow and fast calcium-driven processes can account for observed activation dynamics across all three variants. In this scheme, transitions to high

fluorescence for GCaMP6f and jGCaMP7f require the slow process, and respond maximally to bursts of spikes. In contrast, jGCaMP8f fluorescence is dominated by the fast calcium-dependent process, leading to rapid rise times and near-linear response to sequences of individual spikes. However, spike-history-dependent slowing in jGCaMP8f responses become prominent in response to prolonged activity, which drives entry into the slow process and decreases the effective rate of calcium elimination. We were able to capture these distortions accurately using a generative spike-encoding model. We further demonstrate that standard spike deconvolution approaches predict spurious spikes in jGCaMP8f recordings taken during periods of elevated activity. We conclude that despite the accelerated speed and reduced nonlinearity of jGCaMP8f, it is still necessary to consider biophysical model-based inference for accurate decoding of high-frequency spikes during sustained activity.

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Poster

413. Computational Tools for Calcium Imaging Experiments

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Program #/Poster #: 413.07

Topic: I.07. Data Analysis and Statistics

Support: Human Frontier Science Program RGP0008/2017

Title: Vodex: volumetric data and experiment manager, an open-source tool for large-scale brain-wide functional imaging studies

Authors: *A. NADTOCHIY[1], P. LUU[2], S. E. FRASER[3], T. V. TRUONG[3];
[1]Quantitative and Computat. Biol., [2]Mol. Biol., [3]Translational Imaging Ctr., USC, Los Angeles, CA

Abstract: Recent advances in fluorescent microscopy and genetically-encoded calcium indicators made it possible to acquire large-scale 3D-time series datasets of brain activity. During these recordings, experimental conditions can vary: subjects can be presented with different stimuli and/or demonstrate different behavior. It is then required to annotate the data based on these experimental conditions and select the recording time of interest for the subsequent analysis. Data annotation is usually done manually or using a custom code deeply integrated with the analysis pipeline. Manual annotation is prone to error and is hard to document. Custom code often requires loading the whole dataset into the memory or depends on the exact file representation of data on a disc, which is not optimal for large datasets. We introduce VoDEx, volumetric data and experiment manager, a data management tool that integrates the information about the individual image frames, volumes, volume slices, and experimental conditions and allows retrieval of sub-portions of the 3D-time series datasets based on any of these identifiers. It is implemented as a napari plugin for interactive usage with a GUI and as an open-source Python

package for easy inclusion into analysis pipelines. We demonstrate the features and performance of VoDEx by presenting how we utilize it within a custom analysis pipeline as a part of a larger project aiming to identify the neural correlates of numerosity in zebrafish. The goal of the analysis is to identify the neurons that are uniquely tuned to specific numerosity. In the experiment, brain-wide functional imaging of larval zebrafish labeled with fluorescent calcium indicators was recorded while the animal was presented with the visual stimuli: 2, 3, and 5 dots varying in pattern, size, timing, and other geometric controls. VoDEx is used to automate the data annotation, manage the complex stimuli pattern, including the geometric controls, and document the exact data subset that is analyzed. We show how VoDEx allows to abstract away the data representation as a set of files on a disc and to focus on the development of analysis tools that operate directly on brain volumes and stimuli.

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Poster

413. Computational Tools for Calcium Imaging Experiments

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Kavli Foundation
UCL Bogue Fellowship

Title: Adapting and comparing 2-photon data processing pipelines for large-scale volumetric recordings of the entire visual cortex

Authors: *A. HAYDAROGLU[1], M. KRUMIN[1], J. GUO[2,3], A. VAZIRI[2,3], K. D. HARRIS[1], M. CARANDINI[1];
[1]Univ. Col. London, London, United Kingdom; [2]Lab. of Neurotechnology and Biophysics, [3]The Kavli Neural Systems Inst., Rockefeller Univ., New York City, NY

Abstract: *[Aims]* The Light Beads Microscope (LBM, Demas et al. 2021) enables volumetric 2-photon imaging across large areas of the mouse cortex, recording up to one million neurons simultaneously. However, the unique recording conditions of the LBM present challenges for extracting cell activity. These challenges include low SNR, large data rates, inter-plane cross-talk, and low temporal and spatial resolution. Do these challenges present issues for the current pipelines for extracting cell activity? Can we improve those pipelines to increase the quality and

quantity of cells detected?
[Methods] We used the LBM to record 60+ hours of activity in mice expressing GCaMP6s in all cortical excitatory neurons (n = 7). The recordings capture an area up to 4x4 mm encompassing the entire visual cortex with a frame rate of 2.8 Hz and contain 20-30 horizontal planes separated by 15 μm. The spatial resolution varies between 0.5-5 μm/pixel, and high resolution recordings are used to validate results on low resolution recordings in the same field of view.
[Results] To pre-process the large raw movies produced by the LBM recordings (12 GB per minute), we developed highly-parallelized programs. We modified the DeepInterpolation denoiser algorithm (Lecoq et al., 2021), trained it on LBM data, and incorporated it in the analysis pipeline. For cell detection and signal extraction, we compared the performance of two commonly-used packages: CaImAn (Giovannucci et al., 2019), which uses nonnegative matrix factorization, and Suite2p (Pachitariu et al., 2017), which uses model-based source-extraction. To measure the quality of the results, we used functional metrics such as receptive field location and orientation tuning of the identified cells. Preliminary results indicate that the deep convolutional denoiser improves the quality and quantity of identified cells, and that there are substantial differences in the cells identified by the two cell-detection algorithms. We are currently working to compare the quantity of cells found by the two algorithms, and evaluate their quality based on the reliability of sensory responses.
[Conclusions] The pipelines that are currently available for two-photon data pre-processing and analysis require some modifications to be optimized for the Light Beads Microscope. The comparison of widely-used cell detection algorithms can also be informative for selecting the most appropriate tool for other experiments conducted at low resolutions.

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Poster

413. Computational Tools for Calcium Imaging Experiments

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Program #/Poster #: 413.09

Topic: I.07. Data Analysis and Statistics

Support: European Union's Horizon 2020 Excellent Science 692943
European Union's Horizon 2020 Research and Innovation Programme 785907
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Title: Latency correction in sparse neuronal spike trains

Authors: *T. KREUZ[1], F. SENOCRATE[2], G. CECCHINI[3], C. CHECCUCCI[2], A. ALLEGRA MASCARO[4], E. CONTI[5], A. SCAGLIONE[6], F. S. PAVONE[7];
[1]Natl. Res. Council, Inst. For Complex Systems, Sesto Fiorentino, Italy; [2]Dept. of Physics and Astronomy, Univ. of Florence, Florence, Italy; [3]Dept. of Mathematics and Computer Sci., Univ. of Barcelona, Barcelona, Spain; [4]Natl. Res. Council, Natl. Res. Council, Pisa, Italy; [5]European

Lab. for Non-linear Spectroscopy, European Lab. for Non-linear Spectroscopy, Sesto Fiorentino, Italy; [6]Univ. Degli Studi Di Firenze, Univ. Degli Studi Di Firenze, Sesto Fiorentino, Italy; [7]LENS, LENS, Sesto Fiorentino, Italy

Abstract: Introduction: In neurophysiological data, latency refers to a global shift of spikes from one spike train to the next, either caused by response onset fluctuations or by finite propagation speed. Such systematic shifts in spike timing lead to a spurious decrease in synchrony which needs to be corrected **[1]**.
Methods: We propose a new algorithm of multivariate latency correction suitable for sparse data for which the relevant information is not primarily in the rate but in the timing of each individual spike. The algorithm is designed to correct systematic delays but to maintain all other kinds of noisy disturbances. It consists of two steps, spike matching **[2]** and distance minimization between the matched spikes using simulated annealing.
Results: We show its effectiveness on simulated and real data: cortical propagation patterns recorded via calcium imaging from mice before and after stroke **[3]**. Using simulations of these data we also establish criteria that can be evaluated beforehand in order to anticipate whether our algorithm is likely to yield a considerable improvement for a given dataset.
Conclusions: For any given dataset the criterion for applicability of the algorithm can be evaluated quickly and in case of a positive outcome the latency correction can be applied easily since the source codes of the algorithm are publicly available.
Software: The three measures ISI-distance **[4]**, SPIKE-distance **[5]** and SPIKE-Synchronization **[2]** and their adaptive versions **[6]** as well as the directional measure SPIKE-Order **[7]** are implemented in the Matlab-based graphical user interface SPIKY **[2]**, the Matlab command line library cSPIKE, and the Python library PySpike **[8] [9]**.
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[9] http://www.thomaskreuz.org/source-codes/SPIKY, http://www.thomaskreuz.org/source-codes/cSPIKE and https://github.com/mariomulansky/PySpike

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Poster

413. Computational Tools for Calcium Imaging Experiments

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Program #/Poster #: 413.10

Topic: I.07. Data Analysis and Statistics

Support: PARI-CNRS 980229

Title: Embedding behavior into the analysis of in-vivo recordings

Authors: *E. MARTIANOVA[1], M. IHIDOYPE[2], C. D. PROULX[2];
[1]Doric Lenses Inc., Quebec, QC, Canada; [2]Cervo Brain Res. Centre, Laval Univ., Quebec, QC, Canada

Abstract: Nowadays, in-vivo imaging and recordings are an essential part of neuroscience research. One of the main obstacles to using these techniques is the tedious analysis that usually takes weeks to make even a preliminary conclusion about experiments. Many tools exist that allow users to process data, e.g., transform a stack of images into neural signals. However, these tools often require programming skills, do not embed behavioral data, and spread results across multiple files. Here, we introduce a data structure and processing pipeline of in-vivo experiments from raw data to final plots for publishing. The data structure allows to combine in one file the initial recordings from one or several recording devices, behavior videos, events (e.g., consumption, freezing bouts, mobility onsets) and stimuli (e.g., laser stimulation, shock, air puff), measurements (e.g., speed, freezing score), any steps of data processing, and combined analysis of neuronal signal and behavior. Besides, the data structure contains experiment meta-data and analysis parameters. We propose a user-friendly graphic interface to visualize the files and their content, as well as convenient way to manipulate the files by adding new and removing unnecessary data. A user would typically use multiple libraries and software to process the data from different in-vivo experiments such as recordings from miniature fluorescent microscope, fiber photometry, electrophysiology, optogenetic stimulation, and behavior. We suggest that analysis of these data can be combined under the same pipeline, which roughly consists of three steps and differs only in the first step of the processing depending on the data type. First, the data is processed to ΔF/F signals, spikes, or other signals. Second, a user defines meaningful events and stimuli during their experiment. Third, the signals from step one and events from step two are combined to create a result array. For example, an array can represent an average signal before and after an event, or an area under curve of the signal at specified time windows relative to the event. Using the data structure and the pipeline, we will present analysis of recordings from different devices. Overall, we aim to demonstrate that our data structure and analysis pipeline saves time and resources that a user would spend on analysis of in-vivo recordings.

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Poster

414. Electrical Stimulation: Human and Non-Human Primates

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Program #/Poster #: 414.01

Topic: I.08. Methods to Modulate Neural Activity

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NIH BRAIN Initiative U24NS113637
National Science Foundation Graduate Research Fellowship Program

Title: Artifact characterization and mitigation techniques during concurrent sensing and stimulation using bidirectional deep brain stimulation platforms

Authors: *M. E. ALARIE[1], N. R. PROVENZA[2], M. AVENDANO-ORTEGA[3], S. MCKAY[3], A. S. WAITE[1], R. K. MATHURA[2], J. A. HERRON[4], S. A. SHETH[2], D. A. BORTON[1], W. K. GOODMAN[3];
[1]Engin., Brown Univ., Providence, RI; [2]Neurosurg., [3]Menninger Dept. of Psychiatry and Behavioral Sci., Baylor Col. of Med., Houston, TX; [4]Neurolog. Surgery, Univ. of Washington, Seattle, WA

Abstract: Bidirectional deep brain stimulation (DBS) platforms have enabled a surge in hours of recordings in naturalistic environments, allowing further insight into neurological and psychiatric disease states. However, high amplitude, high frequency stimulation generates artifacts that contaminate neural signals and hinder our ability to interpret the data. This is especially true in psychiatric disorders, for which high amplitude stimulation is commonly applied to deep brain structures where the native neural activity is miniscule in comparison. Here, we characterized artifact sources in recordings from a bidirectional DBS platform, the Medtronic Summit RC+S, with the goal of optimizing recording configurations to improve signal to noise ratio. Data were collected from three subjects in a clinical trial of DBS for obsessive-compulsive disorder. Stimulation was provided bilaterally to the ventral capsule/ventral striatum (VC/VS) using two independent implantable neurostimulators. We first manipulated DBS amplitude within safe limits (2-5.3 mA) to characterize the impact of stimulation artifacts on neural recordings. We found that high amplitude stimulation produces slew overflow, defined as exceeding the rate of change that the analog to digital converter can accurately measure. Overflow led to expanded spectral distortion of the stimulation artifact, with a sixfold increase in the bandwidth of the 150.6 Hz stimulation artifact from 147-153 Hz to 140-180 Hz. By increasing sense blank values during high amplitude stimulation, we reduced overflow by as high as 30%, and improved artifact distortion, reducing the bandwidth from 140-180 Hz artifact to 147-153 Hz. We also identified artifacts that shifted in frequency through modulation of both Summit RC+S system's telemetry mode and ratio parameters. Therefore, we demonstrate that there exists configurable parameters unrelated to stimulation, having unexpected implications for data quality. Overall, the artifact characterization methods and results described here enable increased data interpretability and unconstrained biomarker exploration using data collected from bidirectional DBS devices.

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Poster

414. Electrical Stimulation: Human and Non-Human Primates

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 414.02

Topic: I.08. Methods to Modulate Neural Activity

Title: Closed-loop neuromodulation for hippocampal oscillatory activity using deep brain stimulation applied to the posterior cingulate cortex

Authors: *D. X. WANG[1], C. E. DAVILA[2], E. FORBES[1], J. L. KRIEGEL[1], B. C. LEGA[1]; [1]Dept. of Neurolog. Surgery, UT Southwestern Med. Ctr., Dallas, TX; [2]Electrical and Computer Engin., Southern Methodist Univ., Dallas, TX

Abstract: The past two decades have witnessed the rapid growth of therapeutic brain-computer interfaces (BCI) targeting a diversity of brain dysfunctions. Deep brain stimulation (DBS) with neuromodulation technique has emerged as a fruitful treatment for neurodegenerative disorders such as epilepsy, Parkinson's disease, and amnesia. In parallel to the open-loop neuromodulation strategies, closed-loop BCIs have shown empirical success in targeting memory disorders. Our efforts are focused on applying control theoretical principles to achieve closed-loop neuromodulation of hippocampal memory-relevant oscillatory activity, namely, theta and gamma oscillations. We adapt and apply a binary-noise (BN) DBS paradigm to the posterior cingulate cortex (PCC) in 12 human subjects for developing a closed-loop BCI system using an innovative nonlinear ARX neural network (NARXNN) paired with a proportional-integral-derivative (PID) controller (NARXNN-PID) for delivering a precise DBS pattern to achieve desired hippocampal theta and gamma power. We compare our NARXNN-PID framework to a benchmark system based on linear state-space modeling (LSSM), and we not only demonstrate the significantly superior performance of our NARXNN modeling but also show the greater capability of NARXNN-PID architecture in controlling both hippocampal theta and gamma power, compared to the benchmark system (9 out of 12 subjects, $p<0.05$). We outline further experimentation to test our BCI systems and compare our findings to emerging closed-loop neuromodulation strategies.

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Poster

414. Electrical Stimulation: Human and Non-Human Primates

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Program #/Poster #: 414.03

Topic: I.08. Methods to Modulate Neural Activity

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Title: Beta-based adaptive deep brain stimulation in chronic at-home setting: a case study

Authors: L. ROMITO[1], M. ARLOTTI[2], V. LEVI[1], S. RINALDO[1], R. ELEOPRA[1], T. MANDAT[3], M. LANOTTE[4], M. ZIBETTI[4], L. LOPIANO[4], C. FONDA[4], E. PIROLA[5], M. LOCATELLI[5], A. LANDI[6], A. ANTONINI[6], M. L. F. JANSSEN[7], Y. TEMEL[7], L. ACKERMANS[7], A. PRIORI[8], ***S. MARCEGLIA**[9];
[1]Fondazione IRCCS Inst. Neurologico Carlo Besta, Milan, Italy; [2]Newronika SpA, Milan, Italy; [3]Narodowy Instytut Onkologii im. Marii Skłodowskiej-Curie, Warsaw, Poland; [4]Univ. degli Studi di Torino, Torino, Italy; [5]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy; [6]Univ. of Padua, Dept. of Neurosci., Padova, Italy; [7]Maastricht Univ., Maastricht, Netherlands; [8]Univ. degli Studi di Milano, Milano, Italy; [9]Univ. Degli Studi Di Trieste, Trieste, Italy

Abstract: Chronic application of adaptive deep brain stimulation is today the objective of the translational efforts of the neuroscience community including academic scientific groups and industrial partners. The path to the clinical translation needs to solve interdisciplinary challenges and open issues with focus on technological feasibility and clinical efficacy.Here we report the experience collected in the management of a single patient treated chronically with adaptive DBS for up to 7 months. A male subject (48 years old) with bilateral DBS leads implant in the subthalamic nucleus (Model 3389, Medtronic, Minneapolis, US) underwent surgery for implantable pulse generator (IPG) replacement 3 years after the first implant. Two single channel IPGs (Model Activa SC, Medtronic) were replaced by the AlphaDBSipg (clinical trial n. NCT04681534). After completing the clinical study protocol, at day 32 after the implant, the device was programmed in adaptive mode for chronic at home treatment. Neurophysiological and stimulation data were collected 24/7 by the device, reporting beta band amplitude time series for the hemisphere showing greater activity and the time-frequency data in the range 5-35 Hz. In aDBS, stimulation amplitude was linearly adapted to the normalized beta oscillation power within the patient's therapeutic window. The patient specific beta band used for adapting DBS was 11-16 Hz. To drive aDBS, beta amplitude was normalized by the total spectral power in the 5-35Hz band, to mitigate unwanted movements artifacts. The range of variability of the normalized beta activity was stable for the full period of at home setting. The minimum and maximum value of stimulation amplitude was fine-tuned at two consecutive follow-up visits together with the medication daily dosage. At day 32 the therapeutic range for aDBS was set between 2.2 mA and 3 mA for the left STN and 2.3mA and 2.8 mA for the right STN, 80 μs and 130 Hz for both sides. At month 3 follow-up visit, the parameters of the Left STN were refined to 2.3 mA and 3.4 mA 80 us and 130 Hz. The patient is currently continuing aDBS treatment and data are still under collection. This case report shows a practical framework for aDBS long term management which ultimately adds evidence to support the feasibility and clinical practice translation of aDBS.

Disclosures: **L. Romito:** 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.; Newronika SpA. **M. Arlotti:** A. Employment/Salary (full or part-time):; Newronika SpA. **V. Levi:** None. **S. Rinaldo:** None. **R. Eleopra:** 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.; Newronika SpA. **T. Mandat:** 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.; Newronika SpA. **M. Lanotte:** 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.; Newronika SpA. **M. Zibetti:** None. **L. Lopiano:** 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.; Newronika SpA. **C. Fonda:** None. **E. Pirola:** 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.; Newronika SpA. **M. Locatelli:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Newronika SpA. **A. Landi:** 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.; Newronika SpA. **A. Antonini:** None. **M.L.F. Janssen:** 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.; Newronika SpA. **Y. Temel:** None. **L. Ackermans:** None. **A. Priori:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Newronika SpA. **S. Marceglia:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Newronika SpA.

Poster

414. Electrical Stimulation: Human and Non-Human Primates

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Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 414.04

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH R01 NS111019

Title: In vivo application of electrical rejuvenation pulses to chronically implanted neural macroelectrodes in nonhuman primates for regulation of interface properties

Authors: *K. P. O'SULLIVAN[1], J. L. BAKER[3], B. PHILIP[1], M. E. ORAZEM[4], K. J. OTTO[5], C. R. BUTSON[2];
[1]Biomed. Engin., Univ. of Utah, SALT LAKE CITY, UT; [2]Norman Fixel Inst. for Neurolog. Dis., Univ. of Utah, Gainesville, FL; [3]BMRI, Cornell University: Weill Cornell Med. Col., New York, NY; [4]Chem. Engin., [5]Biomed. Engin., Univ. of Florida, Gainesville, FL

Abstract: Chronically implanted neural electrodes exhibit distinct changes in electrode-tissue interface properties over time, characterized by the development of a semicircular arc ("tissue component") in electrical impedance spectroscopy (EIS) measurements. This impedance change can impact clinically relevant stimulation as well as the signal-to-noise ratio (SNR) of single-unit recordings, and may be an important consideration in maintaining interface consistency for applications of closed-loop neuromodulation. Previous work has demonstrated the potential for direct-current electrical rejuvenation to reduce the impact of "tissue component" impedance on measured EIS spectra in microelectrodes chronically implanted in rodents. Our aim here is to further investigate this phenomenon using scaled versions of human deep brain stimulation (DBS) and electrocorticography (ECoG) electrodes that were chronically implanted in two adult male rhesus macaque non-human primates (NHP). Both direct-current (DC) and alternating current (AC) rejuvenation strategies were tested on segmented and unsegmented DBS electrodes implanted in the animals' thalami, as well as epidural single-row (8 channel) and grid style (32 channel) ECoG devices. Rejuvenation pulses were applied, and included a 1 V DC pulse for a duration of 4 seconds per electrode contact, as well as AC strategies under voltage control at 1 V and current control at 1 mA spanning a range of frequencies (1 Hz-10 kHz). *In-vivo* electrical impedance spectroscopy was performed pre- and post- rejuvenation, measured between 10 Hz and 100 kHz using a Palmsens4 Potentiostat. Results showed a consistent reduction in measured impedance and in observed "tissue component" across the EIS spectrum for all rejuvenation strategies, with current controlled rejuvenation producing the largest and longest-lasting effect. Identical electrodes to those used *in-vivo* were characterized *in-vitro* using the same protocols, and equivalent circuit models were created for both scenarios to aid in comparison and interpretation of results. This analysis demonstrates the potential for electrical rejuvenation as a strategy for regulating electrode-tissue interface changes over time, which may be a useful approach in future applications to stabilize closed-loop neuromodulation interfaces and to reduce electrical impedance for improved recording characteristics.

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Poster

414. Electrical Stimulation: Human and Non-Human Primates

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Topic: I.08. Methods to Modulate Neural Activity

Support: SNSF Sinergia // Grant CRSII5_183519

Title: Assessing hearing acuity in non-human primate: from frequency discrimination task to auditory cortex recordings using a novel soft ECoG technology

Authors: *E. REVOL[1], A. TROUILLET[1], F. V. COEN[1], F. FALLEGGER[1], M. DELACOMBAZ[2], A. CHANTHANY[2], F. LANZ[2], D. J. LEE[3], J. BLOCH[4], S. P. LACOUR[1]; [1]Lab. for Soft Bioelectronic Interfaces, Ctr. for Neuroprosthetics, Ecole Polytechnique Fédérale de Lausanne (EPFL), Geneva, Switzerland; [2]Dept. of Neurosci., Platform of Translational Neurosci., Fribourg, Switzerland; [3]Otology and Laryngology, Harvard Med. Sch., Boston, MA; [4]Dept. of Neurosurg., Ctr. Hospitalier Universitaire Vaudois (CHUV), Lausanne, Switzerland

Abstract: In the field of auditory neuroprosthetics, it is crucial to be able to evaluate a subject's hearing acuity in order to develop relevant prosthetic solutions, and notably to compare acuity in artificial versus natural hearing. In this study, we are aiming at measuring the hearing acuity of two non-human primates (NHP) using different metrics: an auditory behavioral task and a passive measure of cortical activity, using a soft electrocorticography (ECoG) neurotechnology, based on platinum/polyimide stretchable serpentines embedded in silicone and soft platinum-silicone electrode coating. Two rhesus monkeys were trained in a discrimination task, in which the animals learned using positive reinforcement, to differentiate tones. Auditory stimulation sequences included a series of reference and probe tones, both randomly chosen over each training session (ranging from 800Hz - 10kHz with positive/negative frequency difference from 10Hz - 2000Hz). Acuity was defined at the point when performance in the task drops by more than 15%. One animal was also implanted with a soft microECoG array laid subdurally over the auditory cortex. Auditory evoked potentials (AEPs) were measured in response to two alternating pure tones of different frequencies played in a continuous fashion to the animal. ECoG data were processed to reveal AEPs by synchronizing, filtering and averaging the waveforms over ~100 repeats. Hearing acuity was defined as the frequency difference between two tones below which no more AEP is clearly visible at the frequency switch (signals comparable to the presentation of a continuous tone or average z-scored amplitude of the AEP across the array is below 1.2x corresponding baseline amplitude). One animal displays an average performance of 77.8% (n=122 trials) when presented with two tones which difference was 10Hz (positive difference), while the second animal of 65.4% (n=162 trials). For both, the performance drops by 15% around 2Hz. When measured using ECoG on the second animal, we were able to discriminate responses for a frequency difference of 10Hz (positive difference) with a z-scored amplitude of ≥ 4 (absolute amplitude in the range of 15 µV). These first results suggest that the acoustic protocols in place are suitable to infer auditory acuity from behavioral performance. The next step is to correlate these with ECoG recordings, collect more data and extend to a broader frequency range or smaller frequency differences. In addition, the soft ECoG neurotechnology is sufficiently sensitive to decipher small changes in local field potentials.

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Poster

414. Electrical Stimulation: Human and Non-Human Primates

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Program #/Poster #: 414.06

Topic: I.08. Methods to Modulate Neural Activity

Support: SNSF Sinergia Grant CRSII5_183519

Title: A non-human primate &ltNHP&gt model for soft auditory brainstem neuro-prosthesis

Authors: *A. TROUILLET[1], E. REVOL[2], F.-V. COEN[1], F. FALLEGGER[3], A. CHANTHANY[4], M. DELACOMBAZ[4], A. GARCIA[5], L. ANSCHUETZ[6], J. BLOCH[7], D. LEE[8], S. P. LACOUR[9];
[2]STI - LSBI, [1]EPFL, Geneva, Switzerland; [3]École Polytechnique Fédérale De Lausanne, Geneve, Switzerland; [4]UNIFR, Fribourg, Switzerland; [5]Massachusetts Eye and Ear Infirmary, boston, MA; [6]inselspital, bern, Switzerland; [7]CHUV, Paudex, Switzerland; [8]Mass Eye and Ear, Boston, MA; [9]Swiss Federal Inst. of Technol., Lausanne, Switzerland

Abstract: In order to develop an efficient auditory neuro-prosthesis that can generate resolutive hearing percepts we have leveraged advances in the field of soft bioelectronics. Using microfabrication, we manufactured a multichannel, soft auditory brainstem implant(ABI) that conforms to the curved surface of the cochlear nucleus(CN) and delivers selective electrical stimulation. While cochlear implant is a successful auditory neuro-prosthesis, it is not suitable for a category of deaf patients presenting nerve damage, leaving them unaddressed. This study is designed to find an alternative solution to existing suboptimal central auditory neuro-prostheses.In a NHP, we designed electrophysiology and behavioral studies to assess soft ABI function and biointegration. We manufactured macaque sized, soft ABIs(150µm thick, 11 electrodes of 300µm diameter) and successfully implanted one adult macaca mulatta. The animal was first trained to a frequency-based discrimination task. When the animal mastered the task(performance rate above 60%), the soft neuroprosthesis was implanted under anesthesia. A retrosigmoid craniotomy of the left ear was performed and the array was placed under endoscopic visualization at the surface of the CN. Electrically evoked auditory evoked potentials(AEPs) were elicited by stimulation of the ABI intro-operatively, confirming the position and functionality of the ABI. The response was shown to be ABI specific as the eAEPs amplitudes were modulated according to the amplitude of stimulation(ranging from 10 to 40 µV / bipolar stimulation between 0.6 to 1.1 mA). We also determined the activation threshold, which was different from one electrode to the other, demonstrating the spatial resolution of our device. These results were confirmed behaviorally over the course of daily, awake recordings. The animal was presented randomly with purely acoustic sequences(ranging from 0.5 to 20kHZ), with sequences during which stimulation pulses were paired with the probe sound (0.1 to 1.5mA, acoustic 0.4 to 12kHz, several pairs of electrodes), and with purely electrical sequences(0.1 to 1.5mA, bipolar stimulation, several pairs of electrodes). Performance of each of the 3 conditions were then compared. Although there was some variability in its performance from day to day, the results indicate that soft ABI stimulation can evoke auditory percepts and can be used as a cue to perform a behavioral task.These results demonstrate the successful implementation of the first chronic NHP model for soft ABI. The preliminary investigation of this model shows that the soft ABI enable functional and resolutive auditory percepts.

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Poster

414. Electrical Stimulation: Human and Non-Human Primates

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Program #/Poster #: 414.07

Topic: I.08. Methods to Modulate Neural Activity

Title: Stability and sensitivity of cortical evoked potential measures in human stereoencephalography

Authors: *L. H. LEVINSON[1], S. SUN[2], C. J. PASCHALL[2], K. E. WEAVER[3], J. A. HERRON[4], A. L. KO[4], S. I. PERLMUTTER[5], J. G. OJEMANN[4];
[1]Neurosci., [2]Bioengineering, [3]Radiology, [4]Neurolog. Surgery, [5]Dept Physiol. & Biophysics, Washington Natl. Primate Res. Ctr., Univ. of Washington, Seattle, WA

Abstract: When individual pulses of electrical stimulation are applied to a region of cortical or subcortical grey matter through stereoencephalography (sEEG) electrodes, stereotyped responses (cortical evoked potentials; CEPs) are commonly recorded at local and remote sites throughout the brain. CEPs are a well-established metric of effective connectivity between the stimulation and recording sites. However, the number of single pulse trials used in these CEP studies ranges from as few as 10 to over 1000 - variation that does not necessarily reflect experimental needs. Little guidance exists for choosing an appropriate number of trials. Although too few trials may lead to unreliable results, higher trial counts increase the risk of stimulus adaptation, habituation, or temporal conditioning that may impact the very metrics of connectivity being measured. For the first time, we systematically approach the question of CEP experimental power. We quantify variability of CEP magnitude scalars (peak/trough amplitude, component root mean square) and temporal characteristics (peak/trough latency, number and polarity of components identified) as a function of the number of applied pulses. In 6 sEEG subjects, we delivered a minimum of 200 bipolar, biphasic DES pulses at pairs of adjacent electrodes that had been identified as evoking CEPs at one or more additional electrodes. We test the stability of CEP metrics using subsets of these trials, ranging from 10 to over 100, and investigate sensitivity, defined as the capacity to automatically detect CEP presence/absence and differences in CEP magnitude using the same trial subsets. We investigate if and how CEPs scalars change or evolve over the course of many stimulation trials. We conservatively estimate that 50 trials are needed to achieve stable results (variability (IQR) over 100 repetitions of randomly subsampling trials reaches an asymptotic minimum) that are consistent with the results when computed over the full 200+ trials (median RMS of 50 trials is in the 40-60% quantile range of all 200+ trials for all channels in >75/100 repeated random trial selections). When delivered at 0.5-1 Hz with random 25% jitter, collecting 50 trials of CEPs lasts <2 minutes, which is tractable even with stringent clinical time constraints, can be easily scaled to accommodate CEP measurement at multiple stimulation sites,

and ameliorates concerns about stimulation habituation and/or conditioning. These findings will allow us to more effectively use CEP data to characterize effective connectivity in sEEG and identify changes in this connectivity.

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Poster

414. Electrical Stimulation: Human and Non-Human Primates

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Program #/Poster #: 414.08

Topic: I.08. Methods to Modulate Neural Activity

Support: R01 MH 122258
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Title: Electrical stimulation of white matter pathways reveals widespread axonal projection fields

Authors: *D. HERMES[1], T. PAL ATTIA[1], N. GREGG[1], G. A. WORRELL[1], K. MUELLER[4], M.-H. IN[2], J. HUSTON[1], M. BERNSTEIN[1], K. J. MILLER[3];
[2]Neurologic Surgery, [3]Neurosurg., [1]Mayo Clin., Rochester, MN; [4]TU Berlin, Berlin, Germany

Abstract: Electrical brain stimulation is a growing therapy to treat neurological and neuropsychiatric disorders at the network level. Whereas some disorders involve a pathology in small, highly localized regions, others involve large networks that can span multiple distant territories. Understanding how electrical stimulation can influence activity in such networks is essential to developing novel stimulation therapies. In this study, we quantify the extent to which white and gray matter stimulation could drive the human brain.Diffusion MRI (dMRI) and T1 weighted images were collected on the high-performance compact 3T MRI scanner (Foo et al. 2018) at Mayo Clinic in three human subjects before stereo EEG (sEEG) electrodes were implanted for epilepsy monitoring purposes. During the subsequent sEEG monitoring period, we stimulated electrode pairs with a single biphasic pulse. We measured stimulation evoked potentials in all other electrodes and quantified the sites that showed a reliable evoked response. Reliable stimulation evoked potentials were observed in highly varying numbers of electrodes: some sites elicited many evoked potentials whereas others elicited few. To understand which stimulation sites drove large networks, we calculated anatomical properties of the stimulated sites. The fractional anisotropy (FA) for each electrode was extracted from the dMRI data. The data show that stimulating electrodes with higher FA values elicited evoked responses in significantly more electrodes compared to stimulating electrodes with lower FA values. The FA was more predictive of the number of outputs from each stimulated electrode pair compared to a simple gray and white matter assignment based on the segmentation of the T1 weighted image. These data indicate that white matter stimulation can drive large networks, whereas gray or white

matter stimulation with less anisotropic tissue drives smaller, more localized networks. Furthermore, electrical stimulation of white matter bundles can be used to map axonal projection fields.

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Poster

414. Electrical Stimulation: Human and Non-Human Primates

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Program #/Poster #: 414.09

Topic: I.08. Methods to Modulate Neural Activity

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Title: Comparison of human local field potential dynamics during different electrical stimulation conditions

Authors: ***S. H. SUN**[1], L. LEVINSON[2], C. J. PASCHALL[1], K. E. WEAVER[3], J. S. HAUPTMAN[6], A. KO[4], J. A. HERRON[4], J. G. OJEMANN[4], R. P. RAO[5];
[1]Bioengineering, [2]Neurosci., [3]Radiology, [4]Neurolog. Surgery, [5]Paul G. Allen Sch. for Computer Sci. and Engin., Univ. of Washington, Seattle, WA; [6]Neurosurg., Seattle Children's Hosp., Seattle, WA

Abstract: A growing area of interest in electrical stimulation for neurorehabilitation is adaptive stimulation, where stimulation parameters are adjusted in real-time, informed by a neural biomarker, to reduce stimulation-induced side effects, account for stimulation adaptation and disease progression, and improve stimulation outcomes. While advancements have been made in identifying neural biomarkers for pain, mood, and other potential applications of adaptive stimulation, little is known regarding how to adjust electrical stimulation to drive specific changes in identified biomarkers. To establish a relationship between electrical stimulation and a target neural biomarker, we must first understand how electrical stimulation interacts with underlying activity to produce our recorded neural response. Our prior work identified that local field potential responses to different electrical stimulation conditions are separable, and these responses dynamically transformed over the duration of stimulation. Here, we present work on temporal dynamics of local field potential response to stimulation and comparison of dynamics between stimulation conditions. We performed stimulation experiments with epilepsy patients (n=4) undergoing clinical monitoring via stereo-electroencephalography. We presented 5-7 stimulation conditions that varied in amplitude and frequency at fixed, 5-second intervals in

random sequence, and this sequence was repeated as time permitted. From our recordings, we removed stimulation artifacts and performed spectrogram analysis to examine time-frequency dynamics during stimulation. We used dimensionality reduction methods, such as principal component analysis, to identify a low-dimensional representation of time-frequency dynamics during stimulation. We compared response trajectories and identified whether there exist common dynamic modes between stimulation conditions, which can inform whether neural responses are driven more by stimulation or by underlying neural activity. We expect these results to reveal how electrical stimulation and underlying activity respectively contribute to local field potential activity, and we hope that these results establish new methods of evaluating neural responses to electrical stimulation.

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Poster

414. Electrical Stimulation: Human and Non-Human Primates

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Program #/Poster #: 414.10

Topic: I.08. Methods to Modulate Neural Activity

Title: Modulation effects of non-invasive transcutaneous auricular vagus nerve stimulation on processing of emotional visual stimuli: a pilot study

Authors: *S. TUKAIEV[1], O. PRAVDA[2], V. KOMARENKO[2], S. DANYLOV[2], V. KRAVCHENKO[1], M. MAKARCHUK[1], K. MASHTALERCHUK[1], N. VYSOKOV[3], D. TOLEUKHANOV[3], A. TARASENKO[3];
[1]Inst. of Biol. and Med., Natl. Taras Shevchenko Univ. of Kyiv, Kyiv, Ukraine; [2]Beehiveor Acad. and R&D Labs, Kyiv, Ukraine; [3]BrainPatch, London, United Kingdom

Abstract: Many neuropsychiatric disorders are accompanied by emotional problems. From a neurophysiological point of view, both depression and burnout can arise from the disturbances in neural networks associated with emotional regulation and recognition of emotional states. Vagus nerve stimulation (VNS) is a promising neuromodulation therapy technique for treatment-resistant depression. Due to reducing anxiety and burnout auricular VNS can improve the cognitive functioning. The aim of the current study was to evaluate the effects of the non-invasive vagus nerve stimulation on emotional burnout and visual emotional information perception. 11 healthy men volunteers (stimulation (6 persons) and Sham/control (5 persons) groups), aged 18 to 22 years (Mage = 19.5, SD = 1.36 years) participated in this study. We used the combination of pleasant meditative classical music and a slow bi-polar wave (0.1-0.2 Hz) of electrical non-invasive transcutaneous auricular VNS for 5 minutes by BrainPatch platform for non-invasive stimulation. The set of 4 VNS was performed with intervals of 3 days. EEG was registered during the rest state (3 min, closed eyes condition). The participants were presented a

set of alarming images, taken from the NAPS database. To measure the severity of emotional burnout we used the 22-item Maslach Burnout Inventory. VNS significantly attenuated the emotional burnout (improvement of the depersonalization and reduction of personal achievements). Changes in the psychoemotional state of the respondents were accompanied by the increase in the theta-Fz/alpha-Pz ratio, that reflects an enhancement of the activation level. An increase in alpha rhythm may reflect internally oriented attention in creative activities. After 1th session of VHS event-related EEG activity analysis detected activation of cortical structures involved in the stimulus processing (verbal memory (Fz) and cognitive processes (P3)) 600-800 ms after visual stimuli exposition. 4th VHS session leaded to changes of the temporal pattern of processing visual emotional information: we observed the activation of processes associated with emotional understanding (750 ms after the stimuli presentation), processes associated with attention, judgments formation and verbal memory (1200 ms). We may conclude that vagus nerve stimulation has enhanced the cognitive processes involved in the processing of stimuli and changed the temporal pattern of processing visual emotional information.

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Poster

414. Electrical Stimulation: Human and Non-Human Primates

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Topic: I.08. Methods to Modulate Neural Activity

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Title: Non-invasive transauricular vagal nerve stimulation mimics invasive vagal nerve stimulation and could act as an alternative treatment option

Authors: ***M. OWENS**[1], L. DUGAN[1], C. COOPER[1], V. JACQUEMET[2,3], V. NAPADOW[4], E. BEAUMONT[1];
[1]Biomed. Sci., East Tennessee State Univ., Johnson City, TN; [2]Dept. of Pharmacol. and Physiol., Univ. of Montreal, Montreal, QC, Canada; [3]Res. Ctr., Canada and Sacred Heart Hosp. of Montreal, Montreal, QC, Canada; [4]Dept. of Physical Med. and Rehabil., Harvard Med. Sch., Boston, MA

Abstract: Vagal nerve stimulation (VNS) is a neuromodulatory therapy under investigation as a treatment option for multiple disorders. Activation of the vagus nerve generates afferent signaling to the nucleus of the solitary tract (NTS) where information is processed and propagated to higher brain regions allowing for sympathetic and parasympathetic control. Studies have shown that NTS has substantial connections with the autonomic system making it a potential target in treating disorders like heart failure and gastric motility disorders. Two VNS

methods are under investigation. Cervical vagal nerve stimulation (cVNS) is an invasive method of activating the vagus by targeting the cervical branch in the neck and is currently FDA approved for drug-resistant epilepsy and depression. The non-invasive transcutaneous auricular vagal nerve stimulation (taVNS) method targets the subcutaneous auricular branch at the cymba concha of the ear. Human fMRI studies have shown that both methods lead to activation of NTS, yet few studies have compared their effects. In this study, we used electrophysiological methods to compare neuronal activity changes elicited by cVNS and taVNS in 12 chloralose-anesthetized rats. A high-impedance tungsten electrode was stereotaxically placed into NTS to record individual extracellular activity from 50-70 neurons following VNS conducted at a frequency of 20Hz. Two stimulation intensities were used for taVNS (0.5, 1.0mA), and bradycardic intensity (the intensity level that elicits a transient 5% decrease in heart rate) was used for cVNS. Changes in neuronal activity from the two methods were analyzed during and immediately following stimulation by comparing firing rates to baseline activity using Spike9.0 software. Neurons were classified as positive or negative responders if firing rates increased or decreased, respectively, by 20% or non-responders if firing rate changes were less than 20%. Our data suggests that both taVNS and cVNS conducted at 20Hz generates a comparable response with no significant differences in the number of responders generated ($p=0.45$). Both methods resulted in a greater number of positive responders as compared to negative responders ($p<0.03$). Additionally, similarities in the pattern of activation show an immediate increase in firing rate at stimulation onset that remains weakly elevated following the cessation of stimulation. The identification of taVNS stimulation parameters that mimic cVNS activation could allow for a non-invasive treatment option in many chronic disorders.

Disclosures: **M. Owens:** None. **L. Dugan:** None. **C. Cooper:** None. **V. Jacquemet:** None. **V. Napadow:** None. **E. Beaumont:** None.

Poster

414. Electrical Stimulation: Human and Non-Human Primates

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 414.12

Topic: I.08. Methods to Modulate Neural Activity

Support: FWO SB fellowship 1S32421N
FWO-Project G0B4520N
NIH 1R01MH123508-01

Title: Investigate tDCS neurophysiological mechanisms using a motor sequence learning paradigm in healthy volunteers

Authors: *S. KERSTENS[1], L. VAN BOEKHOLDT[1], H. VANDERHEYDEN[1], N. SEMINCK[1], T. VAN BOGAERT[1], L. DE SMEDT[1], G. ALBOUY[4], B. R. KING[2], J.-J. ORBAN DE XIVRY[3], M. MC LAUGHLIN[1];

[1]Dept. of Neurosciences, KU Leuven, Leuven, Belgium; [2]Dept. of Hlth. & Kinesiology, KU Leuven, Salt Lake City, UT; [3]KU Leuven, Leuven, Belgium; [4]Univ. of Utah, Salt Lake City, UT

Abstract: Transcranial direct current stimulation (tDCS) is a noninvasive neuromodulation method that aims to modulate cortical excitability by applying a direct current via scalp electrodes. It is generally accepted that tDCS modulates brain activity by direct polarization of cortical neurons. However, recent studies show that the resulting electric field in the cortex is relatively weak and suggest that instead the high electric field in the scalp may stimulate peripheral nerves and cause the observed tDCS effect via an indirect peripheral mechanism. The trigeminal and occipital nerve in the scalp ascend information to the somatosensory cortex via the brainstem, where they give input to the locus coeruleus (LC) and other brain regions. The LC is a key nucleus of the sympathetic nervous system and controls synthesis and release of norepinephrine in the brain. Interestingly, indirect stimulation of the LC during tDCS can also affect cortical excitability and plasticity. Previous research has shown that tDCS can modulate human behavior, such as the ability to learn sequences of movements. To investigate whether the effects of tDCS on motor sequence learning are caused by increased cortical excitability due to direct polarization of neurons or by stimulation of peripheral nerves in the scalp, we developed a novel tDCS stimulation condition in which the peripheral input is blocked using a topical anesthetic. In a double-blinded healthy volunteer study (99 subjects, M and F, age 18 - 40y), we use a serial reaction time task (SRTT) to compare the effect of standard tDCS, sham stimulation and tDCS plus topical anesthetic (a-tDCS) on motor sequence learning. Stimulation was applied over the left primary motor cortex with the reference in the contralateral supraorbital position. In the SRTT, subjects learned a motor sequence in three sessions on consecutive days. Progress in motor sequence learning was assessed at the end of each session, as well as 24h later to include the effect of consolidation. Preliminary results (currently n = 20) indicate all subjects significantly improved in motor sequence learning ($p < 0.0001$, 2-way-RM-ANOVA) over time. In a preliminary post-hoc analysis using two-sample t-tests, we found no significant differences between tDCS and sham ($p = 0.7619$), nor between tDCS and a-tDCS ($p = 0.8246$), or between sham and a-tDCS ($p = 0.8543$) after three days of learning. Initial results indicate that subjects significantly learned the sequence, but tDCS had no significant effect on this learning process, neither via direct polarization of cortical neurons nor via the peripheral mechanism. The full group of subjects should be tested to confirm these preliminary findings.

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Poster

414. Electrical Stimulation: Human and Non-Human Primates

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 414.13

Topic: I.08. Methods to Modulate Neural Activity

Support: NRF-2015M3C7A1031969
NRF-2021R1F1A1052020

Title: Impacts of transcranial direct current stimulation on the temporal and spatial profiles of fMRI activity in the human visual cortex

Authors: *J. AHN[1], J. RYU[1], S. LEE[2], C. LEE[3], C.-H. IM[4], S.-H. LEE[1];
[1]Seoul Natl. Univ., Seoul, Korea, Republic of; [2]Univ. of Minnesota, Minneapolis, MN; [3]Korea Brain Res. Inst., Daegu, Korea, Republic of; [4]Hanyang Univ., Seoul, Korea, Republic of

Abstract: Transcranial direct current stimulation (tDCS) is a widely used, non-invasive brain stimulation method that applies a weak direct current to the brain through the scalp. Despite its popularity in clinical application and cognitive intervention, the exact impacts of tDCS on brain activity remain elusive. Animal studies reported that tDCS affects membrane potentials during stimulation and this effect lasted for several minutes after stimulation ceased. Recent neuroimaging studies on human brains also measured the impacts of tDCS on cortical excitability but reported inconsistent results. Being motivated to detail the impacts of tDCS on brain activity reliably and rigorously, we acquired functional magnetic resonance imaging (fMRI) measurements from the human visual cortex with the following strategies. First, to control for the tDCS-irrelevant changes of fMRI signals that are idiosyncratic to individuals, and even daily sessions, we adopted a sham-controlled crossover design in which each subject participates in all the three types of daily tDCS sessions—the 'anodal', 'cathodal', and 'sham' sessions. Second, to apply electrical signals effectively and to minimize the differences across individual brains, the stimulation was tailored for each individual via electric field simulation. Third, we chose the early retinotopic visual cortex as a testing site because its well-established functional architecture and the advanced experimental and analysis protocols allow us to detect the impacts of tDCS in diverse aspects. Specifically, to inspect how tDCS affects the temporal and spatial dynamics of cortical activity, we measured fMRI responses to brief (3 s) whole-field stimuli and traveling-wave stimuli. Then we characterized the temporal dynamics by estimating the baseline, response amplitude, and sustained response, and quantified the spatial tuning width by fitting a population receptive field model. We found the significant impacts of tDCS on the baseline measure and the spatial tuning width *only after* (i.e., offline), but not during (i.e., online), anodal tDCS. The offline anodal tDCS increased the baseline of the fMRI time course ($z = 7.00$, FDR-adjusted $p < 0.05$ across voxels) and decreased the spatial tuning width ($z = -5.19$, FDR-adjusted $p < 0.05$ across voxels). Our results corroborate the findings in the animal studies by demonstrating the robust impact of tDCS on the baseline fMRI activity, which is known to be closely associated with that of membrane potentials in the intracellular recording. Furthermore, the current work goes beyond previous work by discovering a novel impact of tDCS on the tuning properties of the human visual cortex.

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Poster

414. Electrical Stimulation: Human and Non-Human Primates

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Program #/Poster #: 414.14

Topic: I.08. Methods to Modulate Neural Activity

Support: European Union's Horizon 2020 research and innovation programme 2021-2024 under grant agreement number 101017716 ("Neurotwin")

Title: Induction and stabilization of gamma oscillations in the human brain

Authors: *B. GLINSKI[1,2], M. SALEHINEJAD[1], K. TAKAHASHI[1,2], M.-F. KUO[1], M. A. NITSCHE[1,3];
[1]The Leibniz Res. Ctr. for Working Envrn. and Human Factors (IfADo), Dortmund, Germany; [2]Dept. of Psychology, Ruhr-University Bochum, Bochum, Germany; [3]Dept. of Neurol., Univ. Med. Hosp. Bergmannsheil, Bochum, Germany

Abstract: Alzheimer's disease (AD) constitutes a serious burden on the global health system. According to the World Health Organization (WHO) dementia cases, including AD, will double by 2030 and triple by 2050. However, treatment approaches tackling underlying causes of AD are unavailable. Accumulated evidence suggests non-pharmacological interventions such as non-invasive brain stimulation (NIBS) technologies e.g., transcranial alternating current stimulation (tACS) or transcranial magnetic stimulation (TMS), especially in the gamma frequency range, as a potential treatment approach for underlying causes of AD. The efficacy of these techniques is however unclear, and optimizing studies for maximizing therapeutic effects are required. The present within-subject, randomized, crossover, single-blinded study, aimed to develop novel NIBS protocols for the induction and stabilization of gamma oscillations in the human cortex of 30 young healthy participants. In total five NIBS protocols were explored. These protocols include (1) a customized gamma intermittent theta burst protocol (gTBS), (2) a gamma tACS protocol, (3,4) two gTBS protocols phase-locked to either the peak or the trough of the tACS (Peak/Trough X-tACS) and (5) a Sham protocol. The effects of these protocols were examined on resting-state electroencephalography (EEG). Preliminary results show varying effects of the different NIBS protocols on resting-state EEG activity. The application of Trough X-tACS generated strong short-lasting effects on oscillatory activity in the gamma range (up to 30 minutes), whereas tACS alone produced a linear increase of gamma oscillations by time, peaking two hours after the stimulation. The Peak X-tACS protocol led to a short-lasting inhibition (up to 30 minutes) of gamma oscillations before an increase was seen. The administration of the gTBS protocol showed a minor facilitatory effect on gamma oscillatory activity compared to Trough X-tACS. No effects were observed for the sham stimulation. The results suggest the effectiveness of different non-invasive brain stimulation protocols for the facilitation of gamma oscillations in the healthy human cortex. These effects nominate NIBS as a promising approach in pathological populations.

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Poster

414. Electrical Stimulation: Human and Non-Human Primates

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 414.15

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant UL1TR002003

Title: Transcranial electrical stimulation on heart rate variability during emotion regulation in individuals with internalizing psychopathologies

Authors: *J. MCALEER, L. STEWART, R. SHEPARD, M. SHEENA, S. KABIR, I. SWANK, J. P. STANGE, A. LEOW, H. KLUMPP, O. AJILORE;
Univ. of Illinois at Chicago, Chicago, IL

Abstract: Internalizing psychopathologies (IPs) are characterized by disruptions in emotion regulation (ER). A potential target for ER modulation in individuals with IPs is the theta band. We hypothesized that offset theta-tACS (transcranial alternating current stimulation) would result in more enhanced ER, indexed by greater increase in heart rate variability (HRV), than transcranial direct current stimulation (tDCS) in participants with IPs due to tACS's ability to target frequency-specific dysregulation. This pilot study utilized a double-blind, pseudo-counterbalanced design. Participants (n=29) with internalizing psychopathologies, such as depression and anxiety, were randomly assigned to receive either offset theta-tACS or tDCS and underwent four sessions of stimulation (two sham). In both arms, there were alternating iterations of an emotion regulation task (ERT) during or immediately after stimulation and rest. Heart rate data were collected during each ERT and rest iteration, and analyses were completed using high-frequency (HF) and root mean square of successive differences (RMSSD) HRV metrics. tACS participants displayed consistent increases in both HRV metrics from Time 1 to Time 4. Participants receiving tDCS displayed few significant changes in HF-HRV and no significant changes in RMSSD-HRV. The lack of a baseline ERT makes it difficult to determine overall ER improvement, but tACS appears to increase ER capacity as reflected in increased HRV in individuals with IPs, particularly after two sessions of stimulation. Though the small sample size disallowed a direct comparison of tACS and tDCS participants, the significant improvements demonstrated by the tACS participants was not mirrored by the tDCS participants. This study adds validity to the use of tACS as a neuromodulatory technique in cognitive and clinical research. Additional research is required to better understand potential carry-over effects of multiple sessions of stimulation.

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Poster

414. Electrical Stimulation: Human and Non-Human Primates

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 414.16

Topic: I.08. Methods to Modulate Neural Activity

Title: Investigation of the location of action of low-intensity focused ultrasound (TUS) within the human motor cortex using transcranial magnetic stimulation (TMS) - A combined TMS-TUS study

Authors: *T. ARORA[1], C. CUI[2], N. K. DESAI[1], T. C. GRIPPE[1], J.-F. NANKOO[1], Y. DING[1], R. CHEN[1];
[1]Krembil Res. Inst., Univ. Hlth. Network, Toronto, ON, Canada; [2]Inst. of Med. Sci., Univ. of Toronto, Toronto, ON, Canada

Abstract: Low-intensity transcranial ultrasound stimulation (TUS) offers non-invasive targeting of neural structures at different depths with high spatial resolution. In humans, TUS modulates cortical structures; however, the underlying pathways are not well understood. We are using transcranial magnetic stimulation (TMS) to understand the pathways within the human motor cortex (M1) that are targeted by TUS. TMS of M1 generates evoked potential (MEP) in the target muscles, which involves direct (pyramidal axons) and indirect (intracortical) pathways. The direct (D) and indirect pathways (I1 and I3) can be preferentially targeted with TMS coil orientations that induce currents in posterior-anterior (PA), lateral-medial (LM), and anterior-posterior (AP) directions, respectively. Previously we have shown that TUS, when delivered during TMS, can inhibit the amplitude of MEP (online effect). To better understand the pathways targeted by the TUS, we aim to investigate and compare the extent of TUS-based MEP amplitude inhibition during LM, PA, and AP TMS coil orientations. We hypothesize that TUS will lead to different extent of MEP inhibition for different TMS coil orientations and target depth. We studied 16 young (29.1 ± 7.1 years) adults. TMS was delivered to M1 using PA, LM, and AP coil orientations in combination with real (0W) or sham (20W) TUS. For each coil orientation, there were 3 random blocks (20 MEPs/block) of TUS conditions (30 or 50mm depth or sham). The test TMS (sham TUS) was delivered at an intensity that evoked 1 mV MEP (0.5-1.5 mV) amplitude. The MEP amplitudes for TUS conditions were normalized to the test TMS amplitude such that values < 1 indicated inhibition. The latency and the normalized MEP amplitude were compared between different TMS orientations and TUS depths. We investigated the main effects of the TMS orientation, TUS depth, and their interactions on the MEP latency and normalized amplitude using a linear mixed-effect model.For MEP latency, there were significant main effects of TMS orientation [$F(2, 1114) = 17.37$, $p < .0001$]. Follow-up paired *t*-tests revealed significant differences between all TMS orientations; LM (21.1 ± 2.1 ms) < PA (21.7 ± 1.9 ms) < AP (22.5 ± 2.1 ms) confirming activation of D, I1 and I3 pathways with

respective TMS orientations. For normalized MEP amplitude, there were significant main effects of TMS orientation [F (2, 71) = 4.51, p = .014]. Follow-up paired t-tests confirmed significantly greater inhibition for PA and ML in comparison to AP orientations; PA (0.85 ± .36) ~ LM (.90 ± .32) < AP (1.10 ± .36). The results confirm TUS preferentially targeted I1-and D-wave pathways, whereas the I3-wave pathway remained uninhibited.

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Poster

414. Electrical Stimulation: Human and Non-Human Primates

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 414.17

Topic: I.08. Methods to Modulate Neural Activity

Support: MRC MC_UU_0003/3
203139/Z/16/Z

Title: Comparison of plasticity effects of monophasic and biphasic theta-burst stimulation using modulation-based TMS

Authors: *K. WENDT[1,2], M. MEMARIAN SORKHABI[2], J. O'SHEA[3], T. J. DENISON[1,2]; [1]Dept. of Engin. Sci., Oxford Univ., Oxford, United Kingdom; [2]MRC Brain Network Dynamics Unit, Nuffield Dept. of Clin. Neurosciences, [3]Wellcome Ctr. for Integrative Neuroimaging, Oxford Ctr. for Human Brain Activity, Univ. of Oxford, Oxford, United Kingdom

Abstract: Transcranial magnetic stimulation (TMS) is a non-invasive method to stimulate the brain, with repetitive pulse trains changing corticospinal excitability that out-lasts the stimulation period. Theta-burst stimulation (TBS), a protocol that applies gamma-frequency bursts (50 Hz) repeated in the theta-frequency range (5 Hz), is FDA-approved for the treatment of major depressive disorder when applied daily over weeks. TBS is mostly applied using biphasic pulse shapes, owing to the need to recover large amounts of energy after each pulse. However, monophasic pulses have been hypothesised to more selectively recruit cortical neurons compared to biphasic pulses, so monophasic stimulation could increase the effectiveness of TBS. Recent technological advances have allowed different pulse shapes to be applied at faster rates, paving the way for monophasic TBS protocols. In this within-subjects study, we used a custom-made pulse width modulation-based TMS device to apply monophasic and biphasic intermittent TBS to the primary motor cortex of 19 healthy right-handed volunteers and measured changes in motor corticospinal excitability using motor evoked potentials (MEP). Protocols were applied in counter-balanced order in separate sessions, at least one week apart. Each session started with two baseline blocks, consisting of 30 pulses applied at 120% of the resting motor threshold (RMT), followed by one of the TBS conditions (mono/bi-phasic) applied at 70% of the RMT. Over a 1-hour follow-up period MEPs were assessed using the same 30-pulse blocks every 5-10

minutes. The participants were blinded to the TBS condition and the hypothesis. Individual MEPs at each time point were averaged within-blocks for each participant and normalised by the baseline average. For the group analysis, normalized individual block means for each condition were analysed across participants. Our results show that when averaged over the 60 min follow-up period, monophasic iTBS led to a significant increase in MEP amplitude of 25.60% (± 29.72% SD) compared to baseline ($t(18)=3.76$; $p=.00$). Biphasic iTBS led to an increase of 17.42% (± 15.49%) for the same time period ($t(18)=4.90$; $p=.00$). ANOVA revealed no significant difference between monophasic and biphasic iTBS ($F(1,18)=1.58$; $p=.23$) and no interaction effect of the factors time and TBS type ($F(7,126)=0.57$; $p=.78$). In summary, our study demonstrates the application of monophasic and biphasic iTBS with a custom-made pulse width modulation-based TMS device. Over a period of 60 minutes, both pulse shapes lead to an average increase in corticospinal excitability of similar size to conventional TMS devices.

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Poster

414. Electrical Stimulation: Human and Non-Human Primates

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 414.18

Topic: I.08. Methods to Modulate Neural Activity

Title: Priming reduced the interindividual after-effect variability of anodal transcranial direct current stimulation

Authors: *S. FRESNOZA[1], A. TRUMMER[1], M. CHRISTOVA[2,3], A. ISCHEBECK[1];
[1]Inst. of Psychology, Univ. of Graz, Graz, Austria; [2]Otto Loewi Res. Center, Physiol. Section, Med. Univ. of Graz, Graz, Austria; [3]Inst. of Physiotherapy, Univ. of Applied Sci. FH-Joanneum, Graz, Austria

Abstract: Transcranial direct current stimulation (tDCS) involves the application of a weak direct current through scalp electrodes. Early electrophysiological studies showed a polarity-dependent excitability modulation in the motor cortex (M1): anodal tDCS (a-tDCS) increased excitability, while cathodal tDCS (c-tDCS) decreased it. However, recent studies revealed interindividually highly variable after-effects. Some participants showed a decrease or no response in excitability for a-tDCS and reversed effects for c-tDCS. Suggested possible causes for this variability range from skull thickness and morphology, age, gender, genetic variations, working memory capacity, time of day, caffeine consumption, baseline levels of intracortical inhibition and facilitation, as well as sensitivity to transcranial magnetic stimulation (TMS). One additional factor could be the impact of past synaptic activity or “cortical history” on the after-effect of tDCS. Here, we explored the impact of prior “cortical history” on the after-effect of a-

tDCS. We hypothesized that interindividual response variability would be less in a group of subjects with similar cortical history before stimulation. Twenty-eight healthy male participants (18-30 years old) received a-tDCS with standard parameters: 1 mA intensity, 13 min duration with a 10-sec current ramping, and 35 cm² electrodes with a left M1-right supraorbital montage. They were equally assigned to a "primed group" that viewed a video as priming protocol and a control group ("unprimed") that did not watch a video before stimulation. Corticospinal excitability was measured using TMS-elicited motor-evoked potentials (MEPs) before, immediately after (0 min), and 5, 10, 15, 20, 25, and 30 min after stimulation. The analysis performed on raw MEPs showed a significant increase in corticospinal excitability in both groups compared to baseline. Pairwise comparisons revealed greater excitability in the primed than the unprimed group at 5, 20 and 25 minutes after stimulation. Clustering the participants based on the grand average of normalized MEP values (0 to 30 min) revealed that all participants in the primed group responded (>1 mV, facilitation) to a-tDCS stimulation compared to five responders and nine non-responders (<1 mV, inhibition) in the unprimed group. These results show that simple priming interventions can improve reliability of the stimulation effects , which is crucial for therapeutic applications.

Disclosures: **S. Fresnoza:** A. Employment/Salary (full or part-time):; University of Graz. **A. Trummer:** None. **M. Christova:** None. **A. Ischebeck:** None.

Poster

414. Electrical Stimulation: Human and Non-Human Primates

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 414.19

Topic: I.08. Methods to Modulate Neural Activity

Support: NSF CAREER Award 1845348

Title: Feasibility study of phase-locked transcranial magnetic stimulation of cerebellum for the treatment of essential tremor

Authors: *X. ZHANG[1], R. HANCOCK[2], S. SANTANIELLO[1];
[1]Biomed. Engin. Dept., [2]Dept. of Psychology, Univ. of Connecticut, Storrs, CT

Abstract: Motor cerebellum, in particular Lobule VIII of the cerebellar cortex, has recently been proposed as a potential target for transcranial magnetic stimulation (TMS) to suppress upper extremity tremors in patients affected by Essential Tremor (ET). Early studies using cerebellar TMS showed some positive results, which however lacked robustness and consistency across patient cohorts, and the mechanism of action is poorly understood. To address these issues and assess the feasibility of cerebellar TMS for ET, we developed a computational pipeline in SimNIBS that combines finite element modeling of the human cerebellum, the MagVenture DB80 coil, and the induced electric field, with optimized coil position (maximal field norm) to target the Lobule VIII of the left hemisphere. We estimated the response of Purkinje cells (PCs)

by pairing the induced electric field with multi-compartment models of PCs arranged along the cerebellar cortical surface. We found that, given the optimized coil position, a biphasic TMS pulse with intensity between 60~100A/μs can activate between 16.5%~38.2% of the PCs in Lobule VIII.
Then, we mapped the estimated responses of the PCs onto an established computational model of the cerebello-thalamocortical circuit that has been shown to recapitulate the cellular mechanisms underlying ET, and we varied the frequency and pattern of the TMS sequences to assess its effects on tremor. We found that both regularly spaced (frequency: 3~8Hz) and irregular TMS pulse trains activating up to 40% of the PCs have very low efficacy in breaking tremor oscillations within 5,000ms (<20% success rate in both cases). In contrast, phase-locked TMS with a preferred phase between -0.56π~-0.08π reliably breaks tremor oscillations (tremor survival time since stimulation onset: 774±537ms, mean±S.D.), even when activating only 20% of the PCs.
Altogether, the study indicates that phase locked cerebellar TMS can provide an acute and robust suppression of tremor while operating within the established safety boundaries, even though the preferred phase is likely patient-specific. Mechanistically, our study suggests that cerebellar TMS operates by disrupting the pacemaker mechanism along the olivo-cerebellar loop, and the preferred phase corresponds to the middle of the silent period between complex spikes of the PCs.

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Poster

414. Electrical Stimulation: Human and Non-Human Primates

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 414.20

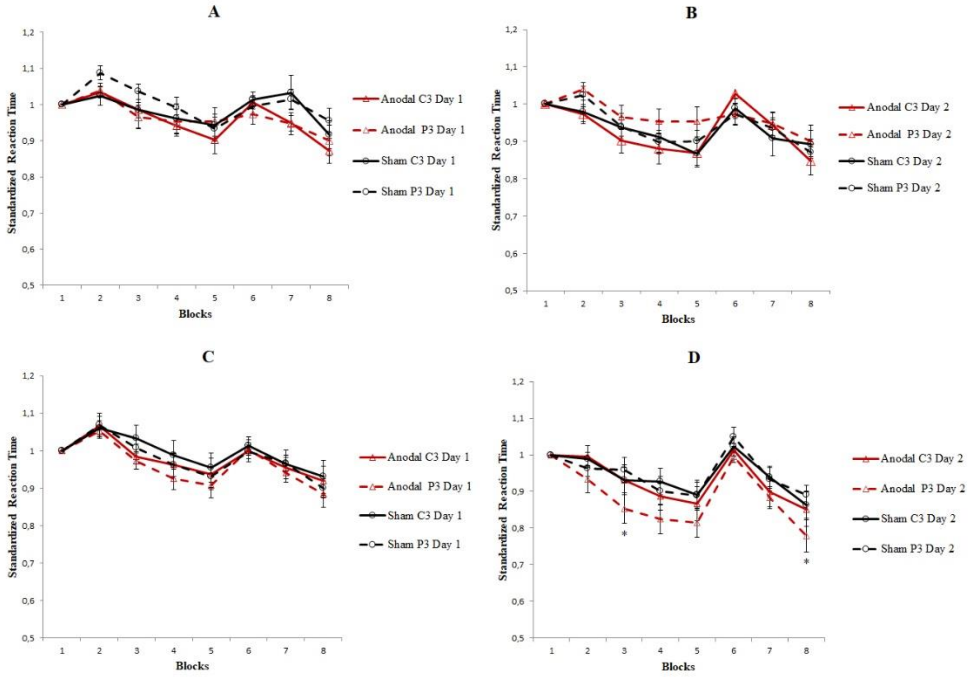
Topic: I.08. Methods to Modulate Neural Activity

Title: Effects of tDCS over the primary motor and posterior parietal cortex on different stages of motor learning

Authors: *G. RIVERA-URBINA[1], A. MOLERO-CHAMIZO[2], M. A. NITSCHE[3];
[1]Autonomous Univ. of Baja California, Ensenada,, Mexico; [2]Univ. of Huelva, Huelva, Spain; [3]Dept. of Neurol. Univ. Med. Hosp. Bergmannsheil, Bochum, Germany., Leibniz Res. Ctr. for Working Envrn. and Human Factors, Dortmund, Germany., Leibniz, Germany, Germany

Abstract: In this study we aimed to explore the efficacy of anodaltDCS applied over the posterior parietal cortex (PPC) compared to primary motorcortex (M1), which is involved in memory motor processes, on serial reactiontime task (SRTT) performance. Specifically, to evaluate the involvement of bothmotor learning network components, we compared the effects of tDCS applied overregions corresponding to M1 and PPC during the early and late stages oflearning. All subjects gave informed written consent before participation. Thisstudy was conducted in conformance with the policies and principles containedin the Federal Policy for the

Protection of Human Subjects and the World MedicalAssociation Declaration of Helsinki. The results revealed a selectiveimprovement of reaction time (RT) during anodal stimulation over the PPC in thelate stage of learning. These findings support the assumption that the PPC isrelevant during specific phases of learning, at least for SRTT performance. Theresults also indicate that not only the target area (i.e., PPC), but alsotiming is crucial for achieving the effects of stimulation on motor learning.



Disclosures: G. Rivera-Urbina: None. **A. Molero-Chamizo:** None. **M.A. Nitsche:** None.

Poster

414. Electrical Stimulation: Human and Non-Human Primates

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 414.21

Topic: I.08. Methods to Modulate Neural Activity

Support: Indiana University Addictions Grand Challenge grant

Title: Temporal interference neurostimulation yields fMRI BOLD activation in humans

Authors: *P. MODAK[1], B. COLON[1], E. NEED[1], J. M. FINE[3], L. HULVERSHORN[2], P. FINN[1], J. W. BROWN[1];
[1]Indiana Univ., Bloomington, IN; [2]Indiana Univ., Indianapolis, IN; [3]Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Temporal interference (TI) electrical neurostimulation (Grossman et al. 2017) is a relatively new approach to non-invasive stimulation of deep brain regions. Due to its non-invasive nature, this method holds promise for treatment of conditions including substance use disorder and Parkinson's disease that have previously been shown to benefit from stimulation of certain brain areas like the nucleus accumbens (NAc) and the basal ganglia, respectively. We tested whether TI stimulation targeted at the right NAc successfully stimulates it. To test this, we collected functional MRI data from healthy subjects (n=8) while they were stimulated using the TI stimulation. TI stimulation utilizes the low-frequency current interference pattern resulting from two slightly different high-frequency currents to stimulate a target region. Since the stimulation depends on the site of interference which further depends on the location of current sources, it is possible to select a certain target location, NAc in our case, and obtain the optimal scalp locations of the current electrodes. We found the optimal electrode locations in a 10-20 system for a standard MNI brain using finite element analysis. Our results from pilot data showed strong stimulation effects in a number of subjects (n=5), with the stimulated region slightly dorsal and anterior to NAc, noting that the exact location of stimulation was different for each subject. The spatial variability may be due to variation in the head geometries of different subjects, as we used only the average MNI template to optimize electrode positions. Further, we also observed that the neighboring regions of the stimulated area often showed deactivation suggesting that TI stimulation not only leads to activation at the location of interference but may also lead to inhibition in certain off-target neighboring regions (Mirzakhalili et al., 2020). We demonstrated that the technique could stimulate deep brain regions but identify potential challenges such as the inhibition in certain off-target regions and the need to optimize the electrode locations for individual subjects. These results contribute to the development of TI stimulation of brain regions as a potential treatment method.

Disclosures: P. Modak: None. **B. Colon:** None. **E. Need:** None. **J.M. Fine:** None. **L. Hulvershorn:** None. **P. Finn:** None. **J.W. Brown:** None.

Poster

414. Electrical Stimulation: Human and Non-Human Primates

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 414.22

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH R01-EB026439
NIH U24-NS109103
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NIH R01-NS101013
NIH R01-CA203861
NIH R01-MH120194

Title: Transcutaneous auricular vagus nerve stimulation triggers a dose-dependent stimulation-evoked response in the brain

Authors: *K. M. DONOVAN[1], T. XIE[2], M. ADAMEK[3], H. CHO[2], J. T. WILLIE[2], P. BRUNNER[2,4], E. C. LEUTHARDT[2];
[1]Biomed. Engin., [2]Neurosurg., [3]Neurosci., Washington Univ. in St. Louis, Saint Louis, MO;
[4]Natl. Ctr. for Adaptive Neurotechnologies, Albany, NY

Abstract: Transcutaneous auricular vagus nerve stimulation (taVNS) is under investigation for several clinical applications ranging from stroke rehabilitation to anti-inflammatory interventions. However, the underlying neuronal response to taVNS, in addition to the effect of stimulation intensity, is not well understood. To better probe potential mechanism(s) of action for taVNS, we administered taVNS across a range of intensities to patients undergoing predominantly fronto-temporal stereotactic electroencephalography (sEEG) procedures. Three invasively monitored human subjects (median of 15 trajectories) participated in the study; across all subjects, sEEG signals from a total of 512 electrode contacts were analyzed. In this study, taVNS delivery alternated between 5 minutes off, 1 minute on, for a total of four stimulation rounds. Three stimulation rounds were administered with electrodes on the tragus and cymba concha (active) at amplitudes of 0.5, 1.5, and 3.0 mA, and one stimulation round was administered with electrodes on the earlobe (sham) at an amplitude of 1.5 mA. All recordings were visually inspected to exclude time windows containing inter-ictal spikes. To quantify the presence of a stimulation-evoked response, power in the 88-92 Hz frequency band was computed across conditions and z-scored to the baseline power prior to the first stimulation round. 90 Hz was chosen as the central frequency due to it being a harmonic of the stimulation frequency and having lower magnitude resting power. We found that administering taVNS at amplitudes of 0.5, 1.5, and 3.0 mA in the active site triggered a significantly greater response across electrodes as compared to sham in all three subjects (Kruskal-Wallis test). Additionally, the responses were stronger at 1.5 and 3.0 mA than at 0.5 mA for all subjects, though the response at 3.0 was only significantly higher than 1.5 mA in two subjects. Across all subjects, the stimulation response was not uniform in all electrodes, with electrodes in middle temporal and orbitofrontal cortices showing stronger power. These results suggest that electrical stimulation delivered to the auricular branch of the vagus nerve triggers a central response in the brain that is both region-specific and dose-dependent. Further, stimulating at the same magnitude in a physically proximal region of the ear (the earlobe) that is not innervated by the vagus nerve does not trigger the same response as the z-scores are concentrated around 0, suggesting specificity in the stimulation-evoked response. Our findings improve our understanding of the effect of taVNS intensity on the central nervous system, as well as the mechanisms of action of taVNS.

Disclosures: K.M. Donovan: None. **T. Xie:** None. **M. Adamek:** None. **H. Cho:** None. **J.T. Willie:** None. **P. Brunner:** None. **E.C. Leuthardt:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurolutions, Sora Neuroscience, Inner Cosmos.

Poster

414. Electrical Stimulation: Human and Non-Human Primates

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 414.23

Topic: I.08. Methods to Modulate Neural Activity

Support: Defense Advanced Research Projects Agency (DARPA) /N66001-17-2-4018
Chilean National Agency for Research and Development (ANID) /BIO-Fulbright Scholarship 2016
GPSA Fall 2021 JumpStart Grant

Title: Effects of online transcutaneous trigeminal nerve stimulation on visuomotor learning

Authors: *D. E. ARIAS, C. A. BUNEO;
Arizona State Univ., Tempe, AZ

Abstract: Stimulation of cranial nerves with low-intensity current represents a novel neuromodulation approach for addressing the symptoms of several neurological disorders. For example, in the rehabilitation setting, vagus nerve stimulation (VNS) combined with conventional therapy results in greater recovery of upper limb function following stroke than conventional therapy alone. Typically, VNS requires a surgically implanted electrode, but this may not be desirable in all circumstances, necessitating non-invasive alternatives. Trigeminal nerve stimulation (TNS) is a potential candidate because it affects similar anatomical areas as VNS, and it can be easily stimulated through the skin via its ophthalmic branches. To assess its potential in rehabilitation, we previously investigated the effects of offline TNS (delivered *prior* to task performance) on motor learning and found evidence for frequency-dependent effects on learning. In this study, we explored the effects of online TNS (delivered *during* task performance). Fifty-one (51) right-handed healthy subjects (10 women, 41 men; 18-31 y/a), were randomly assigned to 3 groups based on the stimulation frequency: 120 Hz (n=18), 60 Hz (n=16) and sham (n=17). Participants performed a visuomotor rotation task that involved goal-directed arm movements to 8 targets arranged in a vertical plane. Four blocks of trials were performed as follows: one baseline block with veridical visual feedback, one adaptation block involving a 30^{o} CCW rotation of hand visual feedback and concurrent TNS (only during this block), another block with rotated feedback, and one washout block where the rotation was removed. TNS was delivered by two electrodes placed on the forehead. Each subject set the current to a sub-maximum tolerable level (group mean current; 120 Hz: 3.16 ± 0.82 mA, 60 Hz: 3.09 ± 0.81 mA). No current was applied to the sham group. The directional error (DE), defined as the angular difference between the hand position and visual feedback, decayed exponentially during the adaptation block. DEs were averaged across groups and fitted to double exponential models to quantify motor learning. Preliminary analyses showed that the 120 and 60 Hz groups demonstrated faster learning rates in the early phase of the adaptation. However, rates converged to the same levels as the sham in the late adaptation. In addition, learning rates with 60 Hz online TNS were markedly faster than those observed previously with 60 Hz offline TNS. This was also shown by the 120 Hz group, but the differences were smaller. The results suggest that the frequency-dependent effects of TNS on visuomotor adaptation depend on when TNS is applied along with the task.

Disclosures: D.E. Arias: None. **C.A. Buneo:** None.

Poster

414. Electrical Stimulation: Human and Non-Human Primates

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 414.24

Topic: I.08. Methods to Modulate Neural Activity

Title: Transcutaneous electrical stimulation alters physiological biomarkers of stress activation and marksmanship performance during high cognitive load

Authors: *C. RIDGEWELL, W. H. NEUMEIER, D. J. MERULLO, A. HILDEBRANDT, N. EKON, V. PECORELLI, D. ZEPPETELLI, K. J. HEATON;
USARIEM, Natick, MA

Abstract: Military training and operations place heavy demands on service members' cognitive and physical resources, contributing to poor health and performance. Transcutaneous electrical stimulation (TES) is a non-invasive neuromodulatory method with purported cognitive benefits. However, the effects of TES on cognition and performance in healthy adults, as well as its impact on the human stress response, remain unclear. A single-blind, cross-over study design with repeated measures was used to evaluate the effects of TES compared to sham on physiological and biochemical markers of stress and cognitive and marksmanship performance in 30 healthy volunteers (5 women; mean ± SD, age 25.20 ± 8.43 years). Participants completed a marksmanship task following 20-minutes of TES of the trigeminal nerve or sham using a commercially-available device (Thync One, Cerevast Therapeutics), on each of two consecutive days, in counterbalanced order. The marksmanship test was a friend vs. foe discrimination task using an Engagement Skills Trainer (EST 2000) with a modified M4 rifle. Computer-based measures of attention, working memory, and response inhibition, as well as salivary cortisol and α-amylase and subjective workload, were collected before, during and after marksmanship; electrocardiography (ECG) and photoplethysmography (PPG) were monitored continuously. Linear mixed models analyzed the effect of TES on marksmanship, cognitive, physiological, and salivary outcomes. Effects of TES were found for heart rate variability measured with ECG and PPG ($p < 0.01$ - 0.048), and marksmanship shot accuracy ($p = 0.01$) and distance of shots from the targets' center of mass ($p = 0.02$). TES was associated with greater sympathetic (SNS) and parasympathetic (PNS) nervous system activity and poorer shot accuracy compared to sham. A condition-by-time interaction effect on target detection latency ($p = 0.04$) was also observed. There were no effects of TES on accuracy or response times for cognitive tasks. No effects of stimulation were found for salivary stress biomarkers or for cognitive or physical workload. Results indicate that TES influences stress physiology and performance. However, the heightened SNS and PNS responses and impaired shot accuracy observed in this study may reflect the specific stimulation parameters used; different parameters may produce different results. Overall, these results provide support for the use of TES for neuromodulation but its use for performance optimization warrants further investigation.

Disclosures: **C. Ridgewell:** None. **W.H. Neumeier:** None. **D.J. Merullo:** None. **A. Hildebrandt:** None. **N. Ekon:** None. **V. Pecorelli:** None. **D. Zeppetelli:** None. **K.J. Heaton:** None.

Poster

414. Electrical Stimulation: Human and Non-Human Primates

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 414.25

Topic: I.08. Methods to Modulate Neural Activity

Title: The evaluation of safety and efficacy of a novel electrode configuration targeting specific auricular vagal dermatomes to improve opioid withdrawal symptoms: A pilot study

Authors: C. TIRADO[1], S. WASHBURN[2], M. MCWADE[2], C. BENNER[2], C. HEDENBERG[1], A. COVALIN[2], ***N. KHODAPARAST**[2];
[1]CARMAhealth, PLLC, Austin, TX; [2]Spark Biomedical, Inc., Dallas, TX

Abstract: The United States is experiencing an epidemic for prescription and non-prescription opioids. In 2020, the Center for Disease Control reported 93,331 substance use overdose deaths. The continuing increase in opioid-related deaths from 2015 (18%) to 2020 (60%) may partly be attributed to the mental health crisis during the Covid-19 pandemic. The emergence of opioid withdrawal syndrome (OWS) can be a significant barrier for dependent individuals to cease opioid consumption. There is a need for a non-opioid intervention to mitigate symptoms of OWS. Recently, neurostimulation approaches have earned scientific interest and demonstrated treatment efficacy in reducing opioid withdrawal symptoms. However, these neurostimulation therapies vary in degree of effectiveness and invasiveness - from deep brain to percutaneous stimulating electrodes.
Spark has developed a non-invasive transcutaneous auricular neurostimulation (tAN) system, called the Sparrow Therapy System, for the treatment of OWS. The first-generation Sparrow tAN System was designed to target the auricular branch of the vagus nerve (ABVN; via cymba concha region) and the auriculotemporal nerve (branch of trigeminal nerve; temporomandibular region). This prospective, open-label pilot trial studied a novel electrode configuration designed to target the vagus nerve via the tragus dermatome as an alternative to the cymba concha to compare its safety and efficacy in reducing opioid withdrawal symptoms.
Six participants with OWS entering acute detox treatment were enrolled at a single site and received tAN therapy with the novel tragus electrode configuration. The Clinical Opiate Withdrawal Scale (COWS) was used to determine withdrawal level, and participants were required to have a baseline COWS score $\geq$ 13 before enrollment. Results demonstrated a mean (SD) COWS reduction of 6.7 (6.0) points (37.6% decrease) from baseline to 60 minutes after start of tAN therapy in the patients. In comparison, prior data on the Sparrow tAN targeting the ABVN via the cymba concha demonstrated a mean (SD) COWS reduction of 7.7 (4.52) points (50.4% decrease) from baseline to 60 minutes. Two adverse events were reported related to discomfort of the earpiece clip on the tragus.

In conclusion, this study implicates that activating the ABVN via the tragus dermatome is inferior to the cymba concha dermatome for alleviating opioid withdrawal symptoms. The results of this trial informed the next-generation earpiece design of the Sparrow tAN system, which received FDA clearance in 2020 (K201873).

Disclosures: **C. Tirado:** F. Consulting Fees (e.g., advisory boards); Spark Biomedical. **S. Washburn:** A. Employment/Salary (full or part-time):; Spark Biomedical. **M. McWade:** A. Employment/Salary (full or part-time):; Spark Biomedical. **C. Benner:** A. Employment/Salary (full or part-time):; Spark Biomedical. **C. Hedenberg:** None. **A. Covalin:** A. Employment/Salary (full or part-time):; Spark Biomedical. **N. Khodaparast:** A. Employment/Salary (full or part-time):; Spark Biomedical.

Poster

414. Electrical Stimulation: Human and Non-Human Primates

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 414.26

Topic: I.08. Methods to Modulate Neural Activity

Support: Medical University of South Carolina National Center for Neuromodulation Pilot Grant
NIH P2CHD086844

Title: Transcutaneous Spinal Cord Stimulation to Reduce Phantom Limb Pain

Authors: *A. N. DALRYMPLE[1], L. E. FISHER[2], D. J. WEBER[1];
[1]Mechanical Engin., Carnegie Mellon Univ., Pittsburgh, PA; [2]Physical Med. & Rehabil., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Phantom limb pain (PLP) is debilitating and affects up to 85% of amputees. Neuromodulation of the spinal cord can be used to reduce chronic pain in a variety of conditions; however, traditional spinal cord stimulation requires a surgical implant. Here we explore the use of a non-invasive neuromodulation method, transcutaneous spinal cord stimulation (tSCS), to reduce PLP in lower-limb amputees.In chronic pain conditions, plastic changes may occur in neural circuits of the spinal cord resulting in increased excitability, which has been linked to pain syndromes. Altered spinal excitability can be measured using recordings of evoked reflexes, such as the posterior root-muscle (PRM) reflex. PRM reflexes are considered to be similar to the Hoffman reflex (H-reflex) but are elicited through stimulation of sensory axons at the spinal roots.We have recruited three lower limb amputees, two males (5- and 9-years post-amputation, traumatic) and one female (3 months post-amputation, diabetic dysvascular) for this 5-day study. We measured their pain using the McGill Pain Questionnaire (MPQ) and the Pain Pressure Threshold Test on days 1 and 5. Each day, we measured spinal reflex excitability using PRM reflexes. We provided neuromodulation using tSCS at 30 Hz with a carrier frequency of 10 kHz for 30 minutes/day for each of the 5 days. We hypothesized that the spinal cord will be

hyperexcitable, indicated by low reflex thresholds, and that tSCS can reduce the reflex hyperexcitability towards normal. We also hypothesized that tSCS could reduce pain scores and increase pain pressure thresholds.Here we show that tSCS can reduce phantom limb pain over 5 days. Mean MPQ scores decreased from 34.0 (± 7.0) on day 1 to 18.3 (± 6.8) on day 5, which was a clinically meaningful difference. Two subjects had increases in their pain pressure thresholds across several locations of their residual limb (Day 1: 5.4 ± 1.6 lbf; Day 5: 11.4 ± 1.0 lbf), demonstrating an increased tolerance of mechanical stimuli. Surprisingly, PRM reflex thresholds were much higher in the amputees (60.0 ± 5.0 μC) than in controls (35.6 ± 11.6 μC), suggesting that in PLP, reflexes are hypoexcitable, not hyperexcitable. However, throughout the 5 days of tSCS, the reflex thresholds for all subjects significantly decreased towards control thresholds (50.3 ± 6.8 μC; $p = 0.02$).The mechanisms of PLP and how it affects spinal cord excitability appears to be different from other chronic pain syndromes. tSCS is a comfortable, effective, and non-invasive neuromodulation method that can reduce PLP in lower-limb amputees and should be explored by further study.

Disclosures: **A.N. Dalrymple:** None. **L.E. Fisher:** None. **D.J. Weber:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bionic Power, Inc., BlackFynn, Inc., Iota Biosciences, Inc., Neuronoff, Inc., Neuroone, Inc., Reach Neuro, Inc.. F. Consulting Fees (e.g., advisory boards); Neuronoff, Inc., Neuroone, Inc..

Poster

414. Electrical Stimulation: Human and Non-Human Primates

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 414.27

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant UH3NS100541

Title: Restoring sensations by percutaneous spinal cord stimulation in people with lower limb amputation

Authors: *R. BOSE[1,3], A. NANIVADEKAR[1], B. PETERSEN[1,3], D. SARMA[4], E. OKOROKOVA[5], S. J. BENSMAIA[6], E. HELM[2], M. BONINGER[4,2], D. J. WEBER[7], L. E. FISHER[2];
[1]Rehab Neural Engin. Labs, [2]Physical Med. and Rehabil., Univ. of Pittsburgh, Pittsburgh, PA; [3]Ctr. for Neural Basis of Cognition, Pittsburgh, PA; [4]Univ. of Pittsburgh, Rehab Neural Engin. Labs, Pittsburgh, PA; [6]Dept. of Organismal Biol. and Anat., [5]Univ. of Chicago, Chicago, IL; [7]Mechanical Engin. and Neurosci. Inst., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Sensory feedback is crucial for tasks involving the lower limbs such as balance and gait. People with lower-limb amputation lack feedback from the missing limb, which contributes to abnormal gait patterns, impaired balance, and increased risk of falling. In addition, nearly 85%

of amputees experience phantom limb pain, which may be tied to the lack of sensory feedback from the limb. A major goal of our research is to restore sensations in the missing limb, reduce phantom limb pain and improve lower-limb functions using electrical stimulation of the spinal cord. In this context, we evaluated (1) the locations and stability of percepts evoked by stimulating the ipsilateral lumbar spinal cord, (2) the perceptual quality (i.e. modality and intensity) of these percepts, (3) functional assessment of balance and gait with restored sensory feedback, and (4) changes in subjects' phantom limb pain. We implanted two or three 8- or 16-contact spinal cord stimulation leads in the epidural space near the lumbar spinal cord in three human subjects with transtibial amputation over a period of 29 days (for Subject 1 and 2) and 90 days (for Subject 3). We delivered stimulation through monopolar and multipolar combinations of contacts over a wide range of stimulation parameters (e.g. amplitude, frequency) and recorded the location, modality, and intensity of the evoked sensations. We conducted standard psychophysical assessments to measure the just noticeable difference and threshold for evoking a percept. We also performed clinical functional assessment of gait and balance while giving real-time sensory feedback with spinal cord stimulation. The subjects also reported their phantom limb pain across the implant days using a visual analog scale and the McGill pain questionnaire. In all subjects, spinal cord stimulation elicited sensations localized to the ankle and foot of the missing limb along with sensations in the residual limb. The threshold for evoking sensations in the missing limb decreased over time but was always higher than the threshold for evoking sensation in the residual limb. The evoked percepts were described as a combination of both naturalistic and paresthetic. Additionally, we observed in Subject 3 that a short duration (~10 minutes) of tonic stimulation facilitated the perception of evoked sensation in the missing limb. We also observed clinically meaningful improvement in their balance and gait measures. Further, in all subjects, there was also a clinically meaningful reduction in phantom limb pain across the duration of the implant. These results demonstrate the potential of using spinal cord stimulation to restore sensation in lower limb amputees.

Disclosures: **R. Bose:** None. **A. Nanivadekar:** None. **B. Petersen:** None. **D. Sarma:** None. **E. Okorokova:** None. **S.J. Bensmaia:** None. **E. Helm:** None. **M. Boninger:** None. **D.J. Weber:** None. **L.E. Fisher:** None.

Poster

414. Electrical Stimulation: Human and Non-Human Primates

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 414.28

Topic: I.08. Methods to Modulate Neural Activity

Support: UH3NS100541

Title: Evaluating the myoelectric effects of epidural stimulation for the restoration of physiologic function in trans-tibial amputees

Authors: *D. SARMA[1], B. PETERSEN[4], R. BOSE[5], A. C. NANIVADEKAR[6], A. N. DALRYMPLE[2], E. HELM[7], M. CAPOGROSSO[8], L. E. FISHER[7], D. J. WEBER[3]; [1]Mechanical Engin., Carnegie Mellon Univ., PITTSBURGH, PA; [2]Mechanical Engin., [3]Mechanical Engin. and Neurosci. Inst., Carnegie Mellon Univ., Pittsburgh, PA; [4]Rehab Neural Engin. Labs, [5]Bioengineering, [6]R, [7]Physical Med. and Rehabil., [8]Neurolog. Surgery, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Sensory inputs to the lower-limb evoke coordinated spinal reflexes, mediating gait transitions and maintaining stability after unexpected perturbations. Without these sensory inputs, lower-limb amputees experience double the annual incidence of falls as non-amputees. Lumbar epidural spinal cord stimulation (eSCS) modulates dysregulated lower-limb sensorimotor function and augments residual motor capacity. In three below-knee amputees, eSCS was delivered through commercial percutaneous leads implanted (<90 days) over the lumbar enlargement and dorsal rootlets. eSCS was delivered at pulse widths of 0.2 to 1ms at 1-50Hz across amplitudes ranging from 0.1mA to 6mA. Participants reported any stimulation-induced sensations. Surface electromyographic (EMG) activity was recorded bilaterally. Evoked compound muscle action potentials (CMAP) were calculated via stimulation-triggered averaging. For the two participants whose amputations were related to complications from diabetes, the mean stimulation threshold for sensory percepts (1.5mA +/- 0.5mA) was lower than the mean threshold for evoking CMAPs (2mA +/- 0.5mA). For the other participant, whose amputation was due to trauma, there was no significant difference in the mean stimulation thresholds for sensation (1.25mA +/- 0.25mA) and evoked reflexive responses (1mA +/- 0.25mA). For all three participants, CMAPs increased with increasing stimulation amplitudes with an overall rostral to caudal recruitment of muscles aligning to the rostral-caudal orientation of the electrodes. Using non-negative matrix factorization, up to five muscle synergies, aligned to anatomical groups, were calculated from the evoked CMAPs. For a subset of caudal contacts, stimulation >50Hz evoked percepts in participants' missing feet. The reported locations of the percepts evoked in the missing limb as compared to the residual limb were correlated ($r > 0.85$) to an increased EMG response in all residual muscles. During ambulation, supra-threshold eSCS (between 1.5-3mA) on electrodes evoking sensations in the missing foot increased the EMG amplitude of the residual quadriceps and lateral gastrocnemius during the stabilization period of the stance phase. In order to develop a clinically relevant stimulation protocol that effectively restores foot and leg sensations, the normal activity of the leg muscles needs to be preserved within a physiological range. These early results show that restoring sensation by eSCS can produce effects on muscle recruitment during locomotion. Further work is needed to understand the relationship between sensory and motor responses evoked by eSCS.

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